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Breeding strategies for wine grapes: From genetic analysis of agronomic traits to wine sensory evaluation
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Breeding strategies for wine grapes:

"From genetic analysis of agronomic traits to wine sensory evaluation"

Cristina Manso Martínez

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Breeding strategies for wine grapes: "From genetic analysis of agronomic traits to wine sensory evaluation"

Memoria presentada por:

Cristina Manso Martínez

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Dra. María del Mar Hernández Alamos

Departamento de Agricultura y Alimentación

Las doctoras Cristina Menéndez Menéndez y María del Mar Hernández Álamos, investigadoras del Instituto de Ciencias de la Vid y del Vino (Universidad de La Rioja, CSIC, Gobierno de la Rioja,) y profesoras de la Universidad de La Rioja

INFORMAN:

Que la presente memoria titulada "Breeding strategies for wine grapes: from genetic analysis of agronomic traits to wine sensory evaluation" ha sido realizada por Cristina Manso Martínez en el Departamento de Agricultura y Alimentación de la Universidad de La Rioja bajo nuestra dirección, y reúne las condiciones exigidas para optar al grado de Doctor.

Lo que hacen constar en Logroño, a 19 de junio de 2020

Dra. Cristina Menéndez Menéndez   Dra. María del Mar Hernández Álamos

List of publications obtained from the PhD research

This PhD thesis has resulted so far in the following scientific papers:

1. Manso-Martínez, C., Sáenz-Navajas, M. P., Hernández, M. M., & Menéndez, C. M. (2020). Sensory profiling and quality assessment of wines derived from Graciano × Tempranillo selections. *LWT - Food Science and Technology.* https://doi.org/10.1016/j.lwt.2020.109394.

2. Manso-Martínez, C., Sáenz-Navajas, M. P., Hernández, M. M., & Menéndez, C. M. (2020). Wine quality and berry size: a case study with Tempranillo progenies. *LWT - Food Science and Technology* (under revision).

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Abbreviations

Abbreviation	Full name
a_{10}/b^*_{10}	Red / yellow ratio
ANT	Anthocyanin content
AFLP	Amplified fragment length polymorphism
BD	Berry diameter
BL	Berry length
BS	Berry shape
BW	Mean berry weight
c	century
CAPS	Cleaved amplified polymorphic sequence
Ce	Expected genome map coverage
CI	Colour intensity
cM	centi Morgan
CN	Cluster number
cv.	Cultivar
CW	Mean cluster weight
DNA	Deoxyribonucleic acid
EV	End Veraison date
F	Flowering
FAM	6-carboxy-fluorescine
FAO	Food and agriculture organisation
FD	Flower diameter
FI	Fertility index
F-V	Flowering - Veraison interval
GAR	Grenache
GBS	Genotype-by-sequencing
Ge	Estimated genome length
Go	Observed genome length
GRA	Graciano
KW	Kruskal-Wallis test
LG	Linkage group
LOD	Logarithm of odd ratio
L^*	Lightness
MA	malic acid

MAS	Marker assisted selection
Mbp	Megabase pair
NGS	Next generation sequencing
OIV	International Organisation of Vine and Wine
OL	Ovary length
OS	Ovary shape
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
pH	Power of hydrogen
PL	Pistil length
PS	Pistil shape
Q	Quality
QTL	Quantitative trait loci
r	Coefficient of correlation
RD	Ripening
S	Sprouting
SD	Standard deviation
S-F	Sprouting-Flowering interval
SN	Mean seed number
SNP	Single Nucleotide Polymorphism
SO_{2F}	Free dioxide sulfur
SO_{2T}	Total dioxide sulfur
Spp.	Species
SSR	Single Sequence Repeat
SV	Start Veraison date
SW	Mean seed weight
TA	Total acidity
TE	Tempranillo
TPI	Total phenol index
TSS	Total soluble solid
V	Veraison
VA	Volatile acidity
var.	Variety
VIVC	Vitis International variety catalog
VMC	Vitis Microsatellite Consortium
VL	Veraison length

V-R	Veraison - Ripening interval
Y	Yield
º B	Degree Baumé
$\chi 2$	Chi - square
% ETH	% Ethanol

Summary

Grapevine (*Vitis vinifera* L.) is one of the most widely cultivated and highest value horticultural crops in the world due to the economic value of wine, being one of the most valuable agricultural products in Europe. Current wine grape breeding programmes are focusing on wine quality, disease resistance and climate change adaptation which should also bring about new varieties that match new consumer preferences in the market. Therefore, relevant traits in wine grape breeding include berry weight and composition, high phenolic content, high acidity, early or late phenology, and moderate productivity with low alcohol content. Small berry size is a key factor that influences grape composition and presumably improves wine quality due to the higher concentration of aromatic and phenolic compounds in the grape skin. On the other hand, final berry shape is thought to be predictable at the ovary stage being that final berry size is correlated with flower morphology which could be also influenced by flower sex.

The main goal of this research was to develop strategies for wine grape breeding. Two specific objectives were considered: 1) analysis the genetic basis of target traits such as berry size and morphology, flower sex, production and phenological periods 2) assessment of the influence of berry size in the oenological composition of Tempranillo segregating progenies and Pinot Noir clones and the sensory evaluation of the wines derived from Tempranillo selections.

Two segregating progenies derived from Grenache × Tempranillo (130 genotypes) and Graciano × Tempranillo (151 genotypes) were evaluated for 26 traits, including berry, flower, seed, phenology and productivity for up to four years and the influence of sex was assessed at the phenotypic level. When compared with hermaphrodites in both genetic backgrounds, female plants showed rounder flower shape, larger flower diameter, lower number of seeds, and a delay in flowering and veraison onset dates.

The Grenache × Tempranillo progeny was genotyped using GBS methodology with 4452 polymorphic SNP (Single Nucleotide Polymorphism) markers. A consensus genetic map was constructed with 1296 SNP and 4 SSR markers covering 1540 cM distributed in 19 linkage groups, with an average interval length of 1.2 cM between markers. The Grenache map consisted of 1011 markers spanning 1364.5 cM while the Tempranillo map covered 1237.7 cM with 826 markers and an average distance of (1.4) and (1.5) cM respectively.

Quantitative Trait Loci (QTL) analyses of 26 traits including berry and flower morphology, must composition, productivity and phenological parameters were conducted in the Grenache × Tempranillo progeny in two to four seasons from 2014 to 2017; the influence of *Sex* locus was also assessed. In addition, QTL analyses were conducted for berry, flower, and seed traits in the Graciano progeny for two seasons. A QTL region in LG17 was found in Grenache × Tempranillo progeny significantly associated to berry size, productivity traits and phenology stages; whereas in LG7 and LG13 QTL for flower morphology and flowering date suggest close linkage or pleiotropic effects. In Graciano × Tempranillo population, regions in LG3 and LG5 were associated mainly to berry size and seed traits. QTL on LG5 for berry, seed and flower traits in Graciano × Tempranillo progeny covered the region of *FERONIA* locus, and a QTL on LG18 found for seed traits resulted associated to locus *SDI*. In relation to flower morphology, the QTL region on LG11 had the strongest and most stable effect over the two years in both genetic backgrounds and a candidate gene VIT_11s0016g03650 with a function associated to pollen morphology was proposed. Highly significant QTL were found for total acidity on LG4, LG12, LG13, LG14 and LG17 in Grenache × Tempranillo progeny. Concerning phenological traits, in Grenache × Tempranillo progeny, veraison dates showed significant associations with genomic

regions in LG11 and LG17; ripening dates resulted to be significantly associated to LG8, LG11 and LG13. In Graciano × Tempranillo progeny, co-localizations of QTL for flower morphology, seed traits and phenology events were detected in LG3 and LG11. Moreover, a QTL region in LG2 was detected for flower-morphology, seed, productivity traits, and phenological stages (flowering date, veraison), confirming the influence of flower sex in the genetic determinism of these characters.

Influence of berry size on wine composition was studied in two Tempranillo segregating progenies and in Pinot noir clones. Consistently, wines obtained from small berry genotypes presented higher proportions of phenolic compounds and deeper colour. Higher quality scores were obtained for small berry size wines regardless of genetic background and vintage; the wines were described as sweeter, fruitier and with greater astringency. Pinot Noir clones presented differences in berry morphology and nitrogen compounds independently of the subregion studied. Environmental conditions and rootstock were found to influence parameters such as berry size, nitrogen compound accumulation, and phenolic composition in Pinot Noir clones.

All traits except berry shape showed transgressive segregation and large phenotypic variability in both progenies, these are essential for the selection of new genotypes with improved attributes. Eleven red and eleven white genotypes were pre-selected in the Grenache × Tempranillo population based on berry weight, cluster weight, acidity and ripening date. Whilst in Tempranillo × Graciano population, evaluation of twelve pre-selected hybrids including physicochemical and sensory properties of wines, were conducted in 2017 and 2018. Two early ripening selections, TG8 and TG63 were consistently perceived as higher quality than Graciano and Tempranillo, in two very different vintages. Moreover, TG129, a late ripening selection, was perceived as a good option for future climatic conditions. Wines from TG35 or TG128 genotypes provided distinct sensory characteristics (roasted notes) which are valuable for the necessary diversification of the wine market.

Results of this research reveal novel insights into the genetic control of relevant traits for wine grapes, and will be useful for breeding new genotypes with better quality features and adaptation to new consumption patterns. This is the first physicochemical and sensorial evaluation of young red wines elaborated with Tempranillo intraspecific hybrid grapes. Despite an important effect of vintage on sensory properties of wines, selected genotypes were able to produce quality wines with great sensory variability, therefore, confirming that intraspecific hybridization is a useful tool to improve traditional varieties and meet new consumer demands.

Keywords: Tempranillo progenies, QTL, berry size, flower sex, *Vitis vinifera* L.

Resumen

La vid (*Vitis vinifera* L.) es uno de los cultivos más extendidos y de mayor valor económico, debido a su principal producto, el vino, siendo uno de los productos más valiosos en la agricultura en Europa. Los principales ámbitos de mejora para la uva de vinificación son la calidad del vino, la resistencia a enfermedades y la adaptación al cambio climático, debiendo además estas nuevas selecciones satisfacer las nuevas preferencias del consumidor en el mercado. Entre los parámetros más relevantes en las uvas para vinificación están el peso y la composición de las bayas, alto contenido fenólico, alta acidez, fenología temprana o tardía, y productividad moderada con bajo contenido alcohólico. Un menor tamaño de baya es un factor clave que influye en la composición de la uva y presumiblemente mejora la calidad del vino obtenido, debido a la mayor concentración de compuestos aromáticos y fenólicos en la piel de la uva. Además, se estima que la forma final de la baya es predecible en la etapa de desarrollo del ovario, correlacionándose el tamaño final de la baya con la morfología de la flor, que a su vez podría estar influenciada por el sexo de la flor.

El objetivo principal de esta investigación fue desarrollar estrategias para la mejora de la uva de vinificación. Se consideraron dos objetivos específicos: 1) analizar la base genética de los caracteres de interés, como el tamaño y la morfología de las bayas, el sexo de las flores, la productividad y los estadíos fenológicos 2) evaluar la influencia del tamaño de la baya en la composición enológica de progenies segregantes de Tempranillo y clones de Pinot Noir, así como evaluar sensorialmente los vinos derivados de las selecciones de Tempranillo.

Se evaluaron dos progenies segregantes derivadas de Garnacha × Tempranillo (130 genotipos) y Graciano × Tempranillo (151 genotipos) para 26 parámetros relacionados con la baya, flores, semillas, fenología y productividad en hasta cuatro años, evaluándose además la influencia del sexo a nivel fenotípico. Las plantas femeninas mostraron una forma de flor más redondeada, un diámetro de flor más grande, un menor número de semillas y un retraso en la floración y en las fechas de inicio del envero en comparación con los hermafroditas en ambos fondos genéticos. La progenie de Garnacha × Tempranillo se genotipó utilizando la metodología GBS con 4452 marcadores polimórficos tipo SNP. Se construyó un mapa genético consenso con 1296 SNP y 4 marcadores SSR con una distancia de 1540 cM distribuidos en 19 grupos de ligamiento, con un intervalo promedio de 1,2 cM entre marcadores. El mapa de Garnacha constaba de 1011 marcadores que abarcaban 1364,5 cM, mientras que el mapa de Tempranillo resultó ser de 1237,7 cM con 826 marcadores y una distancia promedio de (1,4) y (1,5) cM respectivamente.

Se realizaron análisis de QTL de 26 caracteres, incluyendo morfología de la baya y de la flor, composición del mosto, productividad y parámetros fenológicos en la progenie Garnacha × Tempranillo a lo largo de 4 años 2014-2017, donde también se evaluó la influencia del locus del sexo. Además, a lo largo de dos años se realizaron análisis QTL para caracteres de baya, flor y semilla en la progenie Graciano × Tempranillo. Se encontró una región QTL en el GL17 en la progenie de Garnacha × Tempranillo asociada significativamente con el tamaño de la baya, los rasgos de productividad y los estadíos fenológicos, y en GL7 y GL13 QTL para la morfología de la flor y la fecha de floración, lo que sugiere una estrecha asociación o efectos pleiotrópicos. En la población Graciano × Tempranillo, regiones en GL3 y GL5 resultaron asociadas principalmente al tamaño de la baya y caracteres de semilla. Un QTL en GL5 fue encontrado relacionado con parámetros de baya, semilla y flor en la progenie Graciano × Tempranillo, cubriendo la región del locus *FERONIA*. En la misma población, se encontró un QTL en el GL18 para parámetros de semilla asociado al locus *SDI*. En relación con la morfología de las flores, una

región en el GL11 resultó altamente significativa y estable durante los dos años en ambos fondos genéticos, y se propuso un gen candidato VIT_11s0016g03650 con una función asociada a la morfología del polen. Se encontraron también regiones QTL altamente significativos para la acidez total en GL4, GL12, GL13, GL14 y GL17 en la progenie de Garnacha × Tempranillo. Con respecto a los estadíos fenológicos, en la progenie de Garnacha × Tempranillo, el periodo de envero mostró asociaciones significativas con las regiones genómicas en GL11 y GL17, y la fecha de maduración resultó significativamente asociadas a GL8, GL11 y GL13. En la progenie Graciano × Tempranillo, se detectaron co-localizaciones de QTL para morfología de la flor, caracteres de semilla y estadíos fenológicos en GL3 y GL11. Además, se detectó una región QTL en GL2 para la morfología de la flor, parámetros de semilla, rasgos de productividad y etapas fenológicas (fecha de floración, envero), lo que confirma la influencia del sexo de la flor en la determinación genética de estos caracteres.

La influencia del tamaño de la baya en la composición del vino se estudió en dos progenies segregantes de Tempranillo y en clones de Pinot noir. Consistentemente, los vinos obtenidos de genotipos de tamaño de baya pequeño presentaron una mayor concentración de compuestos fenólico, así como un color más intenso. Se obtuvieron mayores puntuaciones de calidad para los vinos de tamaño pequeño de baya, independientemente del fondo genético y de la vendimia en las progenies de Tempranillo, siendo descritos sensorialmente estos vinos como más dulces, frutales y con una mayor astringencia. Los clones de Pinot Noir presentaron diferencias en la morfología de la baya y en la acumulación de compuestos de nitrogenados en el mosto independientemente de la subregión estudiada. Se demostró que las condiciones ambientales y el portainjerto influyen en parámetros como el tamaño de la baya, composición fenólica y nitrogenadas en los clones de Pinot Noir.

Todos los caracteres estudiados, excepto la forma de la baya, mostraron segregación transgresiva y gran variabilidad fenotípica en ambas progenies, factores esenciales para la selección de nuevos genotipos con características mejoradas. Se seleccionaron once genotipos de uva tinta y once de uva blanca en la población Garnacha x Tempranillo estableciendo como criterios el peso de la baya, el peso del racimo, la acidez y la fecha de maduración, mientras que en la población Graciano × Tempranillo, se evaluaron durante 2 años, 2017-2018, las propiedades fisicoquímicas y sensoriales de los vinos de doce híbridos preseleccionados. Dos genotipos de maduración temprana, TG8 y TG63 fueron percibidos consistentemente como de mayor calidad que Graciano y Tempranillo, en dos añadas muy diferentes. Además, TG129, una selección de maduración tardía, se considera una buena opción dentro del contexto de cambio climático. Los vinos de los genotipos TG35 o TG128 proporcionaron perfiles sensoriales distintos (notas tostadas) interesantes para la necesaria diversificación del mercado del vino.

Los resultados de esta investigación revelan nuevos conocimientos sobre el control genético de caracteres relevantes para la uva de vinificación, y serán útiles para generar nuevos genotipos con una mejor calidad y adaptación a los nuevos patrones de consumo. Esta es la primera evaluación fisicoquímica y sensorial de vinos tintos jóvenes elaborados con uvas híbridas intraespecíficas de Tempranillo. A pesar del importante efecto de la añada en las propiedades sensoriales de los vinos, los genotipos seleccionados pudieron producir vinos de calidad con una gran variabilidad sensorial, lo que confirma que la hibridación intraespecífica es una herramienta útil para mejorar las variedades tradicionales y satisfacer las nuevas demandas de los consumidores.

Palabras clave: poblaciones Tempranillo, QTL, tamaño de baya, sexo de la flor, *Vitis vinífera.*

CHAPTER 1.
GENERAL INTRODUCTION

1. General Introduction

1.1. Economic importance

Grapevine (*Vitis vinifera* L.) is one of the major fruit crops, cultivated along 7,4 mha, (OIV 2019) through temperate and tropical regions around the world. Success of grapevine is partly due to its great adaptability to a wide range of different climates (from oceanic, cold continental, Mediterranean, subtropical, to hyper arid), and latitudes (Schultz & Stoll 2010). However, the ideal growing conditions are met between 30 and 50 degrees, latitude North and 30 and 40 degrees in the Southern Hemisphere, where the most famous and extensive grape producing regions are located (Reisch et al. 2012). Winemaking is the major use of grapes both in terms of quantity and production area, but fresh fruit consume, transformation into raisins and unfermented juice, production of vinegars, spirits, grape concentrates, jams, jellies and grapeseed oil are other of their uses (Myles et al. 2011, Reisch et al. 2012).

Mediterranean countries, where grapes have been grown for thousands of years, are still the world leading wine production and grape cultivation area, being Spain, France and Italy the three major grape growing countries, with 969, 789 and 702 Mha, respectively, representing a third of worldwide production (7400 Mha, OIV 2018). The expansion of Asian vineyards leaded by China which with a production of 875 Mha (OIV 2018) is threating the hegemony of Europe as the first continent in production for first time in the history. Other important regions are eastern regions of Turkey (448 Mha), western regions of the United States of America (430 Mha), and temperate areas of Argentina (219 Mha), Chile (212 Mha), South Africa (125 Mha), being situated New Zealand in the low part of the list (39 Mha) (OIV 2018). In total, around 75 million Tons of grapes were produced worldwide in 2018, and 292,3 Mill hl of wine (OIV 2018). In Spain 92 Denominations of Origin are recognized by the European Union, being DOCa (Denominación de Origen Calificada) Rioja with 65001 ha cultivated and a total of 339290 tons (296174 tons of red and 43116 tons of white grapes) produced, one of the most internationally renowned wine producing regions.

1.2. Taxonomy of Vitis

The Eurasian wild grape (*Vitis vinifera* ssp. *sylvestris*) is a dioecious, perennial, forest vine extensively grown in the Near East and the northern Mediterranean before its domestication (McGovern et al. 2017). Cultivated grapevines (*Vitis vinifera* ssp. *sativa*) were domesticated from wild populations of *Vitis vinifera* ssp. *sylvestris* which still grown along riverbank forests from Western Europe to central Asia and North Africa (Arroyo-Garcia et al. 2006, Reisch et al. 2012).

Grapevines are members of the *Vitaceae* family, which belongs to *Rhammales* order in the subclass *Rosidae* of Eudicots (Bouquet 2011). *Vitaceae* family is formed by around 1000 different species grouped into 17 different genera, from which only the genus *Vitis*, with two subgenera *Euvitis* and *Muscadinia*, have real agricultural interest (Reisch et al. 2012). *Euvitis* Planch and *Muscadinia* Planch. subgenus *Euvitis* (2n = 2x = 38) contains around 70 different species that are the most important in viticulture (Keller 2010). This subgenus is divided into three major groups of species that widely differ in their utility in agronomy, including Asian and American groups with around 30 species each, and the European or central Asian group that contains the widely cultivated *V. vinifera* L. species (Owens 2008). Among the Asian species, only *V. amurensis* has been domesticated and used for fresh fruit, juice wine and jelly production,

and although it contains high-yielding species they are mostly disease-susceptible. (Keller 2010). The American species, including *V. aestivalis, V. cinerea, V. labrusca, V. riparia, V. rupestris, or V. berlandieri* have been extensively used to produce rootstocks and fruiting cultivars characterized by pest and disease resistance but producing low yield and low-quality fruits (Owens 2008).

Although hybrids between the subgenera are usually sterile due to the difference in chromosome number (Reisch et al. 2012); hybrids between species within a subgenus are normally fertile and many interspecific hybrids between *Euvitis* species have been developed as scion and rootstock cultivars. In fact, most commercial grape cultivars belong to the species *V. vinifera*, cultivated grafted on varieties or hybrids of American *Vitis* species used as rootstocks due to their tolerance to diseases or cold temperature (Keller 2010). Few interspecific hybrid cultivars, obtained from crosses of *V. vinifera* with other species (e.g.: *V. labrusca, V. amurensis, V. riparia, V. rupestris, V. aestivalis*), are important in some local regions (Reisch et al. 2012), for cold and humid climates, or for disease resistance, but are generally considered lower quality specimens.

1.3. Historical origin and cultivar evolution

The cultivated grapevine (*Vitis vinifera* ssp. *sativa*) comes from its wild ancestor (*Vitis vinifera* ssp. *sylvestris*) after several domestication events (McGovern et al. 2017). Current cultivated grapevine shows important modifications compared to its predecessor, including the change from dioecy to hermaphroditism, the increase in the number and size of berries per bunch and modifications in seed morphology (This et al. 2006, Picq et al. 2014, Houel et al. 2013). Zhou et al. 2019, found that the diversity level of *V. v. sativa* samples is 94 % that of *V.v. sylvestris*, a far higher ratio of cultivated-to-wild diversity than in other species such as maize (83 %), rice (64 %), soybean , cassava (71 %) , or tomato (54 %).

Chemical findings in pottery fabrics of Georgia in the South Caucasus region, belonged to the early Neolithic period provide the first biomolecular archaeological evidence for viticulture and wine from the Near East, at 6000 – 5800 BC. The discovery was also confirmed by climatic, botanical and environmental analysis, being grape pollen and epidermal remains associated with that date and the pots found (McGovern et al. 2017). Humans spread cultivars first to close regions such as Egypt (Myles et al. 2011) and later to distant Mediterranean regions like Greece, both coasts of the Italian and Iberian peninsulas and the north of Africa (This et al. 2006). Secondary events of domestication and spontaneous hybridizations took place among selected individuals and wild progenies (Arroyo-García et al. 2006, Sefc et al. 2003), increasing crop variability. Spanish, Dutch and French missionaries introduced the European varieties in America as seeds or cuttings around the 16th century, and varieties also reached South Africa, Australia and New Zealand at the beginning of the 19th century (This et al. 2006). Most of the crop diversity generated after all this process of expansion was drastically reduced in European vineyards due to the arrival of phylloxera aphid. Differential selection of genotypes for table and wine during domestication led to a significant phenotypic diversity of current varieties, being cultivars with large, fleshy berries and loose bunches selected for their use as table grape varieties, whereas cultivars with smaller, more compact bunches bearing smaller and juicier berries were preferred for winemaking (Bacilieri et al. 2013, This et al. 2006). Thus, genetic stratification of modern cultivars has been related to human interests and geographical factors, (Wolkovich et al. 2018)

by linking morpho-geographic grouping and haplotypes defined by nuclear and chloroplast DNA (Arroyo-García et al. 2006).

The first classification made by Negrul (1946) differentiated cultivars into three groups: *proles occidentalis*, *pontica* and *orientalis*, attending to bunch and berry morphological differences and geographical origin. The *proles occidentalis* was characterised by wine cultivars of Western European origin with compact and small bunches and berries such as “Riesling”, “Pinot “, “Sauvignon”. The *proles* o*rientalis* consisted of table cultivars from Central Asia with large and loose bunches and fleshy berries including “Muscat d”Alexandrie”, “Sultanine”, whilst *proles pontica* included a group with intermediate characteristics as displayed in “Vermentino” or “Clairette” varieties (Levadoux 1956). Bacilieri et al. (2013) also identified different levels of stratification attending to geographic origin (Iberian Peninsula, West and Central Europe, Balkans and East Europe) and use (wine and table cultivars). In a similar approach, Emanuelli et al. (2013) classified 1659 *V. sativa* into four different groups with a set of SSR markers; the first integrated by Italian / Balkan wine cultivars, the second with Mediterranean table/wine cultivars, the third with the Muscats varieties, and the last group including Central European wine grapes.

DNA fingerprinting allowed to estimate the number of different *V. vinifera* genotypes cultivated across the globe (This et al. 2006), in between 6000 and 10000, being many of them closely related (Myles et al. 2011). The real number is difficult to determine due to the existence of many synonyms (different names for the same cultivar, like “Sultanina” and “Thompson seedless”) and homonyms (identical name for different varieties; such as Malvasía) (Cattonaro et al. 2014). Genetic variability among cultivars is related to variation in agronomical traits such as ripening time, yield, berry size or resistance/tolerance to biotic and abiotic stresses (Duchene 2016).

Winemaking varieties are the result of hybridization (spontaneous or artificial) or somatic mutations. Mutations affecting only some of the cell layers of plant tissues give rise to "chimeras" such as Pinot Gris and Pinot Meunier (Franks et al. 2002). Moreover, somatic mutations caused by small changes in the genome generate new varietal forms within a variety, differing agronomically or morphologically from the original (“Grenache Blanca”, “Grenache Gris”, and “Grenache Peluda” or “Tempranillo Royo” and “Tempranillo Blanco”) (García-Brunton et al. 2018). However, the most famous *V. vinifera* cultivars are the result of intraspecific hybridizations being “Müller-Thurgau”, “Alicante H. Bouschet”, “Cabernet Sauvignon”, “Chardonnay”, or “Merlot” in fact descendants of other known varieties (Duchene 2016). Tempranillo, the most relevant variety in Rioja´s viticulture, is also the result of hybridization between “Albillo Mayor” and “Benedicto” (Ibañez et al. 2012) like other well-known Spanish varieties such as “Palomino“, “Malvasia”, “Moscatel”, “Torrontés”, or “Hebén”. (García-Brunton et al. 2018).The extent of parentage among grapevine cultivars is as surprisingly high that in the study of the relationship among 2344 unique genotypes of the INRA "Domaine de Vassal" grape germplasm repository with molecular markers, Lacombe et al. (2013) identified only 276 genotypes with no direct relationship with any other genotype in the collection. Nevertheless, they could elucidate the complete parentage of 828 cultivars, indicating that sexual reproduction, due to chance or controlled by man, is a major driver of genetic diversity in cultivated grapevine (Duchene 2016).

Unfortunately, wine market globalization, variety-oriented wine labelling and the increasing demand of healthy plant material have led to genetic erosion of landraces in the cultivated grapevine (Wolkovich et al. 2018), with many of the traditional and local cultivars almost disappeared, and some of them only found in germplasm collections (This et al. 2006). In the current scenario, only five highly appreciated wine cultivars: “Cabernet Sauvignon”,

“Merlot”, “Tempranillo”, “Syrah”, and “Grenache Tinta”, make up for the production of red wines, while “Airén”, “Chardonnay”, “Sauvignon Blanc”, “Trebbiano Toscano” and “Welschriesling” (syn. “Grasevina”) are the basis for white wine production (Anderson 2013). On top of that, New World regions are also engaged with those “international varieties” that represent only 1 % of the total genetic diversity but cover more than 80 % of the planted hectares in Australia and New Zealand, being 78 % in Chile and 70 % United States (Wolkovich et al. 2018). In Spain, “Tempranillo” cv. represents 21 % of the vineyard surface-area (201081 has) and 41 % of the area dedicated to red varieties, increasing the surface planted between 2000 and 2012 by 75 % from around 90000 to 200000 ha (MAPA 2016). Besides Tempranillo, other six red varieties, “Bobal” (60301 has), “Grenache” (61372 has), and “Monastrell” (42500 has) followed by “Cabernet Sauvignon”, “Syrah” and “ Grenache tintorera” account for 90 % of the total global surface of red varieties and 46 % of the total vineyard area (García-Brunton 2018). In Rioja, the number of cultivated varieties have decreased from 44 in 1912 to 7 in 2000, with only three varieties (Tempranillo, Grenache, Graciano) covering 90 % of the total area under cultivation in 2019 (Riojawine https://www.riojawine.com).

1.4. DOCa Rioja varieties: Tempranillo, Grenache and Graciano

Rioja total winegrape vineyard area is 65001 ha, of which Tempranillo, a native cultivar to La Rioja (and Aragón) covers 79.8 %, followed by Grenache 7.8 % and Graciano 2 % (www.riojawine.com 2019).

Tempranillo is oenologically very versatile, capable of producing wines that can withstand long ageing periods, with a good balance of alcoholic strength, colour and acidity. From a sensorial point of view, Tempranillo aroma is normally characterized by banana, clove, toasty notes and roses (Ferreira et al. 2000). In ampelographic terms, Tempranillo shares characteristics of Albillo Mayor and Benedicto, being more similar to Benedicto in global terms (Ibañez et al. 2012). The three cvs, are characterized by mature leaves with seven lobes, a pentagonal blade shape, green shoots with red lines on both sides, and globose berries with low weight and neutral flavour (Ibañez et al. 2012). It is also defined by uniform fruit set and dark-blue berries with thick skin (Cervera et al. 2002). Agronomically, it sets well but is quite susceptible to pests and diseases and performs poorly under drought and high temperature conditions. Its name comes from the Spanish *temprano* (early) because of its short ripening cycle (www.riojawine.com). Among the synonyms, complete similarity with Cencibel, Tinto de Madrid, Tinto del País and Tinto Fino and more distant resemblance with Tinto de Toro and Ull de Llebre have been reported (Maul et al. 2014, http//: www.vivc.de).

Grenache is also a variety native to Spain, being also the most extensively grown variety in the world. According to OIV description, it is characterised by a medium-short length of leaf, with 1-2 clusters per shoot, medium cluster length and weight. Clusters contain a medium number of berries of small size and length, very slightly coloured flesh and neutral flavour. Grenache musts are characterised by high sugar content and medium total acidity (OIV 2018). Sensorially, it produces very aromatic wines characterised by floral scents as violet and fresh fruit notes as strawberry or plum (Ferreira et al. 2000). It is considered a robust variety able to withstand periods of drought, and moderately resistant to pests and major vine diseases, such as grape rust mite and powdery mildew, which explains its popularity among growers all over the world, despite being a shatter-prone variety. Synonymies found in ampelographic collections around the world include: Abundante, Alicante, Cannonaddu, Cannonaddu Nieddu, Cannonao, Cannonau

Selvaggio, Canonazo, Carignane Rosso, Garnaccho Negro, Garnatxa País, Gironet, Granaccia, Granaxa, Grenache Rouge, Lladoner, Retagliad Nieddu, Rivesaltes, Rousillon Tinto, Rousillon, Tinto Aragonés, Tinto Navalcarnero, Uva di Spagna.(Maul et al. 2014, http//:www.vivc.de).

Grenache complements Tempranillo cv. with its robustness, intense aroma and freshness, its resistance to drought and pests or diseases, whilst Tempranillo presumably improves Grenache in agronomic features: yield or fertility and oenologically versatility: deep wine colour and phenolic content, and a good balance between alcoholic strength and aging potential.

Graciano is a red grape cultivar native to La Rioja region whose cultivation is very restricted in other areas. Agronomically, shows low productivity and long ripening cycle, being quite resistant to downy and powdery mildew (www.riojawine.com). Among the synonyms found Morratel (France), Xeres (California) and Tinta Miúda (Portugal) (www.riojawine.com) with 78 synonyms gathered in the Vitis International Variety Catalogue (VIVC) (Maul et al. 2014). It delivers vivid red colour wines with a marked acidity and polyphenolic content, very aromatic ideal for ageing, and normally used to improve the characteristics of Tempranillo, giving higher colour intensity and aroma to the mixture (Escudero-Gilete et al. 2010).

1.5. Breeding evolution

The heterozygous nature of grapevine is a complicating feature for any effective breeding program (Adam-Blondon et al. 2004), on the other hand that enables producing offspring with a wide range of variability from crosses between different parental varieties. Old grape varieties carry deleterious alleles that exhibit pronounced inbreeding depression after selfing or sibling mating (This et al. 2011).

Grape breeding is a double face effort, whilst table and raisin grape markets are very receptive to new cultivars, wine industry is highly traditional. Breeding wine varieties is very restricted in European viticulture, especially in the Mediterranean, due to different regulations that ban the introduction of new wine making varieties in the *Denominaciones de Origen* system. Furthermore, grapevine is a perennial crop with a short juvenile period that requires time and space for phenotypic evaluation. In wine grapes, single seedling vines produce small quantity of fruit that needs to be transformed into wine before being evaluated, which complicates the process. Breeding efficiency depends on the screening methods used for fruit quality, yield and disease or climatic resilience. Moreover, little is known about the inheritance of wine-quality parameters, probably quantitative in nature and strongly influenced by environmental conditions (Riaz et al. 2007). However, the need for genotypes able to face new challenges such as plant diseases and climate change, has prompted recently the development by hybridization of new selections with optimal agronomic and oenological characteristics that maintain wine typicity. Since 2013, registration of new wine grape varieties has focused on disease-resistant genotypes, such as "Solaris" or "Cabernet Cortis" in Italy, implying a step forward in the regulation of hybrid varieties.

Plant breeding and genetics research is transitioning from a data-poor to a data-rich environment. The long and cost-consuming process of obtaining a new variety based in a conventional breeding program is being overcome by alternative methods in the recent years seeking to identify genes for desirable traits. These techniques are based on the use of marker-assisted selection (MAS) (Töpfer et al. 2011), genomic selection (GS) (Fodor et al. 2014) or Next generation sequencing (NGS) technologies. NGS of crop plant genomes, is revolutionizing the

field as newly abundant data enable and facilitate the discovery and use of millions of single nucleotide polymorphisms (SNPs) in diverse genomes (Huang et al. 2012, Xu et al. 2012). An important landmark in grapevine genetics was the complete sequencing of two grapevine genomes: the near homozygous "Pinot noir"-derived inbred line PN40024 (Jaillon et al. 2007) and its update by Canaguier et al. (2017); and the heterozygous cultivar "Pinot noir" clone ENTAV115 (Velasco et al. 2007). The publication of the grapevine reference genome sequence has enabled the prediction of gene sequences, the annotation of the grapevine genes (Grimplet et al. 2012) and the identification of single nucleotide polymorphisms (SNPs), which have become the most widely used on high-quality genetic map construction (Zhang et al. 2015). These SNP markers can be identified from short reads generated by NGS, either by aligning to a reference genome or by de novo assembly (Nielsen et al. 2011).

Following the publication of the PN40024 genome in 2007, no genome reference of equivalent or greater quality has been released for *V. vinifera*. A *de novo* approach was adopted to assemble the genome sequence of Thompson Seedless, a ubiquitous multipurpose cultivar. The genome of "Sultanina" table variety has also been fully sequenced (Di Genova et al. 2014), representing a new opportunity for the identification of genes related to the historical and morphological divergence existing between wine and table cultivars. In wine grape cultivars, Da Silva et al. (2013), proved a reference-based assembly approach, which resulted successful assembling multiple Arabidopsis genotypes, but failed to reconstruct specific sequences with Tannat variety and over 10 % of the gene space was not represented in the assembly, illustrating that the genomic sequence of one cultivar is not enough to represent the total variability of the species (Minio et al., 2017). Thanks to the discover of the FALCON-unzip diploid-aware software for the genome reconstruction, the Cabernet Sauvignon (Minio et al. 2017), Chardonnay (Chin et al. 2016), and Carmenere (Minio et al. 2019) assemblies are able to represent their haplotype diversity, since this strategy produced genome assemblies more contiguous and complete than PN40024 and include haplotype-specific gene sequences that are endemic to the highly heterozygous species (Cantu & Walker 2019). Besides, recently, a high-quality, diploid-phased Chardonnay genome assembly was produced from single-molecule real time sequencing, and combined with re-sequencing data from 15 different Chardonnay clones (Roach et al. 2018).

Among the NGS technologies the recent development and availability of different genotype by sequencing (GBS) protocols provided a low-cost approach to perform high-resolution genomic analysis of entire populations in different species (Crossa et al. 2013). GBS method is a powerful and useful method to obtain genome-wide variability information for populations composed by hundreds of individuals (Crossa et al. 2013, Spindel et al. 2015, Perea et al. 2016), delivering large numbers of marker genotypes with potentially less ascertainment bias than standard single nucleotide polymorphism (SNP) arrays (Crossa et al. 2013).

GBS protocols start with a digestion of the DNA using one or more known restriction enzymes. Then, fragments of suitable lengths (less than 800 bp) are ligated to adapters, amplified and sequenced in a high throughput Illumina platform. Another advantage of this method is that multiple samples can be sequenced in one single lane adding appropriate barcodes (Elshire et al. 2011). After this step, sequenced reads are ready to be demultiplexed and either analyzed de-novo or aligned to a reference genome if available (Perea et al. 2016). The most interesting characteristic of this protocol is that although a relatively small portion of the entire genome is sequenced, it is reasonably well distributed and reproducible, what enables to identify and genotype thousands of genomic variants across the genome of different samples. For that reason, this technique is becoming the chosen method for several applications in plant genomics and plant

breeding (Myles et al. 2013), such as the construction of high-density genetic maps (Hyma et al. 2015, Smith et al. 2018, Teh et al. 2017), genetic mapping of complex traits through Genome-Wide Association Studies (GWAS) (Crossa et al. 2013) and estimation of breeding values in genomic selection (Spindel et al. 2015).

A key component of any GBS protocols is the bioinformatics pipeline required to analyze the reads and to obtain polymorphic sites within the sequenced population. Custom packages such as Tassel GBS pipeline (Glaubitz et al. 2014) have been developed specifically for analysis of the types of reads produced by GBS technologies Tassel in particular takes advantage of the nature of GBS reads to perform a highly efficient calculation of genomic variants. Widely used packages as Samtools or GATK for variant detection and genotyping have been used to analyse GBS data (Perea et al. 2016). The main advantage of these methods over previous approaches is that they can still work in the absence of a reference genome. NGSEP (Next Generation Sequencing Experience Platform) currently provides a great balance between completeness, accuracy, efficiency and usability (Perea et al. 2016).

GBS holds the potential to close the genotyping gap between references of broad interest and mapping/breeding populations of local or specific interest. Unlike other high-density genotyping technologies which have mainly been applied to general interest “reference” genomes, the lower cost of GBS makes it an attractive tool of saturating mapping and breeding populations with a high density of SNP markers (Spindel et al. 2013). Results have shown that this methodology is efficient for genotyping a variety of species, including those with complex genomes such as barley (Poland et al. 2012), oats, (Huang et al. 2014), onion (Jo et al. 2017) and grapevine (Yang et al. 2016, Smith et al. 2018, Guo et al. 2019).

The first application of GBS in grapevine was done by Barba et al. (2014), constructing high-resolution parental linkage maps in an interspecific *V. rupestris* × *V. vinifera* segregating population. More recently, high density genetics maps have been elaborated merging two (Teh et al. 2017) or more (Tello et al. 2019) populations. The application of GBS and other NGS technologies has enabled the efficient discovery and genotyping of SNPs in grapevine, resulting in the detection of a massive number of markers to detect phenotype – genotype associations in interspecific segregating populations (Chen et al. 2015, Zhu et al. 2018) and in grapevine diversity panels (Guo et al. 2019).

1.6. QTL analysis

The identification of genotype-to-phenotype associations is essential in plant breeding. Genetic maps have been widely used to identify genes responsible for grapevine composition and development. Traditional bi-parental mapping populations continue to play an important role in gene discovery, and both bi-parental and multi-parental breeding populations remain the foundation of many plant breeding programs (Almeida et al. 2013, Zhu et al. 2018).

Considerable progress has been made in the identification of molecular markers and the construction of molecular linkage maps in grapevine. A great step forward has been made between the first molecular map built from a 60 F_1 progeny from the cross “Cayuga White” × “Aurore” generated in 1995 (Lodhi et al. 1995) and the last high density multiparent map developed in 2019 using 10 subpopulations (Tello et al. 2019).

At the beginning qualitative traits were studied, so the first genetic localizations of traits were based on the observation of their segregation as presence or absence. In grapevine, the major genes responsible for qualitative traits are sex determinism (Dalbó et al. 2000, Margueritt et al. 2009, Fechter et al. 2012, Battilana et al. 2013, Zhou et al. 2018), and berry color (Doligez et al. 2002, Mejía et al. 2007). With the application of genome-spanning genetic maps, the polymorphic qualitative traits detected in a segregation progeny can be positioned in relation to molecular markers. However, many agriculturally important traits such as berry weight (Fanizza et al. 2005, Cabezas et al. 2006), seedlessness (Doligez et al. 2002, Costantini et al. 2008), flower morphology (Margueritt et al. 2009), total sugar content and total acid content (Viana et al. 2013, Chen et al. 2015), timing of flowering, veraison, and ripening (Fischer et al. 2004, Costantini et al. 2008, Duchêne et al. 2012) are controlled by many genes and are known as quantitative or polygenic traits. Quantitative Trait Loci (QTL) are the regions within genomes that contain genes associated with those traits, and their identification was enabled by the development of DNA (or molecular) markers early in the 1980s (Collard et al. 2005). Initially, the identification of QTL was mainly based on linkage mapping techniques, where polymorphisms between two parents were detected in a segregating population, and the linkage of a region to a given phenotype was determined by genotyping recombinants exhibiting phenotypic variation for the trait of interest. Thus, the genetic control of major traits in grape, such grape size, phenology stages, must composition, has been explored via simple sequence repeat (SSR) markers in biparental populations (Constantini et al. 2008, Fechter et al. 2014, Ban et al. 2016, Bayo-Canha et al. 2019). Nowadays thousands to millions of markers are developed by the emerge of next generation sequencing technologies improving mapping coverage and resolution (Deschamps et al.2012). As a result, association analyses have been also performed in grapevine using GBS, starting by Barba et al. (2014) in the study of powdery mildew resistance (Guo et al. 2019), downey mildew resistance (Saptoka et al. 2019) or berry weight, cluster size, berry flavour, malic acid, total soluble solids (Yang et al. 2016). These works could establish the genomic regions that influence a particular trait within different grape mapping populations with common markers, being applicable to establish the relationships of QTL in different genetic backgrounds.

Among the methods for detecting QTL, Simple Interval Mapping is one of the most powerful, since instead of analysing single markers, uses linkage maps and interval analyses between adjacent pairs of chromosomes simultaneously (Lander & Botstein 1989). Several public and private software packages are available to perform QTL analysis; among the most used in grapevine are Cartographer (Basten et al. 2003), WinQTLCart (Wang et al. 2004) and MapQTL (Van Ooijen 2009).

1.7. Grapevine reproductive cycle

In temperate regions, grapevine completes the reproductive developmental cycle over two consecutive growing seasons separated by a dormancy period between autumn and spring (Carmona et al. 2008). A typical trait of *Vitis vinifera* is the simultaneous formation of both vegetative and reproductive forming organ primordia by the same apex (Boss et al. 2003). In spring, every sprouting bud gives rise to a stem and the first-formed bud in the leaf axil produces a lateral shoot that will carry bunches in the second season (Carmona et al. 2008). In the axil of that lateral shoot, a latent bud will be formed and there will take place floral initiation and early stages of inflorescence development (Carmona et al. 2008). During the flowering process the first two to three lateral meristems have the potential to differentiate as inflorescences while the

following lateral meristems produced will start differentiation as tendrils (Lebon et al. 2008). By the end of the summer these buds enter in a dormant state, allowing the possibility to resume growth under more favourable conditions the second year (Díaz-Riquelme et al. 2012). The primary latent bud, if fruitful, contains a future shoot with inflorescence meristems, tendril and leaf primordia and in the case of non-fruitful canes (originated from non - fruitful buds), no lateral structures develop (Carmona et al. 2008). At this stage, carbohydrate physiology of the whole vine during the period of inflorescence initiation determines the number of bunches that will emerge the following year. Once winter comes to its end, this dormant period finishes and different developmental processes start generating the elongation of rachis and lateral branches, and the differentiation of secondary and tertiary branches, that form the racemes characteristic of grapevine inflorescences. (Carmona et al. 2008). In this stage the formation of floral meristems also takes place, producing flowers with their sexual organs, completed only a few days before anthesis. Besides, the terminal flower develops first, then the lateral ones and finally, the most basal (Carmona et al. 2008, Keller 2010).

Grapevine reproductive cycle is different in cultivars which present hermaphroditic flowers, and pollination is made by self - fertilization, compared with wild plants that are dioecious, requiring crosspollination (via either wind or pollinators).Thus, *Vitis vinifera* species display three types of flowers: males and females in *V. v.ssp.sylvestris* and hermaphrodites in the cultivated subspecies *V. v. ssp sativa*. Flowers can be perfect (hermaphroditic), imperfect male (female sterile or staminate) or imperfect female (male sterile or pistillate), with fused petals that separate at the base, forming a “calyptra” or cap (Cattonaro et al. 2014). Male flowers are characterized by having long erect stamens and a reduced carpel without style or stigma, but with nectaries and ovaries. Female flowers have a complete carpel with style and stigma but short and reflexed stamens with sterile pollen (Caporali et al. 2003). Berries produced by female plants are described as small, dark in colour, and sweet enough to attract birds, contributing to seed dissemination (This et al. 2006). Male, female, and hermaphroditic flowers are not visually attractive to insects as the flowers are small and the petals drop at anthesis (Carmona et al. 2008). At early developmental stages, male and female flowers are morphologically indistinguishable from a hermaphrodite flower, becoming unisexual only at later development stages (Ramos et al. 2014).

Morphologically, hermaphroditic flowers are formed by sepals, petals, androecium and gynoecium, which arrange in concentric rings (or whorls) from the outside to the inside (Vasconcelos et al. 2009). Sepals (normally five) constitute the calyx, and they are located at the base of the flower to protect it in the early stages of development (Keller 2010), and petals are fused by epidermal cells, forming the calyptra. The androecium is normally comprised of five stamens, each one composed of a long filament ending in a bilocular anther containing pollen sacs, which contain pollen grains. The gynoecium (or pistil) is located on the central part of the flower, its inner cell wall develops into the septum, which is the central part of the style through which the pollen tube will grow. The ovary is the enlarged area at the base of the style, and it protects the ovules (located in the ovary locules) from desiccation and physical injury (Keller 2010, Lebon et al. 2008, Vasconcelos et al. 2009). Pollination usually occurs by pollen grains originated in the flower own anthers (Keller 2010), which are deposited on the stigma, in the upper part of the pistil.

Flower formation happens during spring, bud break is preceded by the activation of all structures in the latent bud, especially the differentiation of inflorescences and the first steps of floral organ development (Lebon et al. 2008). There is an order of organ appearance that is similar

to all angiosperms: five sepals appear first and form the calyx, then five petals form the corolla, followed by five stamens and then two carpels that generate the pistil. The calyx has a ring feature (Gerrath 1993) that protects the internal organs from environmental fluctuations at the early stages of bud break, and the cap (formed by the join of petals and sepals) protects the fertile organs, until it falls at anthesis following the growth of stamen filaments. The gynoecium originates from the fusion of two carpels, and in each locule, two anatropous ovules develop and are inserted into the septum. Inflorescence and flower formation in grapevine are such a complex process that some authors have described it with 22 successive stages encoded by numbers from 0 to 50 based on the external inflorescence characteristics (Lorenz et al. 1994, Coombe 1995). For example, anthesis occurs at stage 19 and continues for about one week, and after cap fall take place the following stages: full bloom (stage 23) is reached when 50 % of flower caps have fallen, whereas stage 25 is reached when 80 % of caps have fallen. Stage 27 marks the onset of berry development from the fertilized ovules, stamens then degenerate and the young berry is then visible (Lebon et al. 2008). Interestingly, the kinetics of male and female reproductive development depend on variety and are not necessarily synchronous with the developmental scale previously mentioned. (Lebon et al. 2008). For example, both male and female meiosis occur one week earlier in Pinot noir than in Gewurztraminer and in Pinot noir, meiosis takes place between stages 12 and 15 in anthers and between stages 15+2 days and 15+8 days in ovules (Lebon et al. 2008). Meiosis is a key point in the accomplishment of sexual reproduction, where anthers and ovules show particular sensitivity to various kinds of stress, and even a lack of sugar could conduct to its abortion (Lebon et al. 2008).

Fruit development is triggered by pollination and fertilization processes. In fleshy fruits, such as tomato or grapevine, berries develop from an ovary after fertilisation. The ovary wall turns into the pericarp, formed by three distinct cell layers: the epicarp (skin); the mesocarp (flesh); and the endocarp (cell layers in contact with the seeds). (Coombe 1976, Ollat et al. 2002). Seeds are in the endocarp and (as in the berry mesocarp) it is possible to distinguish two different seed tissues: an internal hypodermis formed by a few cellular layers and an internal epidermis (Carmona et al. 2008). Berry growth follows a double-sigmoidal pattern with two growth stages (berry formation and berry ripening) separated by a lag phase of slow or no growth (Coombe & McCarthy 2000, Robinson & Davies 2000). The first stage begins immediately after flower pollination, during stage I, berry growth is due to cell mitotic division and cell expansion. Approximately 4 – 6 weeks post-anthesis, cell division ceases and only cell expansion subsists. Coinciding with this rapid growth is the biosynthesis of phenolic compounds, such as tannins and hydroxycinnamates, and organic acids, such as tartaric and malic acid, reaching their maximal concentration at the end of this first stage. (Bindon et al. 2013, Coombe & McCarthy 2000). Besides, at the end of this stage, all seed tissues are formed. Stage II corresponds to a slow growth phase that ends with veraison (the onset of ripening). During this phase, sugars initiate its accumulation reaching its maximal concentration at the end of the third stage. Finally, berry growth restarts in stage III but only through cell enlargement (Coombe 1976, Ojeda et al. 1999). Is in this final stage, when anthocyanins and aroma compounds are accumulated in berry skin (Bindon et al. 2013, Coombe & McCarthy 2000, Zamboni et al. 2010) and the berry experiences a second period of rapid cell expansion as the pericarp grows to its final size. Many changes in berry metabolism happen during this process: accumulation of sugars, decrease in organic acid concentration, and production of secondary metabolites, so berry size and composition will differ depending on the stage of development. (Wong et al. 2016). Grapevine flowering and fruiting developmental processes are not only genetically determined, but are markedly influenced by environmental variations and management practices (Bindon et al. 2008).

1.8. Grape quality and climate change adaptation

The statement that good wine production requires good quality grapes is a crucial dogma in wine industry. Quality is a difficult term to define and is usually linked to ‘grape composition’ as the metabolite composition of grapes and wine can be measured and quantified (Carmona et al. 2008). Thus, berry quality is closely linked with the presence of sugars, acids, anthocyanins, tannins (Holt et al. 2008, Rolle et al. 2015, Gil et al. 2015, Wong et al. 2016), and phenolic and volatile compounds (Gerós et al. 2012), most of them accumulate in the skins and seeds (Barbagallo et al. 2011, Downey et al. 2006).

Skin-to-flesh ratio influences grape composition and quality with higher concentrations of phenolic compounds in small berries (Gil et al. 2015). However, the direct relationship between berry size and wine quality is still highly debated (Friedel et al. 2016, Xie et al. 2018). Several studies reported that berry size had no influence on grape and wine quality, while viticulture practices such as pruning (Holt et al. 2008, Roby & Mathews 2004), and environmental conditions (Van Leeuwen et al. 2017) are major drivers in vine metabolism, hence grape composition (Dai et al. 2011) not berry size per se (Xie et al. 2018).

One of the limitations in the study of berry size and composition is variability. Mean and range values of both parameters are the result of complex interactions among genotype, environmental factors, such as temperature or light, their interactions, and cultural practices (Keller 2010). Variability is present within berries, among berries within a cluster, among clusters on a vine, and among vines within a vineyard (Dai et al. 2011). Sink competition at the tip of a cluster produces lower weight berries than in the centre or shoulder (Tarter & Keuter 2005). Berry weight shows high genetic diversity within the *Vitis* genus, ranging from < 0.5 to > 10 g (Houel et al. 2013).

Among viticultural practices, crop thinning has been found to be a useful tool in the improvement of berry composition by increasing anthocyanin and polysaccharide levels in Syrah (Gil et al. 2013) or increasing color, currant aroma, and astringency of Pinot Noir wines (Reynolds et al. 1996). A decrease in vine vigour is thought to improve final wine quality, by increasing grape-derived compounds, such as anthocyanins (Koundouras et al. 2006, Song et al. 2014), or other phenolics (Schreiner et al. 2013), due to more open canopies and more exposed clusters. Leaf removal is estimated to modify canopy microclimate (sunlight exposure), in a cool-climate Pinot Noir region increasing the levels of grape-derived volatile compounds (Feng 2014). Given that those cultural practices have a repercussion on the final wine aroma and sensory characteristics, wine growers may take advantage of adapting vine management to the specific region and annual weather conditions in order to improve wine quality.

In the last 30 years, a significant change in grape composition has been observed due to climate change. In the future, countries like Spain, and especially warm and semi-arid regions in the south east, will suffer the effects of temperature rise, and the increased atmospheric water deficit and evaporation rate that will make difficult to maintain quality and productivity (Fraga et al. 2013, Resco et al. 2016, Savé et al. 2017). Other visible consequences of climate change will be the advancement in phenology periods like sprouting, veraison and maturation, promoting harvests of up to 20 days earlier in some Mediterranean regions (Webb et al. 2008). Consequently, berry ripening will occur earlier in summer, under higher temperatures, having a significant impact on berry quality. High temperatures accelerate pulp maturity and cause a decrease of grape acidity, mainly because of a faster degradation of malic acid (Sweetman et al. 2014). An excess of sugar content in berries (Fraga et al. 2013), conducts to a higher ethanol content, a greater

aroma volatility, lower anthocyanin content and hence less colour (Resco et al. 2016). In contrast, wine-growing regions at high latitudes where achieving a correct level of ripeness is the limitation for high-quality wines, will be favoured. This challenge can be faced by introducing more variability at the cultivar level with better adaptation to the new climatic conditions.

In 2020, OIV has estimated a total wine production of 260 Mill. hL, meaning a 10 % decrease relative to 2018, that in Spain would reach a 25 % drop, due to the changing climatic conditions. Even though moving vineyards to higher altitude areas seems a good approach, developing varieties better adapted to this new scenario seem to be the best long-term strategy. Therefore, grapevine breeding programs involving the hybridization of heterozygous premium varieties and the further selection for one or a few of the best hybrids using the recently available NGS tools may succeed in developing high - quality wines preserving tipicity in the future climate change context.

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CHAPTER 2. OBJECTIVES

2. Objectives

Wine making is a dynamic process that must be adapted to changes such as global warming and new consumer interests. In the context of breeding for grape quality; two intraspecific populations obtained from crosses between three of the most relevant red wine Spanish varieties, Grenache, Graciano, and Tempranillo were studied with two main objectives:

1. Identification of the major genetic determinants of quality traits such as berry size and shape and the different traits contributing to them.

   - Evaluate phenotypic segregation of relevant traits related to phenology stages, productivity, berry and flower morphology, seed-related traits and must composition in the progenies, and conduct a pre-selection of hybrids with improved characteristics.

   - Construct a high-density genetic map of Grenache ×Tempranillo progeny using GBS technology and identify QTL for berry, flower, seed, productivity, must composition and phenology stages on the genetic maps.

   - Analyze the influence of flower sex in flower morphology, berry size, seed parameters, productivity, must composition and phenological stages.

2. Characterization of oenological composition of Tempranillo segregating progenies and Pinot Noir clones.

   - Assess of the influence of berry size in must and wine composition and quality parameters in two well-known wine regions, La Rioja (Spain) and Marlborough region (New Zealand).

   - Perform sensory profiling of wines derived from twelve Graciano × Tempranillo selections, and identify premium genotypes for a climate change scenario.

CHAPTER 3.

GENETIC ANALYSIS OF MORPHOLOGICAL, AGRONOMICAL AND PHENOLOGICAL TRAITS.

STUDY OF SEX INFLUENCE

3. Genetic analysis of agronomical, must and phenological traits. Study of sex influence

3.1. Flower sex influence in berry and flower morphology traits and phenological stages in two different genetic backgrounds

Abstract

Berry weight is considered one of the most relevant traits contributing to berry quality, both in wine and table grapes. In wine grapes, berry shape may influence phenolic extractability due to its influence in the ratio skin/pulp. In table grapes flower size and sex presumably play an important role in both berry size and shape variability. In this study, two wine grape progenies obtained by crossing Tempranillo (male parent) with Graciano and Grenache as female parents were evaluated for flower sex at the phenotypic and genotypic level with VVIB23 marker. Two alleles adjusting to a dominance segregation model were recorded. The objective of this work was to evaluate the influence of sex and colour in flower, berry, seed, productivity and phenology traits in a.

Female plants presented rounder flower shape, larger flower diameter, lower number of seeds, and a delay in flowering and start veraison dates compared with hermaphrodites in both genetic backgrounds. Colour influenced sex segregation rate, showing a distortion in the proportion of hermaphrodite homozygous plants. Plot and vintage had a strong influence in all the traits studied, especially in phenological stages. Berry length determined berry shape, which is correlated with flower shape in both genetic backgrounds, being associations stronger in hermaphrodite genotypes than in females. Flower morphology, flowering date and seed traits showed moderate correlations (0.3–0.4, $p < 0.01$) in both progenies, suggesting that all these characters are under shared genetic control.

This work reveals the influence of sex in key agronomic traits such as productivity, and phenological periods, but in contrast to table grapes, no clear relationship between berry and flower morphology was found. Berry size and shape are selected by breeders as drivers of consumer acceptance in the table grape market whilst in wine grapes other traits are more relevant.

Introduction

It is widely accepted among wine-growers that smaller and rounder berries will lead to higher quality wines due to a higher concentration of grape skin compounds (Barbagallo et al. 2011, Doligez et al. 2013). In grapevine, like in tomato, an increase in berry weight is correlated with an increase of berry shape diversity, what could be explained by loci having pleiotropic effects for both traits because of domestication (Houel et al. 2013).

Wild grapevine fruits are invariably round and small, whereas cultivated fruits have a large range of sizes and shapes (Tanksley 2004, This et al. 2006, Houel et al. 2013). Thus, *V. vinifera* domestication induced changes in berry size and shape, but also in seed morphology or sugar content (Bowers et al. 1999, Zhou et al. 2017). Observed variation in berry shape and size could be also the result of interactions among genotype, environment, and management practices (Vasconcelos et al. 2009, Dunn & Martin 2000, Gray & Coombe, 2009). Houel et al. (2013) estimated that cell division

before and after anthesis and cell expansion after anthesis are the major determinants of flesh weight variation, what suggests that berry size could be determined already at anthesis. Thus, inflorescence size and flowering length could play an important role in berry size, shape and weight (Barbagallo et al. 2011, Houel et al. 2013). Likewise, variability in flowering time could be also related to cultural practices, hormone factors, carbohydrate supply (May. 2000, Ramos et al. 2016), or weather conditions, especially temperature (Gourieroux et al. 2016), and development at the end of anthesis seems to be critical for the final berry size and shape (Grimplet et al. 2017).

Flower sex presumably plays an important role in both berry size and shape variability and affects other parameters such as number of seeds, flower morphology and flowering period (Constantini et al. 2008, Marguerittt et al. 2009). *V. vinifera* species display dioecious and hermaphrodite sexual systems, in which three types of flowers are observed: males, females and hermaphrodites. Male flowers are characterized by long erect stamens and a reduced pistil without style or stigma, but with a viable ovary. Female flowers have a complete pistil with a large ovary, a short style and stigma but short and reflexed stamens with sterile pollen (Caporali et al. 2003). The hermaphrodite flower displays functional male and female organs, where pistil is perfectly formed and fully functional with style, stigma and ovaries, and the stamens, although shorter than the male ones, are erect and produce viable pollen (Carmona et al. 2008). Ramos et al. (2014), compared the morphology of different flower developmental stages, from B (early) to H (just before blooming) (Baggiolini 1952) in the three flower sex plants, and concluded that at early developmental stages, male and female flowers were morphologically indistinguishable from a hermaphrodite flower, becoming unisexual only later in development (stage G - H).

The molecular basis of the sex trait in *Vitis* remains poorly understood (Ramos et al. 2017). Oberle (1938) proposed that sex expression is controlled by two linked genes with the dominant alleles, *So* (suppressions of ovules) and *Sp* (development of pollen), linked in cis-arrangement. Thus, males would be *SoSp/sosp* and females would be *sosp/sosp*. Interestingly, a rare crossover event would produce a *soSp/sosp* plant that would produce both functional pollen and functional pistils, a possible explanation for the development of hermaphrodites. Nunes-Ramos et al. (2014), considered the model on which females are homozygous for *so* and *sp* genes and hermaphrodites are heterozygous for *sp* (*soSp/sosp*) to be the most likely; suggesting that either a male reversion to hermaphroditic form was selected, or that ancestral hermaphrodite remnants stayed in the population and were later selected to be used in grape industry. Another general model proposed by Levadoux (1946) and supported by Carbonneau (1983) and Antcliff (1980) proposes a single major locus with three different alleles male *M*, hermaphroditic *H* and female *F,* with an *M* > *H* > *F* allelic dominance. Diversity and network analysis indicated that hermaphroditic alleles were more closely related to male alleles than to female and maybe M allele lost the dominant allele for female sterility by mutation, explaining the dominance of the M allele over the H (Picq et al. 2014). In *Vitis* HH genotypes do strive and set seeds, as in the case of certain domesticated grapevines such as Chardonnay, Muscat de Hambourg or Riesling, which produce 100 % hermaphroditic progenies (Picq et al. 2014). Charlesworth et al. (2013) support that sex is controlled by an XY system. The Y chromosome determines male flower development, and the slightly different Yh chromosome determines hermaphrodite flower development.

Several genetic maps mainly based on interspecific crosses have confirmed that sex determinism in the *Vitis* genus is under control of a single genomic region located on chromosome 2, linked to SSR marker VVIB23 (Dalbó et al. 2000, Lowe & Walker 2006, Riaz et al. 2006, Marguerittt et al. 2009). Interestingly, they pointed out VVIB23 as the nearest marker to the QTL detected in LG2 for

inflorescence and flower morphology, indicating that in grapevine as well as in other fruits, flowers also displayed secondary sexual characteristics, such as modified ovary shape and peduncle length, that co-segregated with the *Sex* locus.

The aim of this study was to evaluate the influence of flower sex in flower, berry and seed morphology and in productivity and phenology traits, in two different wine-grape segregating populations, by identifying three types of flowers with the VVIB23 marker.

Material and methods

Plant material

Two segregating populations of 134 and 151 plants obtained from controlled crosses between the wine grape cultivars Grenache and Graciano as female parents and Tempranillo (male parent) were used for our investigation. The individual hybrids (one plant per genotype) have been grown on their own roots since 2004, in a sandy-loam soil with East–West orientation (3 m x 1 m) in double Royat cordon in Varea, La Rioja. Grenache x Tempranillo population (G × T) population was duplicated in an additional plot at the University of la Rioja Experimental field in Logroño in 2012 and G × T progeny was studied in both plots in 2016. Despite both plots are located at a 10 km distance, soil characteristics and geography are different. Varea plot is located in a dip zone, with higher organic matter, and less clay than UR plot.

Standard irrigation, fertilization and plant protection practices for La Rioja region were performed. The plants first flowered and fruited in 2007 in Varea. The G × T population was genotyped for 5 SSRs markers: VMC6, VChr3a, VChr8b, VVIB23, VVIV70 in order to discard individuals resulting from self-pollinations and foreign pollen sources, resulting in a final population of 130 plants. Tempranillo x Graciano progeny (T × G) had been previously genotyped (Song et al. 2014).

Molecular marker analyses

Samples of each genotype consisting of 4 discs (200 mg, 2 cm^2) of young, healthy leaves, were collected in the field in 2 ml Eppendorf tubes, frozen immediately in liquid nitrogen and stored at – 80 ºC until processed. DNA was extracted according to the DNeasy Plant Mini Kit instructions (QIAGEN GmbH, Germany. DNA concentration was measured with a NanoDrop 1000 (Thermo Scientific Inc. USA) and the amount and integrity of resulting genomic DNA was checked on 0,8% agarose gels prepared in 1 x TBE buffer.

Polymerase chain reaction (PCR) amplification was performed in GeneAmp ® PCR System 9700 thermo cycler and VeritiTM 96 Thermal Cycler (Applied Biosystems, USA) in 96 well plates with 20 ng DNA, 0,2 µM of each primer 1x PCR Buffer, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 1-unit Immolase DNA Polymerase (LABOLAN, Navarra, Spain). The PCR was carried out following a ´touch-down´ protocol (Don et al. 1991). Primer sequence and nomenclature for VVIB23 marker was obtained from Merdinoglu et al. (2015), as well as thermal cycling conditions: one cycle at 94ºC for 5 min, followed by 6-touch-down cycles at 92 ºC for 45 s, 60 - 0.5 ºcycle for 1 min, 72ºC for 1 min 30 s, followed by 24 cycles at 92 ºC for 45 s, 57 ºC for 1 min, 72ºC for 1 min 30 s, and a final step of 5 min at 72 ºC. Thermal cycling conditions for Vchrs were: one cycle at 95 ºC for 5 min, followed by 10-touch-down cycles at 94 ºC for 20 s, 55-0.5ºC/cycle for 20 s, 65 ºC for 40 s, followed by 15 cycles at 94 ºC for 20 s, 50 ºC for 20 s, 65 ºC for 40 s, and a final step of 1 hour at 65 ºC. For VMCs the cycling

parameters were: one cycle at 94 ºC for 5 min, followed by 10 - touch - down cycles at 94 ºC for 30 s, 59 - 0.3 ºC / cycle for 30 s, 72 ºC for 45 s, followed by 24 cycles at 94 ºC for 30 s, 56 ºC for 30 s, 72ºC for 45 s, and a final step of 5 min at 72 ºC. The forward primer of each pair was fluorescently labelled with 6-carboxylfluorescein (6-FAM®).

Amplified products were analyzed in 3130 Genetic Analyzer (Applied Biosystems, USA) with the GeneScan-500(-250) LIZ dye size marker, in the Molecular Diagnostics Laboratory of the Center for Biomedical Research (CIBIR), Logroño. The identification and sizing of alleles for each genotype was performed manually with GeneMapper®4.0 Software.

Phenotypic evaluation

Twenty-two berry, flower, seed, productivity and phenology traits were evaluated in two hybrid populations during two seasons (between 2010 and 2017), with distinct weather conditions. Climate data for the vintages studied from April to October are showed in Supplementary material Table S1. In G × T population, data were registered in 2015 and 2016 vintages and in 2016 productivity and phenology traits of G × T progeny were analyzed in both plots (UR and Varea).

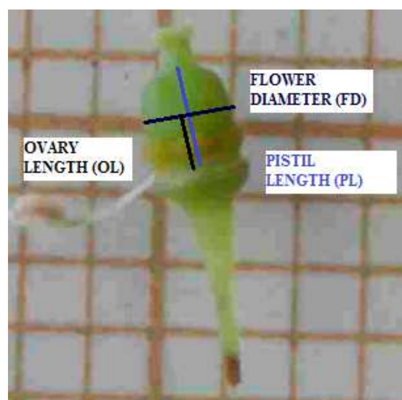
In T × G progeny, all traits were recorded in 2016, whilst the second year of study was completed with phenology and productivity data of 2010 and berry and flower morphology data in 2017. The number of genotypes that bore fruit varied each year due to bird attack during veraison-ripening stages. Thus, in G × T population 111, 117 and were harvested in 2015 and 2016 in UR and Varea plots respectively. In T × G progeny, 102, 114 and 102 genotypes were analyzed in 2010, 2016 and 2017 respectively.

Flower traits measurements

Individuals and parents of both populations were scored for sex phenotype in two years during the flowering period, 2015 and 2016 in Garnacha × Tempranillo progeny and 2016 -2017 in Graciano × Tempranillo population by visual inspection using the descriptor OIV 151 (http://www.oiv.int/). A minimum of 9 inflorescences per individual were harvested, photographed with a digital camera and measured with the image analysis software ImageJ.

To describe inflorescence morphology pistil length (PL, mm), ovary length (OL, mm), flower diameter (FD, mm) were measured and shape coefficients were calculated following Houel et al. (2013). Thus, pistil shape (PS, mm) was calculated as the ratio between pistil length (PL) and flower diameter (FD) and ovary shape (OS) the ratio between ovary length (OL) and flower diameter (FD). All measurements taken are shown in Figure 3.1.1.

Figure 3.1.1. Measurements taken on grapevine flowers with ImageJ software.



Berry and seed parameters

Berries were collected at technological maturity, established as the stage when random grapes picked from the top, medium and bottom of the clusters reached 23.4 ºBrix. At harvest, 200 whole berries from each genotype were sampled from representative clusters and mean berry weight was calculated (BW, g). Additionally, a set of 90 berries were picked and frozen at – 20 ºC to measure berry shape parameters. In three sets of 30 berries per plant, length (BL, mm) and diameter (BD, mm) were measured with a Mitutuyo digital calibre and shape coefficient (BS) was calculated as the ratio between length and diameter (Houel et al. 2013). Seeds were carefully taken out of berries, cleaned with absorbent paper, dried at room temperature for 24 hours, and weighed with an analytical balance. Average seed number per berry (SN) and mean fresh weight (SW, mg) were calculated.

Agronomic traits

Productivity and phenology traits were recorded in 2015 and 2016 for G × T population and in 2010 and 2016 for T × G progeny. For agronomic traits, yield (Y, kg / vine) and number of clusters per vine (CN) were measured at harvest. The fertility index (FI) was scored as the number of inflorescences per young shoot.

For phenology traits, flowering date was recorded as the date when 50 % of the flowers had opened and the anthers were visible (Baggiolini 1952). Veraison start (10 – 20 % of the berries coloured and / or soft) and veraison end (80 – 100 % berries coloured) were also scored. In G×T progeny, segregating for grape colour, red genotypes were harvested at 23.4 °Brix and white at 22 ºBrix. The dates of sprouting (ES when 50% of the buds were in Baggiolini stage H), flowering date (F, 50% flowering in Baggiolini stage I), start veraison (SV, 10-20% berry veraison), end veraison (EV, 80-100 % berry veraison) and ripening dates were scored. Dates were calculated as the number of days from March 1. Length of veraison period (VL) and intervals from sprouting to flowering (ES-F, days), flowering to start veraison (F-SV, days), and end veraison to ripening (EV-R, days) were also registered.

Statistical analysis

Descriptive statistics for all traits were conducted with SPSS Statistics v.25. Normality of each trait distribution was checked by the Kolmogorov-Smirnov test. and traits significantly deviating from normality were analyzed by non-parametric Kruskal-Wallis test. To evaluate the flower sex inheritance model, the goodness of fit segregation ratio with a Chi-square test was performed. ANOVA with LSD

test were carried out to detect differences in morphological, agronomic and phenological traits between parents and progenies in different flower types in each year and between two plots in G × T progeny. Spearman rank-correlation coefficients between years for each trait, and between traits for each flower type (ff, Hf, HH) were calculated ($p < 0.05$). MANOVA was conducted to test interactions between colour, year, plot, and sex factors on all the traits. Principal Component Analyses (PCA), were calculated with mean values (averaged across years) of the berry, flower, seed, productivity and phenology parameters for each progeny using PAST software. R software version 3.6.1 was used to obtain the best linear unbiased predictors (BLUPs) of genetic values. The genotypic, environment and residual variance estimates (σ_g^2, σ_e^2 and σ_r^2, respectively) were used to estimate heritability (H^2) of the inter-environment genotypic mean as $\sigma_g^2 / (\sigma_g^2 + \sigma_e^2 + \sigma_r^2)$.

Results and Discussion

Segregation of flower sex

Segregation analysis identified VVIB23 allelic markers associated with sex alleles in both progenies (Table 3.1.1). These results agree with Marguerit et al. (2009), and Fechter et al. (2012) that reported VVIB23 marker as the closest to *Sex* locus. Tempranillo, Graciano and Grenache cvs. resulted heterozygous (Hf) for *Sex* locus, and in both progenies, hermaphrodite individuals included both homozygous (HH) and heterozygous (Hf) genotypes. The origin of *H* allele is a matter of debate, since Battilana et al. (2013) suggested that the sequence variation responsible for the hermaphrodite flowers should have occurred on *f* allele in dioecious wild plants; contrary to Picq et al. (2014) and Ramos et al. (2014) who stated that the shift may have happened on *M* allele.

In Graciano and Tempranillo *f* and *H* alleles were associated with the same microsatellite sizes, being *f* allele coupled with 307 bp and *H* allele with 289 bp. Instead, in Grenache *f* allele paired with 317 bp and *H* allele with 293 bp. Other fragment sizes have been reported for the same alleles, 288 and 290 bp and 300 bp have been associated to *f* in *V.v.* ssp. *sylvestris* and *V. riparia*; respectively and 288 bp for *H*, in *V. sylvestris*. Besides in *V.v.* ssp. *sylvestris* accessions, the *M* allele fragment size was 304 bp (Battilana et al. 2013, Fechter et al. 2012). Merdinoglu et al. (2005) reported only one allele at 288 bp in Grenache cv., thus homozygous, using VVIB23 marker. In our work Grenache was heterozygous, *Hf*, as proved by visual inspection of flower sex segregation in our progenies and also by VVIB23 marker genotypic results, suggesting a possible Grenache mislabelling in the previously referred work.

Table 3.1.1. Summary of flower sex phenotypic and genotypic segregation and marker-trait linkage in G × T and T × G progenies

Plant material	Flower phenotype	Genotype at the *Sex* locus	Allele sizes at VVIB23 marker (bp)
Grenache (GAR)	H	H/f	H-293 f-317
Graciano (GRA)	H	H/f	H-289 f-307
Tempranillo (TE)	H	H/f	H-289 f-307
GAR x TE population (G×T)	102 H: 28 f	23 HH: 76 Hf: 30 ff	
GRA x TE population (T×G)	115 H: 34 f	46 HH: 72 Hf: 31 ff	

Segregation for flower phenotype, adjusted to the expected 3H:1F ratio for the *Hf* × *Hf* with dominance of H > f in G × T and T × G population, ($p = 0.3$, $p = 0.5$ respectively). The observed segregation supports the single locus theory with the presence of three alleles (Antcliff, 1980) as was previously confirmed by other experiments with different mapping populations (Dalbó et al. 2000, Lowe & Walker 2006, Riaz et al. 2006).

Among hermaphrodite plants, homozygosity rate was 25 % in G × T progeny, as expected from a crossing between Hf × Hf cultivars ($p = 0.26$). However, in T × G population, homozygotes accounted for 31 % of the plants although still adjusting to a 1:2:1 segregation ($p = 0.23$). Deviations from the expected segregation ratios have been only reported in crossings between Muscadine vines, where which 35% of the hermaphrodite plants were homozygous (Conner et al. 2017). However, in two interspecific progenies: V3125 x Borner (Fechter et al. 2012) and Moscato bianco x *V. riparia*, and the intraspecific Muscat Ottonel x Malvasia aromatica di Candia (Battilana et al. 2013) the frequencies of HH genotypes resulted much lower, 7 % and 8 %. Deviation from the expected sex segregation in inter-specific crosses can be attributed to a lower seed germination or to HH genotypes presenting lethal effects as reported in other species (Yu et al 2008).

A little deviation between phenotypic and genotypic segregation according to flower sex was noticed, since 2 and 3 recombinant genotypes were scored in Grenache x Tempranillo and Graciano x Tempranillo progenies, respectively.

Phenotypic evaluation of flower, berry, seed and agronomic traits

Differences between parental genotypes of each progeny are illustrated in Table 3.1.2. Grenache presented significantly larger flower diameter ($p < 0.05$), resulting in rounder pistil shape (PS = 1.0, $p < 0.05$) compared with Tempranillo; contrarily to berry shape, that was rounder in Tempranillo cultivar (BS = 1.0, $p < 0.05$). Regarding phenology stages, significant differences in veraison dates (start and end) were found, with a 5-day delay in average in Grenache compared with Tempranillo ($p < 0.05$), but no differences were reported in ripening date since veraison-ripening resulted longer in Tempranillo cultivar. No differences were found between them in productivity traits.

Graciano and Tempranillo were statistically much more diverse than Grenache and Tempranillo. In flower morphology traits, Graciano had lower pistil and flower diameter resulting in slightly longer pistil shape (PS = 1.14 in Graciano, PS = 1.11 in Tempranillo, $p < 0.05$), and larger flowers than Tempranillo. Despite this, berries from both cultivars resulted spherical (BS = 1.0) but Graciano's were smaller. Besides, Graciano presented lower seed number, lower seed weight and lower productivity; in average 1kg less and 8 fewer clusters per vine, ($p < 0.01$). In phenology traits, Tempranillo presented earlier dates for start and end veraison (17- and 8-days difference, $p < 0.05$), and ripening (12 days of difference, $p < 0.05$), as was previously mentioned by Song et al. (2014).

With the aim of identifying the characters that best described differences between both progenies, a PCA was performed including all the traits analyzed (Figure 3.1.2). The two first dimensions explained 78.4 % and 16.8 % of the total observed variability. Genotypes of each progeny resulted clearly separated in the dimensional plot, G×T in the positive side of the second dimension and T×G in the negative, being mainly influenced by ripening date (positive axis) and seed weight and period between flowering and start veraison (negative axis).

Table 3.1.2. Flower, berry, seed, productivity and phenological parameters for Grenache, Graciano and Tempranillo cultivars

	Grenache		Graciano		Tempranillo	
	N	**Mean ± SD**	**N**	**Mean ± SD**	**N**	**Mean ± SD**
PL	3	0.4 ± 0.1	2	0.3 ± 0.0*	3	0.3 ± 0.0
FD	3	0.4 ± 0.1*	2	0.2 ± 0.0*	3	0.3 ± 0.0
OL	3	0.2 ± 0.0	2	0.1 ± 0.0	3	0.2 ± 0.0
PS	3	1.0 ± 0.0*	2	1.1 ± 0.0	3	1.1 ± 0.0
OS	3	0.6 ± 0.1	2	0.6 ± 0.2	3	0.6 ± 0.2
BL	3	17.1 ± 0.2**	4	11.3 ± 0.4**	3	15.4 ± 0.6
BD	3	15.6 ± 0.2	4	11.2 ± 0.3**	3	15.2 ± 0.5
BS	3	1.1 ± 0.0**	4	1.0 ± 0.02	3	1.0 ± 0.0
BW	3	2.2 ± 0.1	2	1.6 ± 0.1**	3	2.1 ± 0.2
SN	3	2.2 ± 0.1	3	1.3 ± 0.3*	3	1.8 ± 0.8
SW	3	56.1 ± 2.0	3	43.4 ± 1.7**	3	50.4 ± 4.9
Y	2	3.3 ± 0.5	3	2.5 ± 0.7**	5	3.6 ± 0.3
CN	2	24 ± 6	3	15 ± 3**	5	23 ± 2
FI	3	1.3 ± 0.2*	3	0.9 ± 0.2**	5	1.5 ± 0.2
F	2	93 ± 0	3	101 ± 1	5	93 ± 1
SV	2	154 ± 1*	3	164 ± 3*	3	147 ± 2
EV	2	176 ± 1	3	178 ± 4*	3	170 ± 2
VL	2	22 ± 1	3	14 ± 3.3	3	23.7 ± 2
R	2	215 ± 9	3	232 ± 3*	5	215 ± 9
EV-R	2	40 ± 8*	3	53 ± 4.2	3	49.7 ± 9

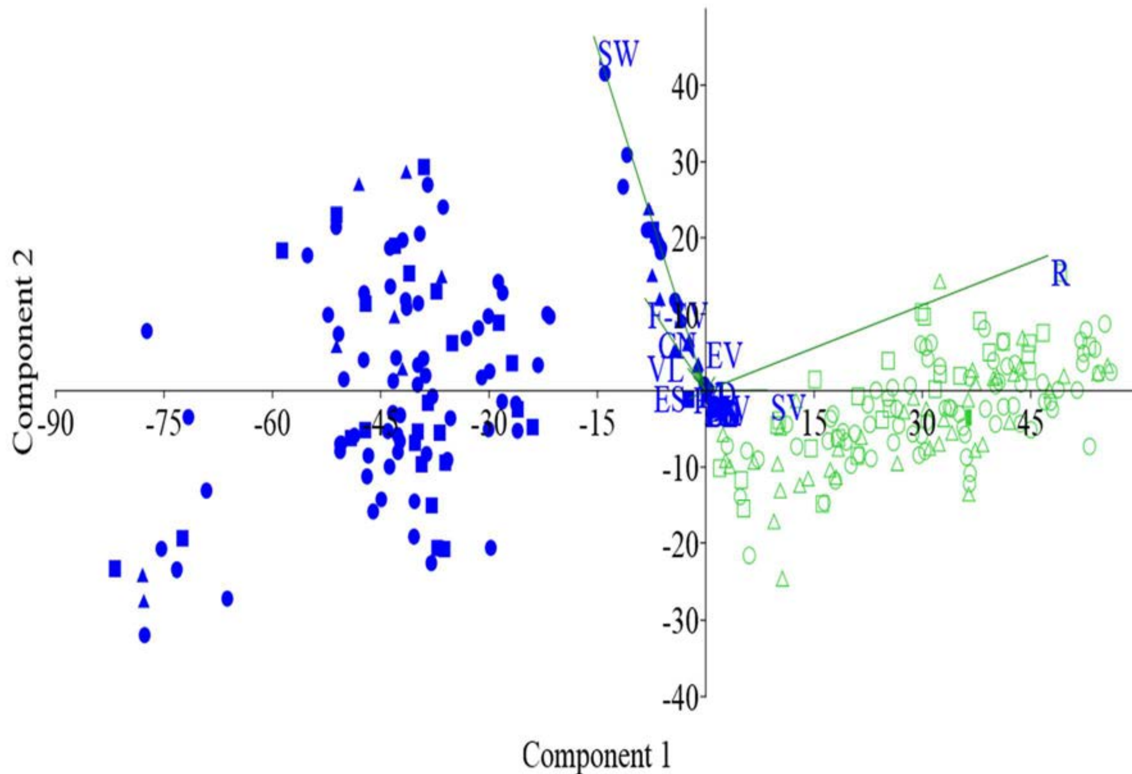
Data expressed as means ± SD. Differences between Grenache or Graciano and Tempranillo values by Tukey test are indicated with *$p < 0.05$; **$p < 0.01$. PL Pistil length, FD Flower diameter, OL Ovary length, PS Pistil shape, OS Ovary shape. BL Berry length, BD Berry diameter, BS Berry shape, BW Berry weight, SN Seed number, SW Seed weight, Y Yield, CN Cluster number, FI Fertility Index, F Flowering date, SV Start Veraison, EV End Veraison, VL Veraison period, RD Ripening date, EV-R End Veraison-Ripening period.

Influence of flower sex on phenotypic traits

Flower and berry morphology, and phenology traits were analyzed in order to assess the influence of flower sex (Table 3.1.2). In both genetic backgrounds, female flowers presented larger flower diameter, and rounder pistil and ovary shape ($p < 0.05$) compared to hermaphrodites in the two years of the study. These results agree with Margueritt et al. (2009), that reported flower diameter (named ovary length by the authors) being larger in female flowers compared with hermaphrodite and male phenotypes.

Female plants also presented lower seed number in both progenies in 2016 and 2017 vintages in G x T and T x G progenies, respectively, explained by a higher rate of parthenocarpic berry set (Doligez et al. 2013). Berry development has been studied extensively with regard to physiological processes associated with ripening but not in reference to flower morphology or sex. Although, in table grapes female plants bear larger berries than hermaphrodites (Boursiquot et al. 1995) the only association found between sex and berry traits was found in berry shape in T × G population in one vintage. Less yield was obtained in female plants (approx 0.5 kg / vine less; $p = 0.1$), but differences were significant only for T x G progeny in 2017 ($p = 0.05$).

Figure 3.1.2. PCA with berry, flower, seed, productivity and phenological data in both progenies



Legend: G x T progeny is represented in blue (filled forms) and T x G population in green (empty forms). □ ff ○ Hf Δ H. SW seed weight, ES sprouting date, F Flowering, SV start veraison, EV End veraison, VL veraison length R Ripening.

Weather conditions varied widely among years. Growing seasons in 2015 and 2017 were abnormally warm, reaching 38 ºC at the end of May and beginning of June corresponding to flowering period. These extreme temperatures triggered almost all flowers opening at the same time, being flowering time concentrated only into 2 days, and hence reducing the possible differences between flower types. In contrast, 2016 year was characterized by a typical flowering season, lasting between 4 and 5 days. Moreover, in 2017 ripening date was between 2 and 3 weeks earlier than usual, with harvest starting in mid-August, an unprecedented time in this region. Variable environmental conditions, mainly due to temperature, presumably affect plant phenology; having differences up to 12 days for flowering time been reported (Constantini et al. 2008, May 2000).

Table 3.1.3. Mean values of flower, berry, productivity and phenological traits according to sex phenotype in both populations and plots (UR and Varea).

	G × T Vintage 2015		G × T Vintage 2016				T × G Vintage 2016		T x G Vintage 2017	
	F UR	H UR	F Varea	H Varea	F UR	H UR	F	H	F	H
PL	2.65 ± 0.4	2.43 ± 0.5	3.14 ± 0.4	3.07 ± 0.3	-	-	**2.81 ± 0.2***	**3.01 ± 0.3**	3.10 ± 0.4	3.25 ± 0.4
FD	**3.07 ± 0.6****	**2.62 ± 0.5**	**3.30 ± 0.5****	**3.06 ± 0.4**	-	-	2.92 ± 0.2*	2.73 ± 0.3	2.94 ± 0.4	2.78 ± 0.4
OL	1.38 ± 0.2	1.30 ± 0.28	1.63 ± 0.2	1.60 ± 0.2	-	-	1.45 ± 0.2	1.51 ± 0.3	1.52 ± 0.2	1.54 ± 0.2
PS	**0.87 ± 0.0****	**0.94 ± 0.0**	**0.96 ± 0.0****	**1.01 ± 0.0**	-	-	**0.96 ± 0.1****	**1.11 ± 0.1**	**1.06 ± 0.1****	**1.18 ± 0.1**
OS	**0.45 ± 0.1****	**0.50 ± 0.1**	**0.50 ± 0.0****	**0.53 ± 0.0**	-	-	**0.50 ± 0.0****	**0.55 ± 0.0**	**0.52 ± 0.0****	**0.56 ± 0.0**
BL	14.6 ± 1.6	14.3 ± 1.2	15.3 ± 1.4	14.8 ± 1.6	-	-	12.7 ± 0.9	13.0 ± 1.1	12.6 ± 1.0	12.9 ± 1.6
BD	14.9 ± 1.5	14.8 ± 1.1	15.0 ± 1.7	14.7 ± 1.5	-	-	12.8 ± 0.9	12.9 ± 1.1	12.8 ± 1.1	12.91 ± 1.4
BS	0.98 ± 0.0	0.97 ± 0.0	1.02 ± 0.0	1.01 ± 0.0	-	-	**0.99 ± 0.0***	**1.01 ± 0.0**	0.99 ± 0.0	1.00 ± 0.0
BW	1.7 ± 0.4	1.7 ± 0.4	1.9 ± 0.4	1.7 ± 0.5	-	-	1.62 ± 0.3	1.7 ± 0.3	1.4 ± 0.3	1.4 ± 0.4
SN	2.4 ± 0.4	2.5 ± 0.5	**1.7 ± 0.4****	**2.3 ± 0.6**	-	-	2.2 ± 0.4	2.2 ± 0.4	**1.6 ± 0.3****	**2.2 ± 0.5**
SW	55.6 ± 16.6	55.5 ± 14.5	54.4 ± 13.9	56.3 ± 16.6	-	-	31.6 ± 5.3	31.7 ± 4.0	**37.3 ± 4.9****	**28.3 ± 4.8**
Y	2.6 ± 1.6	2.9 ± 1.3	2.1 ± 1.4	2.8 ± 1.6	2.1 ± 1.3	2.8 ± 1.3	2.0 ± 1.3	2.6 ± 1.6	**1.8 ± 1.0***	**2.7 ± 1.2b**
CN	20.6 ± 8.5	18.3 ± 6.5	15.2 ± 15.5	13.6 ± 11.4	**18.6 ± 7.5***	**13.5 ± 5.9**	10.8 ± 5.5	10.3 ± 8.2	11.1 ± 5.0	10.5 ± 8.2
FI	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.6	0.9 ± 0.4	**1.0 ± 0.3***	**0.8 ± 0.3**	0.73 ± 0.3	0.6 ± 0.3	0.8 ± 0.2	0.8 ± 0.3
ES	-	-	70 ± 7	72 ± 7	67 ± 6	68 ± 4	72 ± 8	73 ± 8	60 ± 4	61 ± 4
F	94 ± 1	94 ± 1	**102 ± 2***	**100 ± 2**	100 ± 1	100 ± 2	**100 ± 1****	**98 ± 1**	110 ± 3	110 ± 2
SV	**150 ± 8 ***	**144 ± 7**	158 ± 3	158 ± 8	159 ± 3	159 ± 3	160 ± 5	158 ± 5	171 ± 4	169 ± 5
EV	171 ± 5	168 ± 6	174 ± 4	175 ± 5	175 ± 6	176 ± 5	175 ± 4	175 ± 3	-	-
R	216 ± 10	214 ± 11	213 ± 14	213 ± 12	221 ± 10	219 ± 10	206 ± 14	207 ± 14	232 ± 16	235 ± 15
VL	22 ± 8	24 ± 8	**15 ± 4****	**18 ± 4**	15 ± 4	18 ± 4	**15 ± 3***	**17 ± 4**	-	-
S-F	-	-	31 ± 7	28 ± 6	34 ± 7	32 ± 5	31 ± 17	27 ± 10	**50 ± 4***	**48 ± 4**
F-SV	55 ± 9	50 ± 10	58 ± 3	58 ± 8	59 ± 3	59 ± 3	60 ± 4	59 ± 4	61 ± 5	60 ± 5
EVR	45 ± 12	43 ± 11	41 ± 13	41 ± 11	45 ± 13	38 ± 10	32 ± 14	32 ± 14	-	-

Data expressed as means ± SD. Differences between female (F) and hermaphrodite (H) genotypes values by Tukey test are indicated with *$p < 0.05$; **$p < 0.01$, and highlighted in bold. PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, BL berry length, BD berry diameter, BS berry shape, BW berry weight, SN seed number, SW seed weight, Y yield, CN cluster number, , FI fertility index, ES End sprouting date, F flowering date, SV start veraison date, EV end veraison date, R ripening, VL veraison length, S-F sprouting flowering period, F-SV flowering veraison period and EV-R veraison ripening period.

Female plants of both progenies presented a 1-day delay in flowering time compared with hermaphrodites. This delay began at sprouting and lasted until start veraison, and hence veraison length was shorter. The delay experienced by female genotypes at the beginning of the cycle may be related to the development of the ovary and the differential gene expression among the three *Vitis* flower types during flowering developmental stages. Male flowers, that bloom earlier than hermaphrodite and female plants, have a high proportion of up-expressed genes related to hormone control in stage B (early developmental stage), while females have almost twice the genes expressed in H stage (just before blooming) and hermaphrodite plants show reduced gene expression in stage H compared to female and male (Ramos et al. 2014).

QTL analysis for phenology, productivity and seed traits were reported in LG2 close to VVIB23 marker, in a "Syrah x Grenache" progeny (Constantini et al. 2008) and for flower morphology traits in "Cabernet Sauvignon x *V. riparia* Gloire de Montpellier" population (Margueritt et al. 2009); hence, confirming that differences observed in those traits are influenced by the *Sex* locus.

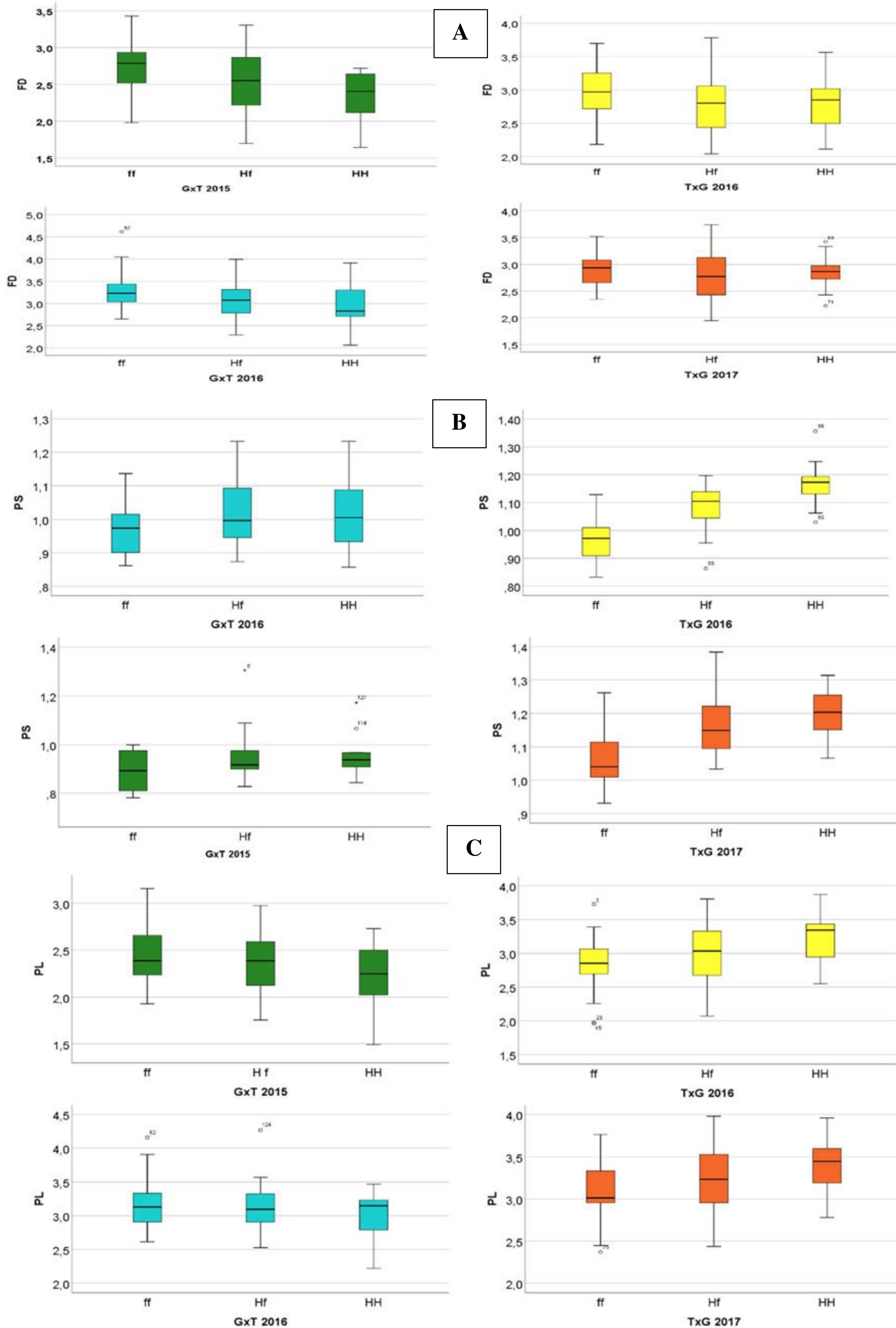
Genotypes were further classified into three sex groups (*HH*, *Hf* and *ff*) based on VVIB23 marker genotyping. Larger differences were found when H and F genotypes were considered, suggesting complete dominance effect of H allele in flower, seed or productivity traits (Table 3.1.3). This result is further confirmed by the fact that differences between Hf and HH genotypes were little and not consistent (Supplementary material 3.1.1) only pistil length (PL) in 2016 and berry length (BL) in 2017 resulted significantly longer in *HH* genotypes than in *Hf* in T × G progeny, and in G × T progeny, veraison ended later for *HH* genotypes than for *Hf* in 2016.

In summary, female plants presented larger flower diameter (FD) than Hf (2015 and 2016) and *HH* (2015) genotypes in G × T progeny and only in 2016 in T × G population (Figure 3.1.3). Pistil shape (PS) was significantly longer ($p < 0.01$) for H than for F genotypes in both progenies across the two years (Figure 3.1.3)., and in T x G 2016 *HH* genotypes presented longer pistil shape than *Hf*. Differences in pistil length (PL) between H and F plants explain the longer pistil shape in H, at least for T x G progeny.

Since G × T progeny segregated for berry colour, associations of colour and sex were investigated. Berry colour adjusted to the expected 3:1 ratio with a dominance of the red allele (98:32 red: white, $p = 0.92$). Considering different flower sex and colour categories, 32 white-berry plants segregated as 16 HH, 13 Hf and 3 ff, representing a segregation distortion favouring HH genotypes ($p = 0.003$). However, of 97 red-berry plants genotyped, 8 were HH, 63 Hf and 26 ff, being HH genotypes underrepresented ($p = 0.0005$). Although, colour presumably triggers a distortion in sex segregation, results are conditioned by limited population size. However, in the intraspecific crossing reported by Battilana et al. (2013) white cultivars also presented a distortion in sex segregation compared to red varieties.

Regarding berry traits, in red-berry genotypes, berry shape was rounder (although significance was low $p < 0.1$) and ripening was delayed compared with white-berry plants ($p < 0.05$) (Supplementary material 3.1.3). Massonnet et al. (2017) compared five red and five white Italian grapevine varieties in different environmental and agronomic conditions finding high transcriptomic variation among red cultivars during ripening. They suggested that colour development affects the maturation process itself by triggering the transcriptional reprogramming of several biological processes, hence, explaining why red genotypes reach ripening later than white ones.

Figure 3.1.3. Flower diameter (FD) (A) pistil shape (PS), (B) and pistil length differences (C) between ff, Hf and HH genotypes in both progenies across two years.



In order to test if different flower types presented differences attending to colour, ANOVA was conducted with 2015 and 2016 data. No consistent differences were found between red female (Supplementary material 3.1.4) and red hermaphrodite genotypes in flower or berry traits. Regarding hermaphrodites (Supplementary material 3.1.5), *Hf* red plants showed longer ripening periods than *Hf* white plants in both vintages (between 8 and 16 days), whilst for *HH* genotypes, only in 2016, and for female plants no difference was found.

Phenotypìc correlations

In order to assess the effect of genetic and environmental factors on the traits studied the vintage (year) and the plot effect, the correlations between years for the same traits and the broad sense heritability were estimated.

A significant year effect was observed for all productivity and phenology traits in both genetic backgrounds. Vintage effect was also observed in both progenies for the berry and flower traits studied except for berry diameter, berry weight and flower diameter. That was expected since cultural practices and environmental factors during floral differentiation and after flowering may influence berry, flower and phenology traits (Dunn & Martin 2000, Gray & Coombe 2009, Barbagallo et al. 2011). In relation to phenology traits, given that Graciano is a late-ripening variety, the fact that T × G progeny showed stronger vintage effects on berry and phenology traits was expected. In G × T, vintage effect was greater in flower parameters but veraison dates and ripening resulted also significant. Climatic data of 2010 year between March and October was characterised by higher pluviometry (almost two-fold) and accumulated radiation than 2016, presumably triggering changes in all phenology stages. Remarkably, for T × G progeny, ripening date suffered a significant delay of approximately two weeks between 2010 and 2016 years.

A MANOVA was conducted in G x T progeny to test the interactions between plot, flower sex and vintage (Table 3.1.4). Plot effect was observed in most phenology traits, mainly in veraison period, from flowering date to start veraison. Interactions among genotypes for sex and fertility index and end veraison date were found. The valley effect and soil characteristics make vines in Varea more productive and earlier in phenology compared to UR plot. Interactions between plot and vintage were observed for fertility index, end veraison and veraison length traits, and as expected not interaction was found between flower sex and vintage. Interaction between vintage and progeny was found only for berry length, berry shape and berry weight traits.

Best linear unbiased predictors (BLUPs) of genetic values were calculated, and broad-sense heritabilities (H^2) of the inter-environment genotypic mean were estimated. Overall H^2 were higher in T × G progeny for flower and berry traits ($0.35 < H^2 < 0.8$) confirming the higher variability present in this progeny compared with G × T progeny. In G × T population, the highest H^2 values corresponded to berry traits (BS, 0.42); whilst in T×G the highest values were registered in the flower parameters 0.7 - 0.8 (for FD and OL).

Table 3.1.4. MANOVA results in G x T progeny with plot, sex and vintage as fix factors.

	G × T progeny					
Factor	**Plot**		**Plot x Sex**		**Plot x Vintage**	
Trait	**F**	**Sig**	**F**	**Sig**	**F**	**Sig**
FI	ns	ns	2.9	0.05	9.9	0.00
F	607.6	0.00	-	-	ns	ns
SV	206.7	0.00	ns	ns	ns	ns
EV	50.6	0.00	3.3	0.04	4.9	0.03
RD	-	-	ns	ns	ns	ns
VL	71.1	0.00	ns	ns	12.4	0.00
F-SV	61.3	0.00	ns	ns	ns	ns
EV-R	-	-	ns	ns	ns	ns

FI fertility index, F flowering date, SV start veraison date, EV end veraison date, RD ripening, VL veraison length, F-SV flowering veraison period and EV-R veraison ripening period.

Table 3.1.5. Broad sense heritability (H^2) and correlations between years in both progenies

	G × T progeny		**T × G progeny**	
	H^2	**r^2**	**H^2**	**r^2**
PL	0.24	0.4 **	0.68	0.8 **
FD	0.35	0.5 **	0.78	0.8 **
OL	0.18	0.3 **	0.79	0.8 **
PS	0.29	0.4 **	0.47	0.7 **
OS	0.17	ns	0.35	0.5 **
BL	0.32	0.7 **	0.54	0.5 **
BD	0.33	0.7 **	0.54	0.6 **
BS	0.42	0.8 **	0.51	0.5 **
BW	0.29	0.8 **	0.46	0.6 **
SN	0.23	0.3 **	0.21	ns
SW	0.28	ns	0.32	0.3**
Y	0.13	0.7 **	0.09	0.6**
FI	0.10	0.2 *	0.20	0.5**
ES	0.00	ns	0.00	ns
F	0.00	ns	0.00	ns
SV	0.00	0.5 **	0.00	0.5**
EV	0.00	0.5 **	0.00	0.5**
VL	0.00	ns	0.02	0.5**
RD	0.07	0.3 **	0.10	0.6**
ES-F	0.00	ns	0.00	ns
F-SV	0.00	ns	0.00	0.1*
EV-R	0.00	0.5 **	0.00	0.5**

** reflects statistical differences at 0.01 level, * at 0.05. PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, BL berry length, BD berry diameter, BS berry shape, BW berry weight, SN seed number, SW seed weight, Y yield, CN cluster number, CW cluster weight, FI fertility index, ES end Sprouting date, F flowering date, SV start veraison date, EV end veraison date, RD ripening.

VL veraison length, ES-F sprouting flowering period, F-SV flowering veraison period and EV-R veraison ripening period.

Berry weight H^2 was low in G × T and moderate in T × G (0.29 and 0.46, respectively) whilst seed number (SN) showed low values ($H^2 < 0.3$) for both progenies. Both traits were reported as high heritability traits by Houel et al. (2015) in a Picovine x Ugni blanc population, where more genetic variance was present. Productivity and phenology traits presented very low heritabilities. in our populations confirming the high genetic complexity and great environmental influence reported by other authors (May 2000, Fechter et al. 2014, Kamal et al. 2017). Traits with high heritability are suitable for indirect selection for other desirable traits with lower heritability in breeding programs. Indirect selection is of great interest particularly in woody species as grapevine that have long generation cycles (Viana et al. 2011).

Correlations between flower berry, productivity and phenology variables

Correlation coefficients (Spearman) between traits for each sex phenotypic class were calculated in both populations with 2016 data (Supplementary material 3.1.5-3.1.8). Estimation of correlations between traits is a key factor in breeding programs especially if traits present negative correlations, low heritability or are difficult to quantify (Viana et al. 2011). As expected, the highest correlation coefficients were obtained between component variables of the same trait in both genetic backgrounds. Thus, berry length, diameter and weight were highly correlated, like flower diameter, pistil length and ovary length ($r = 0.9$, $p < 0.01$). Yield, cluster number and cluster weight were also highly correlated ($r = 0.7 - 0.8$, $p < 0.01$). In G x T progeny, berry weight, seed weight and seed number showed positive and moderate correlations ($r = 0.6$, $p < 0.01$) as previously reported (Walker et al. 2005, Constantini et al. 2008), whilst in T x G progeny a negative correlation was found between seed number and seed weight ($r = - 0.5$, $p < 0.05$), in agreement with other works (Wei et al. 2002, Song et al. 2014). The relationship between seed and berry traits is still debated (Doligez et al. 2013), since positive or negative correlations are reported in different works (Wei et al. 2002, Doligez et al. 2013).

Few significant correlations were found between traits in female genotypes compared to hermaphrodites maybe due to the low number of plants analyzed (around 25 in each progeny) (Supplementary material 3.1.5). In T × G progeny, significant correlations were found between berry and flower traits, being berry weight moderately correlated with flower diameter and ovary length (0.6, $p < 0.01$), whilst in G × T female population only pistil length and flowering date were correlated. Correlations among traits in hermaphrodite plants are presented in Supplementary material 3.1.6. A comparison in the correlations between the two hermaphrodite genotypes was also performed. Remarkably, a high correlation between berry length and berry shape was observed in both genetic backgrounds ($r = 0.6$, $p < 0.01$), being lower in *Hf* plants ($r = 0.4$) and absent in female plants. Similarly, berry and flower shape in both progenies, showed a stronger association ($r = 0.6$, $p < 0.01$) compared to *Hf* plants ($r = 0.4$, $p < 0.01$) and no-significant correlation in females. Start and end veraison dates resulted highly correlated in both progenies ($r = 0.7$-0.9, $p < 0.01$). In relation to *Hf* genotypes (Supplementary material 3.1.8), higher significant correlations were found compared to *HH*, being especially relevant between berry traits, seed traits and productivity traits, which were practically absent in *HH* genotypes. Negative correlations were found between productivity traits and phenology stages, as it was noticed in *Hf* plants, suggesting that apart from environmental factors, the developmental stages had a great influence on yield. Association studies between these parameters according to sex have not been

reported before. Likely, female *ff* and *HH* genotypes are the ones more influenced by different factors as temperature or vigour, resulting in low correlations between characters, maybe because *Hf* genotypes were positively selected for in breeding programs.

Conclusions

This work proved, in two wine-grape segregating progenies, that *Sex* locus influenced flower seed and phenological traits. Female plants presented higher flower diameter that influenced pistil and ovary shape, lower number of seeds and a delay from end sprouting to start veraison along with a shortening of veraison compared with hermaphrodite plants. These differences were consistent in both genetic backgrounds in different vintages and plots for flower shape, being greater between *ff* and *HH* genotypes. VVIB23 marker has allowed the detection of three flower sex types. Vintage and plot had an effect in phenology traits in the early developmental stages. Traits as berry diameter, flower diameter and seed weight seem to present a higher correlation between years, being the correlations higher in hermaphrodite than in female plants especially in T x G progeny. Associations were found in both genetic backgrounds between berry length and berry shape (0.4, $p < 0.01$), and berry shape and pistil shape (0.4, $p < 0.01$). These relationships resulted higher in *HH* genotypes and absent in female plants. Flowering date, seed traits and flower morphology traits were correlated. Colour presumably plays a role in sex segregation distortion especially in white cultivars, and significant differences were reported according to sex and colour in this work, mainly in ripening date. Broad sense heritability estimates for the traits studied varied between low and moderate, except for flower diameter, pistil length and ovary length that were high (0.7 - 0.8).

The interest of this research relies on the fact that it addresses for the first time differences in berry, flower, seed morphology and other productivity and phenological traits between *ff*, *Hf* and *HH* genotypes in *Vitis vinifera* wine progenies, increasing the understanding of the influence of flower sex in relevant traits for grapevine breeding.

Supplementary material

Supplementary material 3.1.1. Mean values of flower, berry, productivity and phenological traits for hermaphrodite plants according to sex genotype in both populations and plots (UR and Varea).

	G × T Vintage 2015		**G × T Vintage 2016**				**T × G Vintage 2016**		**T × G Vintage 2017**	
	H (*Hf*) UR	**H (*HH*) UR**	**H (*Hf*) V**	**H (*HH*) V**	**H (*Hf*) UR**	**H (*HH*) UR**	**H (*Hf*)**	**H (*HH*)**	**H (*Hf*)**	**H (*HH*)**
PL	2.43± 0.4	2.44± 0.7	3.08± 0.3	3.04± 0.4	-	-	2.93± 0.5	3.1± 0.4	3.17± 0.4	3.35± 0.3
FD	2.63± 0.5	2.58± 0.6	3.06± 0.4	3.1± 0.4	-	-	2.7± 0.4	2.8± 0.4	2.75± 0.5	2.81± 0.4
OL	1.3± 0.3	1.3± 0.3	1.6± 0.2	1.58± 0.2	-	-	1.48± 0.3	1.56± 0.2	1.52± 0.3	1.58± 0.2
PS	0.93± 0.1	0.95± 0.1	1.01± 0.1	1.0± 0.1	-	-	**1.08± 0.1***	**1.14± 0.1**	1.2± 0.1	1.2± 0.1
OS	0.5± 0.1	0.51± 0.1	0.53± 0.0	0.5± 0.05	-	-	0.54± 0.0	0.56± 0.0	0.56± 0.0	0.6± 0.1
BL	14.2± 1.2	14.4± 1.1	14.7± 1.7	15.2± 1.4	-	-	12.8± 1.2	13.1± 1.1	**12.7± 1.6***	**13.3± 1.1**
BD	14.7± 1.2	15.0± 0.9	14.6± 1.6	15.1± 1.4	-	-	12.7± 1.1	13.1± 1.1	12.8± 1.4	13.3± 1.0
BS	0.97± 0.0	0.96± 0.0	1.01± 0.0	1.01± 0.04	-	-	1.01± 0.0	1.01± 0.0	0.99± 0.0	1.01± 0.0
BW	1.7± 0.4	1.8± 0.3	1.7± 0.5	1.8± 0.6	-	-	1.7± 0.3	1.7± 0.3	1.4± 0.4	1.5± 0.4
SN	2.5± 0.5	2.8± 0.5	2.3± 0.6	2.3± 0.6	-	-	2.2± 0.4	2.1± 0.5	2.2±0.5	2.2±0.5
SW	54.3± 15.3	58.8± 12.4	56.3± 16.9	55.8± 15.7	-	-	31.4± 4.3	31.7± 3.7	27.8±4.4	29.0±5.3
Y	2.8±1.3	3.2±1.3	2.9±1.8	3.0±1.3	3.0±1.6	2.5±1.6	2.6±1.2	2.7±1.2	2.6±1.5	2.6±1.6
CN	18.6±6.6	17.8±5.8	12.7±8.3	13.7±9.4	13.8±5.9	12.8±6.0	9.7±8.0	11.2±8.5	10.5±9.2	11.5±10.2
FI	1.17±0.4	1.1±0.4	0.9±0.4	0.8±0.4	0.9±0.3	0.83±0.3	0.6±0.3	0.6±0.3	0.7±0.3	0.8±0.3
S	-	-	71.4±6.6	73.7±7.0	67.9±4.5	66.5±3.1	72.8±7.7	72.7±7.2	61.3±4.0	61.6±3.6
F	93.9±0.8	93.7±0.8	99.8±1.8	99.6±1.4	99.7±1.4	99.6±1.5	99.0±1.5	98.6±1.0	109.8±2.1	109.3±2.2
SV	143.9±8.9	141.2±8.4	157.8±8.2	157.7±1.8	158.4±3.2	158±3.0	158.3±3.8	157±2.3	169.7±4.0	168.5±5.1
EV	168.5±5.9	164.6±8.9	**174.6±5.0***	**178.3±2.2**	175.8±4.9	176.5±6.0	175.0±2.8	174.7±3.5	-	-
R	214.5±11.3	214.8±10.3	213.5±12.6	209.6±12.6	217.4±10.1	220.9±7.1	208.3±13.7	204.5±13.8	237.3±13.7	231.7±15.8
VL	25.4±5.0	25.9±4.4	18.0±4.3	20.7±2.7	17.4±3.8	18.5±4.7	16.5±2.7	17.03±2.3	-	-
ES-F	-	-	28.4±5.9	27.1±5.4	31.8±4.7	32.9±3.9	26.5±7.1	28.9±13.4	48.8±4.5	48.4±4.4
F-SV	50.1±9.1	47.4±8.6	58.0±8.4	57.8±1.9	58.7±2.7	60.2±2.2	59.2±3.9	58.7±2.7	60.0±4.2	58.9±4.9
EV-R	43.0±11.2	42.8±11.1	41.4±11.4	40.0±9.8	42.1±10.3	32.6±4.0	33.9±13.7	28.6±14.1	-	-

Data expressed as means ± SD. Differences between female (F) and hermaphrodite (H) genotypes values by Tukey test are indicated with *p < 0.05, and highlighted in bold. PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, BL berry length, BD berry diameter, BS berry shape, BW berry weight, SN seed number, SW seed weight, Y yield, CN cluster number, FI fertility index, ES Sprouting date, F flowering date, SV start veraison date, EV end veraison date, R ripening, VL veraison length, S-F sprouting flowering period, F-SV flowering veraison period and EV-R veraison ripening period. V mean Varea plot.

Supplementary material 3.1.2. Mean values in red and white plants in G x T progeny in both years of the study

	G x T 2015 Vintage						G x T 2016 Vintage					
	Red-berry genotypes			White-berry genotypes			Red-berry genotypes			White-berry genotypes		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
PL	96	2.51	0.46	31	2.38	0.48	82	3.09	0.36	30	3.08	0.32
FD	96	2.75	0.52	31	2.6	0.56	82	3.13	0.43	30	3.08	0.39
OL	96	1.34	0.26	31	1.25	0.29	82	1.61	0.19	30	1.6	0.19
PS	96	0.92	0.09	31	0.92	0.06	82	1.00	0.09	30	1.01	0.09
OS	96	0.49	0.05	31	0.48	0.05	82	0.52	0.04	30	0.52	0.04
BL	81	14.39	1.32	23	14.26	1.02	77	14.87	1.62	24	15.14	1.35
BD	81	14.79	1.23	23	14.89	1.15	77	14.67	1.49	24	15.15	1.35
BS	81	0.98	0.02	23	0.96	0.03	77	1.01	0.03	24	0.98	0.03
BW	97	1.67	0.37	31	1.73	0.36	75	1.75	0.51	26	1.84	0.51
SN	71	2.45	0.51	19	2.64	0.44	74	2.15	0.57	25	2.22	0.62
SW	85	54.92	15.1	26	57.47	14.48	74	56.66	16.76	25	53.44	13.35
Y	98	2.9	1.5	32	2.6	1.1	45	4.93	3.56	22	4.91	2.8
CN	98	18.8	6.9	32	19	7.3	45	14.91	6.96	22	14.64	6.25
CW	98	153.1	68.5	32	145.9	55.9	45	329.9	173.7	22	341.2	149.3
FI	98	1.2	0.4	32	1.1	0.4	98	0.92*b	0.28	32	0.79a	0.23
F	95	101.6	53.1	32	105.5	65.1	88	100	1.82	27	99.7	1.66
RD	**98**	**215.8 ***	**7.1**	**32**	**211.5**	**6.5**	**75**	**215.7*b**	**11.32**	**26**	**204.6 a**	**12.64**

* reflects statistical differences at at 0.05. PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, BL berry length, BD berry diameter, BS berry shape, BW berry weight, SN seed number, SW seed weight, Y yield, CN cluster number, CW cluster weight, FI fertility index, F flowering date, RD ripening date.

Supplementary material 3.1.3. Mean values in red and white plants in G x T progeny according to sex phenotype in both years of the study

	Red F 2015		**White F 2015**		**Red F 2016**		**White F 2016**		**Red H 2015**		**White H 2015**		**Red H 2016**		**White H 2016**	
	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD
PL	23	2.63±0.44	3	2.78±0.47	22	3.16±0.41	4	2.99±0.26	73	2.47±0.47	28	2.33±0.47	60	3.07±0.34	26	3.09±0.33
FD	23	3.06±0.6	3	3.14±0.39	22	3.35±0.47	4	3.04±0.29	73	2.65±0.46	28	2.54±0.55	60	3.05±0.39	26	3.09±0.41
OL	23	1.4±0.25	3	1.28±0.1	22	1.65±0.22	4	1.53±0.07	73	1.32±0.27	28	1.25±0.31	60	1.59±0.18	26	1.62±0.2
PS	23	0.87±0.09	3	0.89±0.1	22	0.95±0.08	4	0.99±0.03	73	0.94±0.09	28	0.93±0.06	60	1.02±0.09	26	1.01±0.1
OS	23	**0.46±0.04***	3	**0.41±0.02**	22	0.5±0.04	4	0.51±0.02	73	0.5±0.05	28	0.49±0.05	60	0.53±0.04	26	0.53±0.04
BL	22	14.61±1.61	2	14.14±0.25	19	15.39±1.31	4	14.67±1.66	59	14.32±1.2	21	14.27±1.06	58	14.69±1.68	20	15.23±1.31
BD	22	14.97±1.58	2	14.48±0.69	19	15.08±1.25	4	14.72±1.53	59	14.72±1.08	21	14.93±1.18	58	14.54±1.54	20	15.24±1.34
BS	22	0.98±0.04	2	0.98±0.03	19	1.02±0.04	4	1.00±0.02	59	0.98 ±0.03	21	0.96±0.03	58	1.01±0.03	20	1.00±0.03
BW	23	1.65±0.42	4	1.65±0.31	18	1.93±0.4	4	1.73±0.45	74	1.68±0.35	28	1.74±0.37	57	1.69±0.53	22	1.86±0.53
SN	17	2.29±0.38	3	2.67±0.58	18	1.73±0.34	4	1.74±0.53	54	2.51±0.53	16	2.64±0.43	56	2.29±0.56	21	2.32±0.61
SW	21	53.8±15.29	3	67.4±24.55	18	55.8±14.4	4	47.9±10.7	64	55.27±15.1	23	56.18±12.97	56	56.94±17.57	21	54.5±13.75
Y	24	2.63±1.69	4	2.35±0.68	13	2.23±1.29	2	1.85±0.68	74	2.93±1.41	28	2.67±1.17	32	5.03±3.53	20	4.95±2.94
CN	24	20.67±8.73	4	20±6.48	13	18.23±9.17	2	16.5±2.12	74	18.15±6.11	28	18.82±7.51	32	13.56±5.45	20	14.45±6.52
CW	24	118.6±51.4	4	123.2±35.2	13	240.6±97.3	2	272.8±8.6	74	164.24±69.95	28	149.09±58.02	32	366.1±185.7	20	348.1±155.2
FI	24	1.13±0.43	4	0.94±0.17	24	0.99±0.32	4	0.9±0.12	74	1.16±0.4	28	1.1±0.46	74	**0.9±0.26***	28	**0.77±0.24**
F	21	94.25±0.6	4	93.75±0.5	23	101±2	3	101±3	74	99±2	28	100±2	65	100±2	24	100±2
RD	24	217±9	4	211±14	18	214±14	4	211±14	74	215±6	28	211±6	57	**216±10***	22	**204±12**

* reflects statistical differences at 0.05 and highlighted in bold. PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, BL berry length, BD berry diameter, BS berry shape, BW berry weight, SN seed number, SW seed weight, Y yield, CN cluster number, CW cluster weight, FI fertility index, F flowering date, RD ripening.

Supplementary material 3.1.4. Mean values in red and white plants in G×T progeny according to sex genotype in both years of the study

	Red *Hf* 2015		White *Hf* 2015		Red *Hf* 2016		White *Hf* 2016		Red *HH* 2015		White *HH* 2015		Red *HH* 2016		White *HH* 2016	
	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD
PL	62	2.44±0.37	13	2.39±0.44	52	3.06±0.34	12	3.15±0.26	8	2.73±0.94	15	2.29±0.51	5	3.04±0.29	14	3.04±0.38
FD	62	2.65±0.45	13	2.58±0.51	52	3.04±0.38	12	3.13±0.35	8	2.72±0.68	15	2.51±0.59	5	3.02±0.5	14	3.05±0.46
OL	62	1.31±0.25	13	1.26±0.3	52	1.59±0.19	12	1.64±0.17	8	1.43±0.33	15	1.23±0.33	5	1.57±0.18	14	1.59±0.24
PS	62	0.93±0.08	13	0.93±0.06	52	1.01±0.09	12	1.02±0.1	8	**1.02±0.08***	15	**0.92±0.05**	5	1.02±0.12	14	1.01±0.1
OS	62	0.5±0.05	13	0.49±0.06	52	0.53±0.04	12	0.53±0.03	8	0.53±0.05	15	0.49±0.04	5	0.52±0.05	14	0.52±0.05
BL	51	14.21±1.21	9	14.38±1.13	49	14.54±1.74	10	15.4±0.93	5	15.03±1.2	12	14.19±1.06	5	15.39±0.88	10	15.06±1.65
BD	51	14.61±1.11	9	15.01±1.41	49	**14.4±1.11***	10	**15.48±0.9**	5	15.41±0.49	12	14.86±1.04	5	15.29±0.85	10	14.99±1.69
BS	51	0.97±0.04	9	0.96±0.03	49	1.01±0.03	10	1.0±0.02	5	0.98±0.05	12	0.96±0.04	5	1.01±0.04	10	1.01±0.03
BW	63	1.64±0.35	13	1.78±0.4	48	1.65±0.56	10	1.93±0.33	8	1.92±0.26	15	1.72±0.36	5	1.91±0.24	12	1.8±0.66
SN	46	2.45±0.52	9	2.65±0.49	47	**2.24±0.34**	10	**2.64±0.45**	5	2.99±0.59	7	2.62±0.37	5	**2.76±0.52***	11	**2.02±0.51**
SW	56	53.6±15.1	10	58.7±16.7	47	55.38±17.63	10	60.72±13.24	5	**69.4±13.3***	13	**54.7±9.7**	5	71.14±11.39	11	48.85±12.11
Y	63	2.84±1.42	13	2.59±1.1	27	4.58±3.78	9	6.04±3.08	8	**3.93±1.14***	15	**2.74±1.26**	3	5.88±2.03	11	4.06±2.62
CN	63	18.16±6.16	13	20.62±8.37	27	12.85±5.81	9	16.44±5.98	8	18.75±4.13	15	17.27±6.56	3	12.67±2.89	11	12.82±6.75
CW	63	158.6±67.7	13	129.8±30.0	27	245.7±97.0	9	260.2±91.1	8	216.9±81.7	15	165.8±71.2	3	264.5±90.0	11	238.1±78.2
FI	63	1.19±0.44	13	1.09±0.42	54	**0.88±0.41***	12	**1.11±0.28**	8	1.11±0.2	15	1.11±0.51	8	**1.00±0.32***	15	**0.74±0.23**
F	63	100±1	13	99±2	57	100±2	11	99±1	8	94±1	15	94±1	5	99±1	13	100±2
RD	63	**216±8*b**	13	**208±8**	48	**216±11***	10	**200±12**	8	**215±9**	15	**215±11**	5	**219±8***	12	**206±13**

* reflects statistical differences at 0.05 and highlighted in bold. PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, BL berry length, BD berry diameter, BS berry shape, BW berry weight, SN seed number, SW seed weight, Y yield, CN cluster number, CW cluster weight, FI fertility index, F flowering date, RD ripening.

Supplementary material 3.1.5. Correlation matrix in female plants for all the traits studied in both progenies in 2016.

F		G x T progeny																			
		PL	**FD**	**OS**	**PS**	**OS**	**BL**	**BD**	**BS**	**BW**	**SN**	**SW**	**Y**	**CN**	**CW**	**DS**	**F**	**SV**	**EV**	**RD**	**VL**
T x G progeny	**PL**	**1**	0.8**	0.9**			0.5*	0.6*		0.6*							0.5*				
	FD	0.8**	**1**	0.9**	-0.5**	-0.5*															
	OL	0.9**	0.9**	**1**				0.6*		0.6*											
	PS	0.5*			**1**	0.8**	0.6*	0.5*		0.6*											
	OS	0.6**		0.6**	0.6**	**1**	0.7**	0.7**		0.6*											
	BL						**1**	0.8**		0.7**		0.5*				0.5*					
	BD						0.9**	**1**		0.9**											
	BS								**1**				0.5*		0.5*						
	BW						0.9**	0.9**		**1**	0.6**	0.6**	0.5*		0.5**			0.5*		0.6*	
	SN										**1**	0.8**	0.5*		0.6**			0.6*			
	SW										-0.5*	**1**			0.5**			0.5*			
	Y												**1**	0.8**	0.7**						
	CN												0.8**	**1**							
	CW												0.8**		**1**						
	ES	0.5*		0.5*												**1**		0.4*			
	F																**1**	0.6**	0.4*		
	SV																	**1**	0.7**		
	EV		-0.5*															0.8**	**1**		0.8**
	RD																			**1**	
	VL					-0.6**					-0.5*										**1**

Colour legend: 0.8-0.9 dark green, 0.6-0.7 medium green, 0.4-0.5 light green.
** reflects statistical differences at 0.01 level, * at 0.05. PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, BL berry length, BD berry diameter, BS berry shape, BW berry weight, SN seed number, SW seed weight, Y yield, CN cluster number, CW cluster weight, FI fertility index, ES end Sprouting date, F flowering date, SV start veraison date, EV end veraison date, RD ripening, VL veraison length.

Supplementary material 3.1.6. Correlation matrix in hermaphrodite *Hf* plants for all the traits in both progenies in 2016.

H	G x T progeny																			
T x G progeny	PL	FD	OS	PS	OS	BL	BD	BS	BW	SN	SW	Y	CN	CW	DS	F	SV	EV	RD	VL
PL	**1**	0.9^{**}	0.9^{**}			0.5^{**}	0.6^{**}		0.6^{**}		0.3^{**}			0.2^{*}		0.2^{*}			-0.2^{*}	
FD	0.9^{**}	**1**	0.9^{**}	-0.6^{**}	-0.5^{**}	0.4^{**}	0.5^{**}		0.5^{**}	0.3^{**}	0.3^{**}				-0.2^{**}				-0.2^{*}	
OS	0.9^{**}	0.9^{**}	**1**			0.5^{**}	0.6^{**}		0.6^{**}	0.3^{**}	0.4^{**}	0.3^{*}		0.2^{*}						
PS	0.3^{**}	-0.6^{**}		**1**	0.8^{**}	0.2^{*}	0.2^{*}		0.2^{**}	-0.2^{*}					0.3^{**}	0.3^{**}				
OS	0.4^{**}	-0.4^{**}	0.5^{**}	0.7^{**}	**1**	0.4^{**}	0.4^{**}	0.2^{*}	0.4^{**}					0.2^{*}						
BL	0.4^{**}		0.4^{**}			**1**	0.9^{**}	0.4^{**}	, 0.9^{***}	0.4^{**}	0.6^{**}		0.4^{**}	0.5^{**}		-0.3^{*}		0.3^{*}		0.3^{*}
BD	0.4^{**}	0.3^{**}	0.4^{**}			0.9^{**}	**1**		0.9^{**}	, 0.5^{**}	0.7^{**}		0.4^{*}	0.5^{**}	-0.2^{*}			0.3^{*}		0.3^{*}
BS		-0.3^{*}		0.4^{**}	0.3^{*}	0.5^{**}	0.3^{*}	**1**											-0.2^{*}	
BW	0.4^{**}	0.2^{*}	0.4^{**}		0.3^{**}	0.9^{**}	0.9^{**}	0.2^{*}	**1**	0.5^{**}	0.7^{**}	0.3^{*}	0.3^{*}	0.6^{**}				0.3^{*}		0.3^{*}
SN		0.3^{*}	0.3^{*}			0.4^{**}	0.5^{**}		0.4^{**}	**1**	0.8^{**}				-0.4^{**}	-0.3^{**}			0.3^{*}	
SW						-0.3^{*}		-0.3^{*}		-0.5^{**}	**1**			0.4^{**}	-0.4^{**}	-0.3^{*}				
Y	0.4^{**}	0.4^{**}	0.4^{**}			0.4^{**}	0.4^{**}				0.2^{*}	**1**	0.6^{**}	0.8^{**}	-0.3^{**}	-0.3^{**}	-0.3^{**}		0.3^{**}	0.4^{**}
CN	0.2^{*}	0.2^{*}	0.2^{*}			0.3^{*}	0.3^{*}				0.2^{*}	0.8^{**}	**1**		-0.3^{**}	-0.3^{**}				0.4^{**}
CW	0.5^{**}	0.4^{**}	0.4^{**}	0.3^{**}	0.2^{*}	0.3^{**}	0.3^{**}		0.3^{**}			0.6^{**}		**1**	-0.2^{*}	-0.3^{*}	-0.3^{**}	-0.2^{*}	0.3^{**}	0.2^{**}
ES	-0.3^{**}	-0.2^{*}	-0.3^{*}						-0.3^{*}						**1**	0.4^{**}			-0.3^{**}	
F	0.4^{**}	-0.3^{*}	-0.3^{**}	-0.5^{**}	-0.4^{**}				-0.2^{*}	-0.3^{*}	0.3^{**}				0.5^{**}	**1**	0.4^{**}			
SV	-0.6^{**}	-0.5^{**}	-0.6^{**}	-0.3^{**}	-0.3^{**}	-0.3^{**}	-0.3^{**}		-0.4^{**}	0.2^{*}					0.3^{**}	0.3^{**}	**1**	0.4^{**}	-0.3^{*}	-0.8^{**}
EV	-0.5^{**}	-0.4^{**}	-0.5^{**}						-0.2^{*}			0.4^{*}	0.4^{*}				0.7^{**}	**1**		0.8^{**}
RD						0.2^{*}	0.3^{**}		0.3^{**}	0.3^{**}		0.3^{**}	0.3^{**}	0.4^{*}			0.2^{*}	0.4^{**}	**1**	
VL	-0.5^{**}	-0.5^{**}	-0.5^{**}		-0.3^{*}	-0.3^{*}	-0.3^{**}		-0.4^{**}	0.3^{**}							0.9^{**}	0.7^{**}		**1**

Colour legend: 0.8-0.9 dark green, 0.6-0.7 medium green, 0.4-0.5 light green, 0.2-0.3 ocher.

** reflects statistical differences at 0.01 level, * at 0.05. PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, BL berry length, BD berry diameter, BS berry shape, BW berry weight, SN seed number, SW seed weight, Y yield, CN cluster number, CW cluster weight, FI fertility index, ES end Sprouting date, F flowering date, SV start veraison date, EV end veraison date, RD ripening, VL veraison length.

Supplementary material 3.1.7. Correlation matrix in *HH* genotypes for all the traits in both progenies in 2016.

HH		G x T progeny																			
		PL	**FD**	**OS**	**PS**	**OS**	**BL**	**BD**	**BS**	**BW**	**SN**	**SW**	**Y**	**CN**	**CW**	**DS**	**F**	**SV**	**EV**	**RD**	**VL**
T x G progeny	**PL**	**1**	0.9^{**}	0.9^{**}			0.4^{*}	0.4^{*}				0.5^{*}						-0.6^{*}			
	FD	0.9^{**}	**1**	0.9^{**}	-0.6^{**}			0.6^{**}	0.6^{**}												
	OS	0.9^{**}	0.9^{**}	**1**		0.5^{*}	0.4^{*}	0.5^{*}	0.6^{**}			0.5^{*}									
	PS		-0.6^{**}		**1**	0.8^{**}	0.5^{**}	0.7^{**}	0.7^{**}									-0.7^{*}			
	OS	0.5^{*}	-0.5^{*}	0.6^{**}	0.7^{**}	**1**	0.7^{**}									0.6^{**}					
	BL					0.6^{*}	**1**	0.8^{**}	0.6^{**}	0.8^{**}											
	BD			0.6^{*}		0.6^{*}	0.9^{**}	**1**		0.9^{**}	0.6^{*}	0.6^{*}				-0.7^{**}			-0.9^{*}		
	BS				0.5^{**}		0.5^{**}	0.9^{**}	**1**												
	BW	0.5^{*}		0.6^{*}			0.9^{**}	0.6^{**}	0.5^{*}	**1**	0.6^{*}	0.7^{**}	0.4^{*}			-0.6^{**}	-0.5^{*}		-0.9^{*}		0.9^{**}
	SN										**1**	0.8^{**}					-0.6^{*}	-0.9^{*}		0.6^{*}	
	SW	0.4^{*}						-0.4^{*}			-0.5^{**}	**1**			0.8^{**}	-0.5^{*}	-0.6^{**}				
	Y	0.4^{*}			0.4^{*}	0.4^{*}	0.4^{*}						**1**	0.6^{*}	0.7^{**}	-0.4^{*}		-0.5^{**}			0.7^{**}
	CN							0.6^{**}	0.7^{**}				0.7^{**}	**1**							0.7^{**}
	CW	0.4^{*}			0.6^{**}	0.5^{**}	0.6^{**}						0.6^{**}		**1**			-0.5^{**}			
	ES	-0.5^{*}	-0.4^{*}		0.4^{*}											**1**				-0.5^{*}	
	F	0.6^{**}			-0.5^{**}			-0.4^{*}	-0.5^{*}		-0.4^{*}	0.4^{*}				0.5^{**}	**1**				
	SV	-0.6^{**}	-0.5^{*}	-0.6^{**}		-0.5^{*}	-0.4^{*}											**1**	0.9^{**}		-0.9^{**}
	EV	-0.5^{**}	-0.5^{**}	-0.6^{**}		-0.4^{*}			0.5^{**}									0.7^{**}	**1**	-0.9^{**}	0.7^{*}
	RD		-0.5^{**}	-0.4^{*}	0.4^{*}															**1**	
	VL				0.4^{*}		0.5^{*}	0.4^{*}	0.4^{*}					0.7^{**}			-0.5^{**}	-0.7^{**}			**1**

Colour legend: 0.8-0.9 dark green, 0.6-0.7 medium green, 0.4-0.5 light green.

** reflects statistical differences at 0.01 level, * at 0.05. PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, BL berry length, BD berry diameter, BS berry shape, BW berry weight, SN seed number, SW seed weight, Y yield, CN cluster number, CW cluster weight, FI fertility index, ES end Sprouting date, F flowering date, SV start veraison date, EV end veraison date, RD ripening, VL veraison length.

Supplementary material 3.1.8. Correlation matrix of HH genotypes for all the traits in both progenies in 2016 year.

Hf		G x T progeny																			
		PL	**FD**	**OS**	**PS**	**OS**	**BL**	**BD**	**BS**	**BW**	**SN**	**SW**	**Y**	**CN**	**CW**	**DS**	**F**	**SV**	**EV**	**RD**	**VL**
T x G progeny	**PL**	**1**	0.9**	0.9**			0.6**	0.6**		0.6**					0.3*						
	FD	0.9**	**1**	0.9**	-0.6**	-0.4*	0.6**	0.6**		0.6**	0.3*				0.2*	-0.3*					
	OS	0.9**	0.9**	**1**			0.6**	0.6**		0.6**	0.3*	0.3*			0.3*						
	PS	-0.3*		-0.5**	**1**	0.8**					-0.3*					0.3*	0.3*			-0.4*	
	OS	0.3*		0.4**	0.7**	**1**														-0.4*	
	BL	0.5**		0.4**			**1**	0.9**	0.3*	0.9**	0.5**	0.6**	0.3*	0.6**	0.5**						0.3*
	BD	0.5**	0.3*	0.5**			0.9**	**1**		0.9**	0.5**	0.7**	0.4**	0.5**	0.5**						0.3*
	BS		-0.3*		0.4**		0.5**		**1**												
	BW	0.4**		0.4**			0.9**	0.9**		**1**	0.5**	0.7**	0.2*	0.6**	0.5**						
	SN	0.4**	0.5**	0.5**			0.6**	0.6**		0.5**	**1**	0.8**	0.3*			-0.4**	-0.4**	-0.3*			
	SW						-0.4*	-0.4**			-0.5**	**1**	0.3*		0.4**	-0.3*	-0.3*				
	Y	0.7**	0.7**	0.7**	-0.3*		0.5**	0.5**	0.3*	0.4*			**1**	0.6**	0.8**	-0.4*	-0.4**	-0.3*		0.3*	0.4*
	CN	0.5**	0.5**	0.5**			0.4*						0.9**	**1**		-0.4**	-0.4*			0.3*	0.3*
	CW	0.6**	0.5**	0.5**			0.4**	0.4*		0.4*			0.7**	0.4*	**1**		-0.4*				
	ES	-0.4**		-0.4**												**1**	0.4**	0.4**		-0.3*	-0.3*
	F	-0.6**	-0.4**	-0.5**	-0.4**	-0.4**										0.5**	**1**	0.6**	0.4**		
	SV	-0.5**	-0.5**	-0.5**						-0.4**				0.5*			0.3*	**1**	0.7**	-0.3*	-0.8**
	EV	-0.4*	-0.4*	-0.4*						-0.3*								0.7**	**1**	-0.3*	0.6**
	RD										0.3*	0.5**	0.4*	0.3*	0.3*			0.4**	0.4**	**1**	
	VL	0.5**	0.4**	0.4**			0.3*	0.4**		0.4**								-0.6**	0.3*		**1**

Colour legend: 0.8-0.9 dark green, 0.6-0.7 medium green, 0.4-0.5 light green, 0.2-0.3 ocher.

** reflects statistical differences at 0.01 level, * at 0.05. PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, BL berry length, BD berry diameter, BS berry shape, BW berry weight, SN seed number, SW seed weight, Y yield, CN cluster number, CW cluster weight, FI fertility index, ES end Sprouting date, F flowering date, SV start veraison date, EV end veraison date, RD ripening, VL veraison length.

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3.2. Genetic linkage map of Grenache x Tempranillo population

Abstract

A genetic linkage map was constructed using a population (130 genotypes) derived from crossing two of the most widely cultivated Spanish wine grapes, Grenache and Tempranillo (*Vitis vinifera* L.). The progeny was genotyped using GBS methodology with 5832 SNP (Single Nucleotide Polymorphism markers), of which 4452 resulted polymorphic for at least one parent, 1011 were filtered to saturate maternal (Grenache) map and 826 were used for the paternal (Tempranillo) map. Five fully informative markers (4 allele SSRs) were also mapped, and the maternal, paternal and integrated maps were generated using JoinMap 4.1. software, following a pseudo-testcross strategy. Grenache map consisted of 1011 SNPs markers integrated into 19 linkage groups covering 1364.5 cM with an average distance between markers of 1.4 cM. Tempranillo map was formed by 826 SNPs markers aligned into 19 linkage groups spanning 1237.7 cM with an average distance between markers of 1.5 cM. Finally, a Consensus map with 1296 markers was obtained covering 1540.8 cM distributed in 19 linkage groups, with an average interval length 1.2 cM between markers. In summary, a high-resolution SNP map for a biparental family derived from the cross of Grenache x Tempranillo was constructed, proving GBS technique is a useful method for high-throughput genotyping.

Introduction

Grapevine is one of most widely cultivated and relevant fruit crops in the world. There are around 5000 known cultivated varieties within the species *V. vinifera*, presenting large differences in morphology and fruit traits due to their extreme heterozygous nature, which is an obstacle for cultivators and breeders (This et al. 2011). Alternative methods to conventional cross-breeding techniques have emerged seeking to identify genes for desirable traits. In the climate change context, there is a growing demand for new wine grape cultivars able to adapt to the emerging scheme (Duchene 2016). The long and cost-consuming process of obtaining a new variety based in a conventional breeding program is nowadays accelerated by the use of marker-assisted techniques, including marker-assisted selection (MAS) (Töpfer et al. 2011), genomic selection (GS) (Fodor et al. 2014) or Next generation sequencing (NGS) technologies.

Considerable progress has been made in the identification of molecular markers and the construction of molecular linkage maps in grapevine. A great step forward has been made between the first molecular map of a 60 F1 progeny derived from “Cayuga White” × “Aurore” (Lodhi et al. 1995) and the last high density multiparent map developed in 2019 using 10 subpopulations (Tello et al. 2019). The publication of the grapevine reference genome sequence (and its update by Canaguier et al. 2017) has had a key role in this progression, assisting the identification of single nucleotide polymorphisms (SNPs), which have become the most widely used on high-quality genetic map construction (Zhang et al. 2015). This SNP markers can be identified from short reads generated by NGS, either by aligning to a reference genome or by de novo assembly (Nielsen et al. 2011).

Plant breeding and genetics research are transitioning from a data-poor to a data-rich environment. Next-generation sequencing of crop plant genomes, is revolutionizing the field as newly abundant data enable and facilitate the discovery and use of millions of single nucleotide polymorphisms (SNPs) in diverse genomes (Huang et al. 2012, Xu et al. 2012). Yet, at the same time, traditional bi-parental mapping populations continue to play an important role in gene

discovery, and both bi-parental and multi-parental breeding populations remain the foundation of many plant breeding programs (Almeida et al. 2013, Zhu et al. 2018).

Among the NGS technologies the recent development and availability of different genotype by sequencing (GBS) protocols provided a cost-effective approach to perform high-resolution genomic analysis of entire populations in different species. The major component of these methods is the digestion of the initial DNA with known restriction enzymes, to generate sequencing fragments at predictable and reproducible sites (Perea et al. 2016). GBS holds the potential to close the genotyping gap between references of broad interest and mapping/breeding populations of local or specific interest. This is the latest application of next-generation sequencing protocols for the purposes of discovering and genotyping SNPs in a variety of crop species and populations. Unlike other high-density genotyping technologies which have mainly been applied to general interest "reference" genomes, the low cost of GBS makes it an attractive tool of saturating mapping and breeding populations with a high density of SNP markers (Spindel et al. 2013). Results have shown that this methodology is efficient for genotyping a variety of species, including those with complex genomes as barley (Poland et al. 2012), oat, (Huang et al. 2014), onion (Jo et al. 2017) and grapevine (Yang et al. 2016, Smith et al. 2018, Guo et al. 2019). The first application of GBS in grapevine was done by Barba et al. (2014), constructing high-resolution parental linkage maps in an interspecific *V. rupestris* × *V. vinifera* segregating population. More recently, high density genetics maps have been elaborated merging two (Teh et al. 2017) or more (Tello et al. 2019) populations. The application of GBS and other NGS technologies has enabled the efficient discovery and genotyping of SNPs in grapevine, resulting in the detection of a massive number of markers to detect phenotype–genotype associations in interspecific segregating populations (Barba et al. 2014, Chen et al. 2015, Hyma et al. 2015, Smith et al. 2018, Zhu et al. 2018) and in grapevine diversity panels (Guo et al. 2019).

This study presents the construction of a genetic map by single nucleotide polymorphisms identified through genotyping-by-sequencing (GBS) technology in an F1 mapping family of 130 individuals derived from an intra-specific cross between the two Spanish cultivars Grenache and Tempranillo.

Material and methods

Plant material

The original mapping population consisted of 134 plants obtained from controlled crosses between the wine grape cultivars Grenache clone – 63 (female parent) and Tempranillo clone – 43 (male parent). The cross between them was developed in Viveros Provedo (Varea, La Rioja, Spain). After SSR analysis, four self-pollinations were discarded and the final population comprised 130 genotypes.

DNA extraction

For DNA extraction about 400 mg were collected from all genotypes in a 2 ml Eppendorf tube, and frozen immediately in liquid nitrogen in the field. Leaf samples were stored at - 80 ºC until extraction. Leaf samples were ground to a fine powder with liquid nitrogen and genomic DNA was extracted using DNeasy plant Mini Kit (QIAGEN GmbH, Germany) following the manufacturer´s protocol. The concentration of the DNA extracted was quantified with a

NanoDrop 1000 (Thermo Scientific Inc. USA). The amount and integrity of resulting genomic DNA was checked on 0.8 % agarose gel prepared in 1 x TBE buffer.

SSR Primer pairs selection

The 134 F_1 population was genotyped for 5 SSRs polymorphic for the parental varieties: VMC6 (Vitis Microsatellite Consortium, AgroGene S.A. Moissy Cramayel, France) (Salmaso et al. 2008, Constantini et al. 2008), VChr3a, VChr8b, (Cipriani et al. 2008), VVIB23, and VVIV70 (Merdinoglu et al. 2005) in order to discard individuals resulting from self-pollinations and foreign pollen sources, resulting in a final population of 130 plants. Besides, Grenache and Tempranillo genetic identity was verified by SNP genotyping (Ibañez et al. personal comunication).

Polymerase chain reaction (PCR) amplification was performed in GeneAmp ® PCR System 9700 thermo cycler and Veriti TM 96 Thermal Cycler (Applied Biosystems, USA) in 96 well plates with 20 ng DNA, 0.2 µM of each primer 1x PCR Buffer, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, and 1 - unit Immolase DNA Polymerase (LABOLAN, Navarra, Spain).

The PCR program of Vchr, VMC and VVI markers was previously described in Chapter 3.1. Amplified products were analyzed in 3130 Genetic Analyzer (Applied Biosystems, USA) in the Molecular Diagnostics Laboratory of the Centre for Biomedical Research (CIBIR), Logroño. The identification and sizing of alleles for each genotype was performed manually with GeneMapper®4.0 Software.

Genotyping-by-sequencing

GBS libraries were constructed in 96-plex, and genomic DNA was digested with the restriction enzyme ApeK1, chosen after a previous coverage analysis. Each library was sequenced on a single lane of Illumina flow cell HS2000, (2 x 100 cycles) at Centro Nacional de Análisis Genómico (CNAG, Barcelona, Spain).

For SNP calling, the raw sequence data for the 130 F1 progeny plus the two progenitors was processed through GATK 12X.v2 *V. vinifera* 'PN40024' accession reference genome assembly (Canaguier at al. 2017). Alignment files were processed using methodology by Picard et al. (2016) to check file validity, to re-order and to add read groups. The Genome Analysis Tool Kit (GATK) (McKenna et al. 2010) was used for local realignment. Variant calling was done using Unified Genotyper from GATK, being the variants filtered on mapping quality and on phred-scaled quality score using GATK, only biallelic SNPs were kept. Additional filter on depth was processed. Variants were annotated using SnpEff with the IGGP_12x.27 database (Cingolani et al. 2013). The initial output of 170312 SNPs was filtered to 150126 after removing SNPs with missingness > 50%. The following step was removing 66923 SNPs not informative in parents (markers homozygous AA x BB, AA x AA, or heterozygous AB x AB), and 15501 SNPs with a missing genotype in at least one parent. The total number after removing incongruent parental SNPs was 67104.

With this data a pre-processing and data cleaning was applied for alignment. In this filtering, SNPs that did not adjust to Mendelian segregation, and with missingness > 5% were removed. Markers with a segregation not compatible in the progeny between observed and expected identified by a chi-squared ($\chi2$) goodness-of-fit test at $\alpha \leq 0.05$ were also discarded. In order to reduce SNP density, SNPs every 100kb with a genotypic correlation > 0.2 were removed. The output contained a file version including 5832 SNPs.

Genetic mapping and Linkage analysis

Construction of genetic maps

Genetic maps for Grenache, Tempranillo and a Consensus linkage map for the cross were independently generated using 130 F_1 individual population and two way pseudo-test cross strategy (Grattapaglia & Sederoff 1994) and JoinMap 4.1 Software (Van Ooijen 2012). An independence logarithm of odds (LOD) score of 4.0 was used to define the linkage groups (LGs), and the map distances were calculated according to the Kosambi mapping function (Kosambi 1944). Markers within the resulting groups were ordered relative to each other by automatic multipoint analyses using the values of JoinMap 4.1 (Mapping threshold LOD > 4, recombination frequency threshold < 0.4). Markers showing distorted segregation by a Chi square test were included for the construction of the maps, unless they were of low quality or they significantly affected the order of their neighbours.

LGs were numbered from 1 to 19 according to the reference map of Doligez et al. (2006) and the international agreement within the IGGP (International Grape Genome Program; http://www.vitaceae.org). In order to construct the maps, <hk x hk> and <lm x ll> type loci were ignored for paternal population and <hk x hk> and <nn x np> type for maternal population.

Estimation of genome length and map coverage

The genome size was estimated according to the method of Hulbert et al. (1988) modified by Chakravarti et al. (1991). Confidence intervals for genome-length estimates were computed according to Gerber and Rodolphe (1994) for a bilateral type-I error rate $\alpha = 5\%$. The expected genome coverage was estimated according to Lange and Boehnke (1982), as a function of the number of mapped markers, genome length and number of chromosomes.

$Ge = N (N-1) X / K$ where N is the number of markers, X is the maximum observed map distance between marker pairs above a threshold LOD Z (Chakravarti et al. 1991), Z = 4 in this study, and K is the number of locus pairs having LOD values above Z. The value of X and K were obtained from JoinMap using Kosambi function.

Results

Construction of genetic maps

For the maternal map (Grenache), 1011 SNP markers were positioned into 19 linkage groups covering 1364.5 cM, with an average number of 53.2 per group (Table 3.2.1). The average length of linkage groups was 71.8 cM, ranging from 35.1 cM (LG5) to 112.9 cM (LG7). The average distance between markers was 1.4 cM, and only 4 gaps larger than 10 cM were present. The largest gap is 13.8 cM between 16_6743459 and 16_14840035 in LG16 (Figure 4.2.1).

The paternal map (Tempranillo) consisted of 826 SNP markers also assembled in 19 linkage groups and covered 1237.7 cM with an average of 43 markers per group. Linkage groups sizes ranged from 39.9 cM (LG10) to 87.2 cM (LG14) with an average length of 65.1 cM. There were 5 gaps larger than 10 cM, being the average distance between markers of 1.5 cM. The largest gap was 21.7 cM between 12_1982474 and 12_9713704 in LG12 (Figure 4.2.1).

The integrated map consisted of 1296 SNP markers integrated on 19 linkage groups with an average of 68.2 markers per group. The map covered 1540.8 cM with an average interval

length of 1.2 cM between markers. Linkage groups size ranged from 58.7 cM (LG15) to 116.3cM (LG4) with an average size of 81.1 cM. There were 4 marker free regions longer than 10 cM, and the largest gap is 15.1 cM between 18_2435617 and 18_7309153 in LG18 (Figure 4.2.1).

Table 3.2.1. Main features of Grenache, Consensus and Tempranillo maps.

	Tempranillo	Consensus	Grenache
Nº of mapped markers	826	1296	1011
Nº linkage groups	19	19	19
Nº markers / LG range	25-60	45-85	29-75
Mean number markers LG	43.5	68.2	53.2
Total map length	1237.7	1540.8	1364.5
Mean LG length	65.1	81.1	71.8
LG length range	39.9-87.2	56.9-116.3	35.1-112.9
Average distance between loci	1.5	1.2	1.4
Nº gaps between 10 - 20cM	5	6	4
Nº gaps > 20 cM	1	0	0

The total number of positioned markers per linkage group were between 29 (LG5) and 75 (LG2) in Grenache, between 25 (LG6) and 60 (LG7) in Tempranillo, and between 45 (LG15) and 85 (LG7) for the Consensus map (Table 3.2.2). The average distance between markers of linkage group ranged 0.8 and 2.3 for Grenache, 0.9 and 2.4 for Tempranillo and between 0.8 and 1.8 in the Consensus map. There were loci that could not be assigned to any linkage group (ungrouped markers), that may be located in not covered regions by the map and un-positioned markers due to insufficient linkage or location conflicts.

In this work, 313 markers firstly assigned to “Unknown” chromosome could be successfully positioned in different LGs, mainly in LG2, LG7, LG10 and LG13. They are named with -1_ suffix in the graphs reported in this work. Their presence supports Canaguier et al. (2017) who estimated that around 9 % of the grapevine reference sequence remains unanchored to linkage groups, and unlinked scaffolds are located in the so-called Unknown chromosome.

Table 3.2.2. Characteristics of Grenache, Consensus and Tempranillo maps.

Map of Tempranillo						Consensus map					Map of Grenache				
Linkage group	**Length (cM)**	**SNP**	**Mean**	**Nº gaps 10-20cM**	**Nº gaps > 20cM**	**Linkage group**	**Length (cM)**	**SNP**	**Mean**	**Nº gaps 10-20cM**	**Linkage group**	**Length (cM)**	**SNP**	**Mean**	**Nº gaps 10-20cM**
LG1	79	55	1.4			**LG1**	76.6	67	1.1		**LG1**	88.5	62	1.4	
LG2	57	56	1.4			**LG2**	56.7	57	1.0		**LG2**	61.7	75	0.8	
LG3	57.5	40	1.4			**LG3**	66.7	58	1.2		**LG3**	60.3	46	1.3	
LG4	48.6	41	1.2			**LG4**	116.3	65	1.8		**LG4**	90	57	1.6	
LG5	81.7	44	1.9			**LG5**	106.9	58	1.8	1	**LG5**	35.1	29	1.2	
LG6	58.9	25	2.4			**LG6**	76	61	1.2		**LG6**	82.3	36	2.3	
LG7	79.6	60	1.3			**LG7**	93.8	85	1.1		**LG7**	112.9	61	1.9	
LG8	59.7	35	1.7			**LG8**	71.4	73	1.0		**LG8**	76.6	69	1.1	1
LG9	65.9	53	1.2			**LG9**	63.6	53	1.2		**LG9**	62.6	49	1.3	
LG10	39.9	43	0.9			**LG10**	73.6	77	1.0		**LG10**	44.7	41	1.1	
LG11	78.4	46	1.7	1		**LG11**	90.9	69	1.3	1	**LG11**	52.8	44	1.2	
LG12	61.4	29	2.1		1	**LG12**	83.3	61	1.4	1	**LG12**	63.6	67	0.9	
LG13	63.9	39	1.6			**LG13**	83.6	73	1.1		**LG13**	77.6	66	1.2	
LG14	87.2	48	1.8	1		**LG14**	95.5	74	1.3		**LG14**	95.6	67	1.4	1
LG15	62.8	37	1.7			**LG15**	58.7	45	1.3	1	**LG15**	70.7	59	1.2	
LG16	72.3	49	1.5	1		**LG16**	76.8	75	1.0		**LG16**	70.8	35	2.0	1
LG17	62.1	37	1.7	1		**LG17**	72.5	81	0.9	1	**LG17**	66.7	44	1.5	
LG18	51.3	39	1.3	1		**LG18**	107.8	81	1.3	1	**LG18**	81.2	62	1.3	1
LG19	70.5	50	1.4			**LG19**	69.9	83	0.8		**LG19**	70.8	42	1.7	
Total	**1237.7**	**826**	**1.5**	**5**	**1**	**Total**	**1540.8**	**1296**	**1.2**	**6**	**Total**	**1364.9**	**1011**	**1.4**	**4**

Among them LG7 and LG10 registered the highest number of these type of markers, 36 and 40 markers, respectively. The relative proportion of “Unknown” markers mapped is higher than in Tello et al. (2019) but in agreement with Houel et al. (2015). As in Tello et al. (2019) unanchored markers in the PN40024 genome were successfully mapped in regions LGs 2, 7 and 10 in our genetic maps, reinforcing the need of improving the current assembly of the grapevine reference genome.

Comparisons of the two parental genetic maps and the Consensus map revealed in general similarity in map length, marker order, and LG sizes, except inversion segments at a few regions, in LG 7, LG10 and LG13, as reported by Teh et al. (2016), and Tello et al, (2019). Presumably, these discrepancies can be attributed to small family sizes and GBS technical challenges. Segregation distorsion was detected on LG6 and LG13, in this work. Other regions with distorted segregation had been reported in LG7 (Constantini et al. 2008, Song 2014) and LG18 (Cabezas et al. 2006, Song 2014).

Comparison with previous Grenache and Tempranillo maps

Compared to a 178 SSR map of Grenache previously reported (Adam-Blondon et al. 2004), this GBS map represented for the same map length (1360 cM) a five-fold improvement in resolution from 7.6 cM to 1.4 cM between markers. This comparison was also found by Yang et al. (2017), obtaining a nine-fold improvement compared with an SSR map for the same population. Besides, in that work fifteen gaps larger than 20 cM were observed, whereas no gap larger than 20 cM has been detected here, only four between 10-20 cM (Table 3.2.3). Both maps show uncovered regions in LG5, presenting in the present study a shorter linkage group length (35 cM).

Song (2014) developed a Tempranillo map with 136 SSR and 491 SNP, resulting in 1220 cM of length. The space between markers was 2 cM, being 1.6 cM n the present work. In that study, gaps between 10 - 20 cM were also found in LG14, LG17 and LG18, and a large gap in LG12 larger than 20 cM, reinforcing the idea of a highly homozygous region in chromosome 12 of Tempranillo.

To our knowledge, this is the first publication of Grenache and Tempranillo maps with SNP markers using GBS method in a population of more than 70 individuals. Tello et al. (2019) used Grenache as parent in 4 out of the 10 subpopulations that merged to build an integrated map, involving Syrah, Pinot Noir, Cabernet Sauvignon and Terret Noir varieties. However, lower number of individuals (between 58 and 67) were used compared with this study and no comparison among parental maps could be done as Grenache individual map is not reported.

Genome length and coverage

The estimated genome length of Consensus map is greater than that of Grenache and Tempranillo maps. The estimated genome length for Tempranillo in this work (1409.7 cM, Table 4.2.3) is lower than Tempranillo map reported by Song (2014), (2763 cM), what could be the reason for the low value of observed map coverage (47 %) found in Song 2014. In the present work, observed coverage in Consensus, Grenache and Tempranillo maps in the present study is around 100 % except for Tempranillo map, which is around 90 %.

Table 3.2.3. Estimated genome length, expected and observed coverage of the maps.

	Tempranillo	Consensus	Grenache
Nº of mapped markers N	826	1296	1011
Max observed distance (X, cM)	21.7	16.5	13.8
Number of strong linkage K	10187	16072	10423
Estimated genome length (Ge)	1409.7	1576.8	1325.3
Confidence interval 95%	1416.8	1583.2	1331.9
Observed genome length	**1237.7**	**1540.8**	**1364.5**
Observed genome map coverage	**87.8**	**97.7**	**102.0**

Table 3.2.4 contains the main features of the Consensus genetic map generated in this work in relation to reference genome. Regarding the reference genome (physical map), all linkage groups (except LG7 and LG10) had an estimated genome coverage around or greater than 80%, being the average genome coverage close to 90%. This value is lower than 95 % reported by Yang et al. (2016) or 98 % coverage found by Tello et al. (2019). Noticeably, Yang et al. (2016) used a F_2 interspecific family and Tello et al. (2019) a Consensus map obtained from 10 subpopulations and 5 different parents. The merging process of the 10 Consensus maps improved coverage of low-density marker regions in specific populations, increasing total genome coverage.

Correlations between genetic and physical maps in LG7 and LG10 were weaker in comparison with the reference genome (Table 4.2.4). That was expected, because most of the unknown markers were mapped to regions in these LGs. These regions might indicate variety-specific genome structure variation, presumably related with chromosome rearrangements, transposable elements or tandem duplication. Although *V. vinifera* is the only species in the *Vitis* complex with a whole genome sequence already published, variety-specifications were reported in other works, regarding segregation distorted regions (Riaz et al. 2008, Song 2014).

Table 3.2.4. Coverage between G x T Consensus and *V. vinifera* 'PN40024' ref genome.

Linkage Group	Physical Length G x T (bp) [a]	Physical length PN40024 (bp) [b]	Coverage (%) [c]
LG1	22837583	24233538	94.2
LG2	14714495	18891843	77.9
LG3	18488522	20695524	89.3
LG4	21649750	24711646	87.6
LG5	24611875	25650743	95.9
LG6	18762904	22645733	82.9
LG7	20760032	27355740	75.9
LG8	21961699	22550362	97.4
LG9	21804133	23006712	94.8
LG10	17275032	23503040	73.5
LG11	19360789	20118820	96.2
LG12	21720497	24269032	89.5
LG13	22753920	29075116	78.3
LG14	30139491	30274277	99.6
LG15	19820380	20304914	97.6
LG16	21514914	23572818	91.3
LG17	16536498	18691847	88.5
LG18	29086411	34568450	84.1
LG19	23854081	24695667	96.6
Total	407653006	458815822	88.8

[a] Physical Length G x T (bp) is chromosome length of Grenache x Tempranillo Consensus map covered by each linkage map.
[b] Physical Length PN40024 (bp) is chromosome length of 12x.2 *V. vinifera* ‘PN40024’ reference genome covered by each linkage map.
[c] Coverage (%) is calculated as the physical length of the linkage group map divided by the total physical length of the chromosome in the ‘PN40024’ reference genome.

Discussion

Grenache and Tempranillo maps obtained with GBS in the present study improved those previously reported by Adam-Blondon et al. 2004 and Song 2014, respectively. Although map lengths are quite similar, 1360 cM and 1240 cM, for each map, a higher resolution was detected, by reducing the average distance between markers and the number of gaps. Thus, GBS allowed the generation of high-density maps for this progeny. In Tempranillo, as previously reported by Song et al. (2014) a highly homozygous region was detected in LG12 map, whilst in Grenache LG5 had a shorter length like in Adam-Blondon et al. (2014). Homozygosity in *V. vinifera* varieties has been observed in previous works (Cabezas et al. 2006; Costantini et al. 2008; Houel et al. 2015) resulting in a lack of polymorphic markers in some regions of progeny maps. This is usually associated to long chromosomal regions with low recombination events per cM (Tello et al. 2019). Adam-Blondon et al. (2014) failed to identify LG 16, whereas in the present work the linkage group was mapped, although a 14cM gap was present. Tello et al. (2019) using Grenache as parental in 4 out to 10 of the crossings also observed that LG16 presented among the lowest number of markers (20), length (53 cM) and largest gaps (14 cM).

The integrated Grenache × Tempranillo Consensus map covered 1540 cM along 19 LGs, the haploid chromosome number of *V. vinifera* L. (Haas & Alleweldt 2000). This work aligns with recently published high density maps (Barba et al. 2014, Teh et al. 2017, Smith et al. 2018, Tello et al. 2019) in contrast with previous experiments (Lodhi et al. 1995; Dalbo et al. 2000, Adam-Blondon et al. 2004, Cabezas et al. 2006, Carreño et al. 2015, Correa et al. 2016, Doligez et al. 2006a, 2006b; Vezzulli et al. 2008) that were based on limited SSR markers with greater inter-marker distance. The use of the GBS technique in an F_1 family from two heterozygous grapevines allows the generation of abundant SNPs, which are evenly spaced and cover a higher proportion of the genome (Saptoka et al. 2019). Map length in the most recent works by Tello et al. (2019) was 1378 cM map, with 4435 SNPS markers; and Zhu et al. (2018) with a Red Globe table grape map of 3172.33 cM. Variability in map length is expected due to the variable chromosome recombination events that occur during sexual reproduction in each subpopulation (Collard et al. 2005).

Using GBS two maps have been reported for other *V. vinifera* varieties like Chardonnay, with 1215 SNPs and 1967 cM map length (Barba et al. 2014), or Cabernet Sauvignon with 1770 markers and 1983 cM length (Saptoka et al. 2019). In general, map length is not significantly enlarged due to marker quality or ordering issues, and the need for reducing the number of markers generated by NGS strategies to a computable dataset that can be processed. Some of the reported strategies for optimal marker selection include the selective elimination of low-informative markers (Gabay et al. 2018, Ji et al. 2018) and co-segregating markers (Wenzl et al. 2016), or discarding adjacent markers in densely genotyped regions (Tello et al. 2019). The average distance between markers in this work (1.2 cM) is within the expected rank obtained by NGS technologies: Chardonnay (0.5 cM) (Barba et al. 2018), Cabernet Sauvignon (1.3 cM) (Saptoka et al. 2019) and Riesling (4.2 cM); (Smith et al. 2018), and the multi-population map

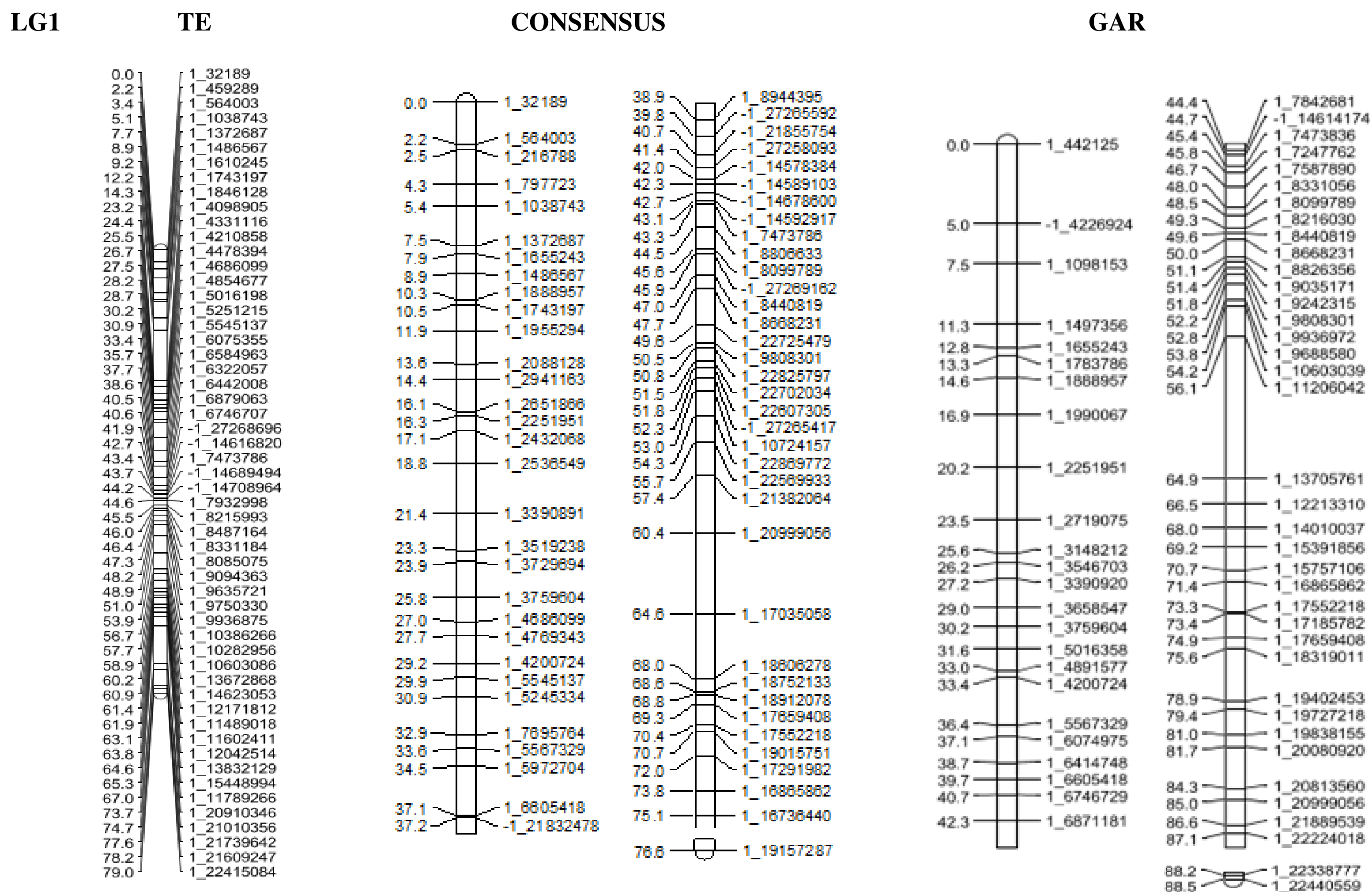
(0.4 cM) (Tello et al. 2019), only Zhu et al. (2018) reported an unusual average distance of 0.02 cM for the table grape cv. Red Globe.

Great coverage (90 %) was found in this study in comparison with PN40024 12x.v2 grapevine reference genome, with the exception of some uncovered regions in LG7 and LG10. The presence of poorly genotyped regions after GBS method is presumably related to both technical and biological reasons (Deschamps et al. 2012) among them the degree of homozygosity in-specific regions and the fact that almost 9 % of the grapevine reference sequence remains unanchored to chromosomes (Canaguier et al. 2017), resulting in the presence of (-1_) markers positioned mainly in LG2, LG7 and LG10. The presence of these markers in those LGs is in agreement with Tello et al. 2019, which found regions in some of the Consensus maps elaborated in LG2, LG7 and LG10, suggesting that these emplacements are their true location. This fact reinforces the necessity of improving the current assembly of the grapevine reference genome.

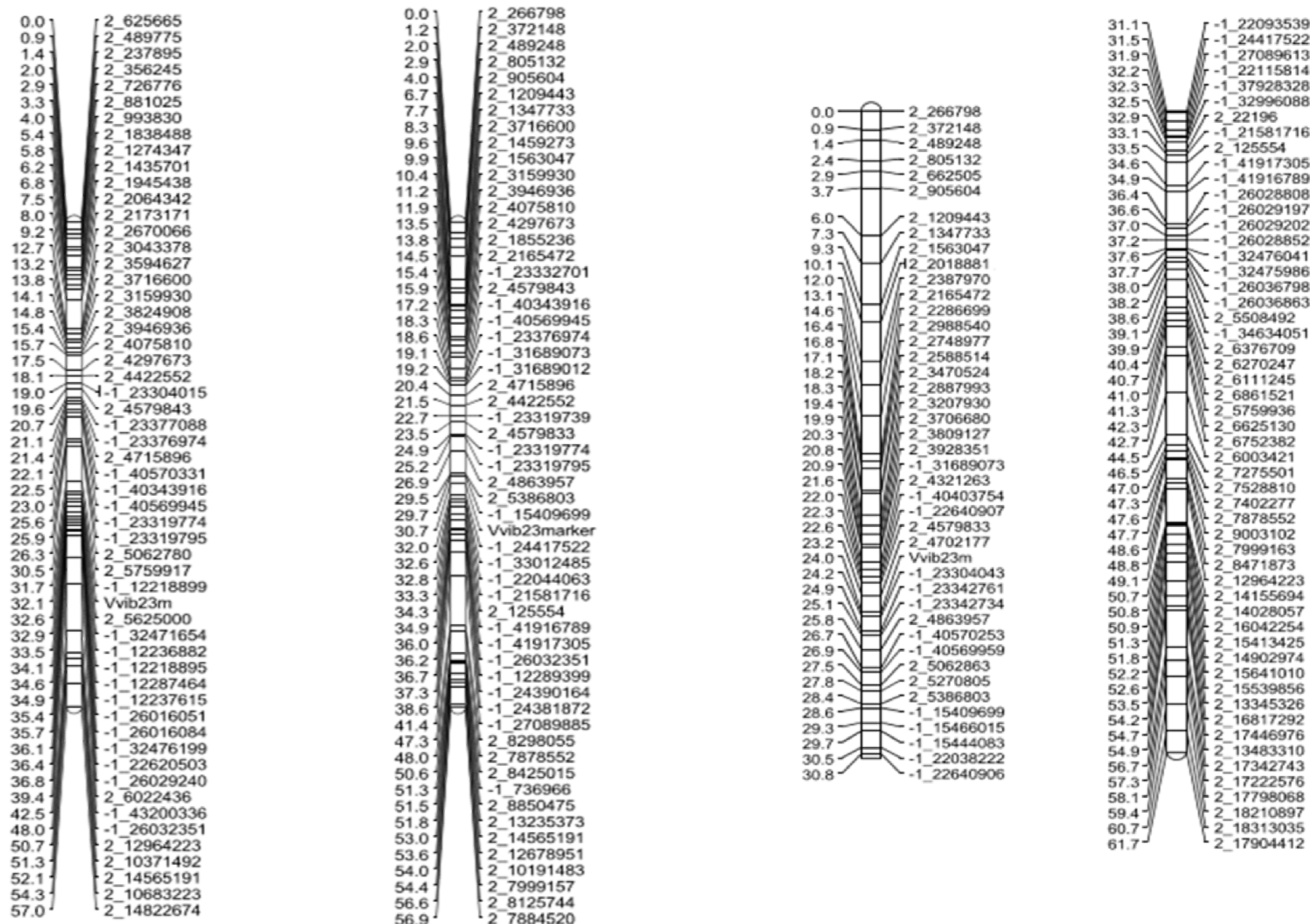
Conclusions

In the present work, a genetic linkage map was constructed using a 130-progeny derived from two Spanish wine grapes Grenache x Tempranillo (*Vitis vinifera* L.) with 1296 SNPs generated by GBS. Maternal, paternal and Consensus maps were assigned to 19 linkage groups, covering 1360 cM, 1240 cM and 1540 cM respectively, confirming the suitability of GBS mapping strategy for an intra-specific *Vitis vinifera* F_1 mapping population in grapevine. The resulted Grenache and Tempranillo genetic linkage maps provide qualitative and quantitative improvements over previous SSR maps in terms of marker density and genome coverage showing the Consensus map a great coverage with the “PN40024” reference genome. Finally, these genetic maps will serve as a valuable tool for QTL analysis for agronomical and oenological key traits.

Figure 3.2.1. Linkage map of F1 population from Grenache x Tempranillo

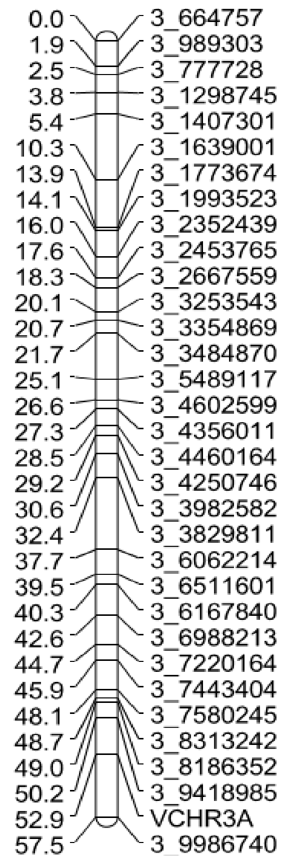


LG2 **TE** **CONSENSUS** **GAR**

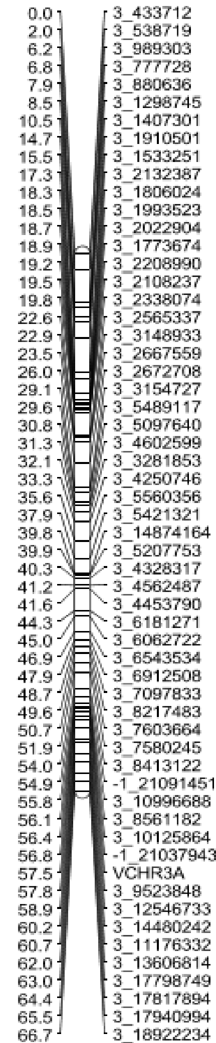


LG3

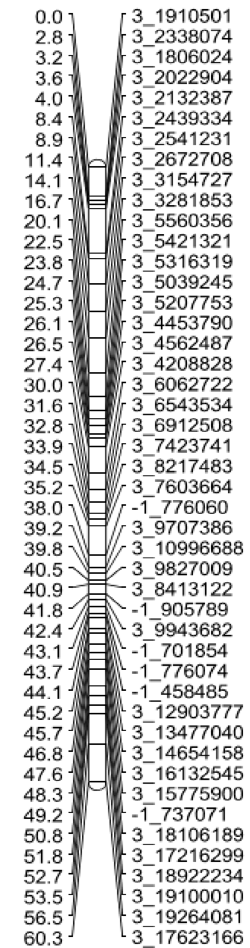
TE



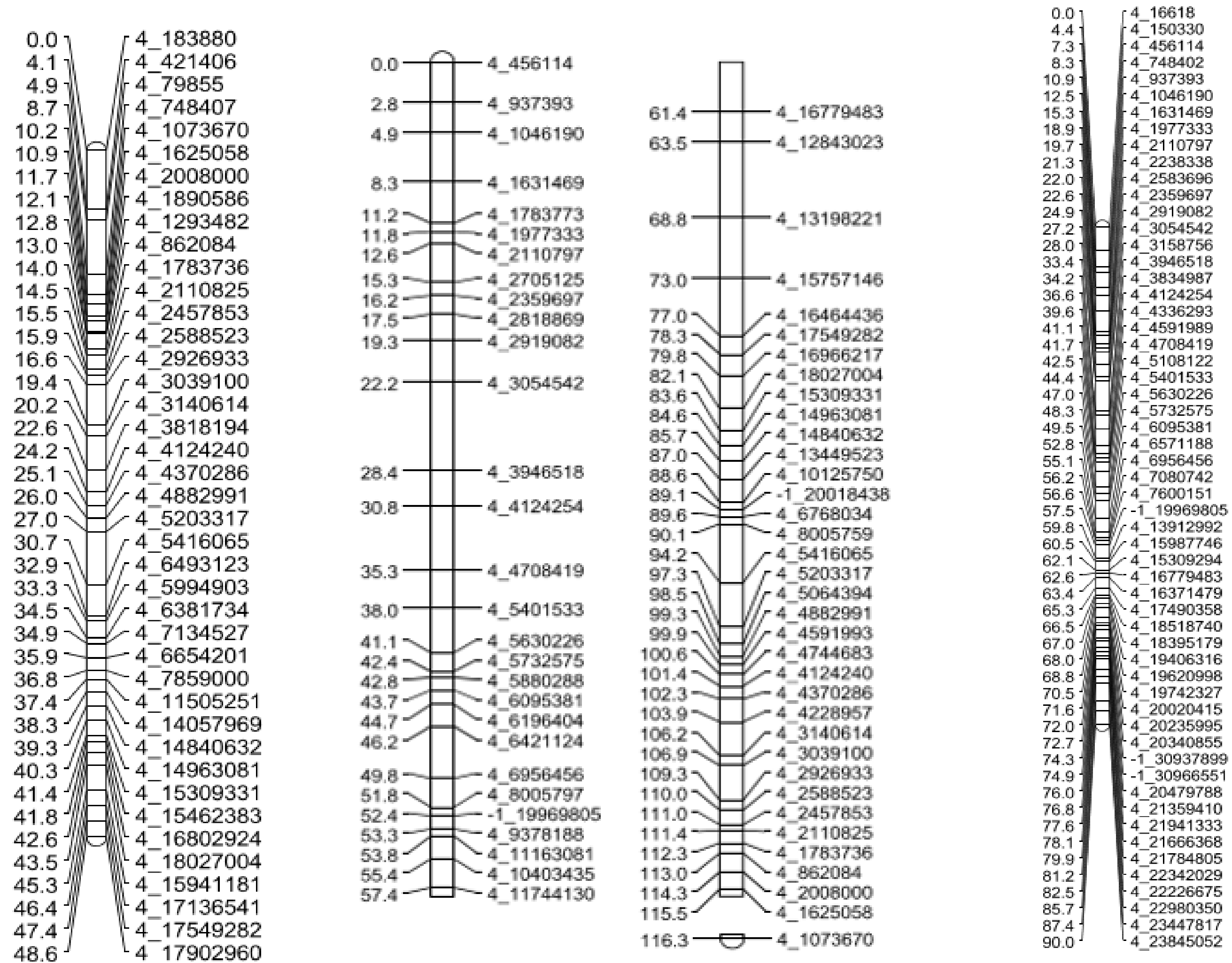
CONSENSUS

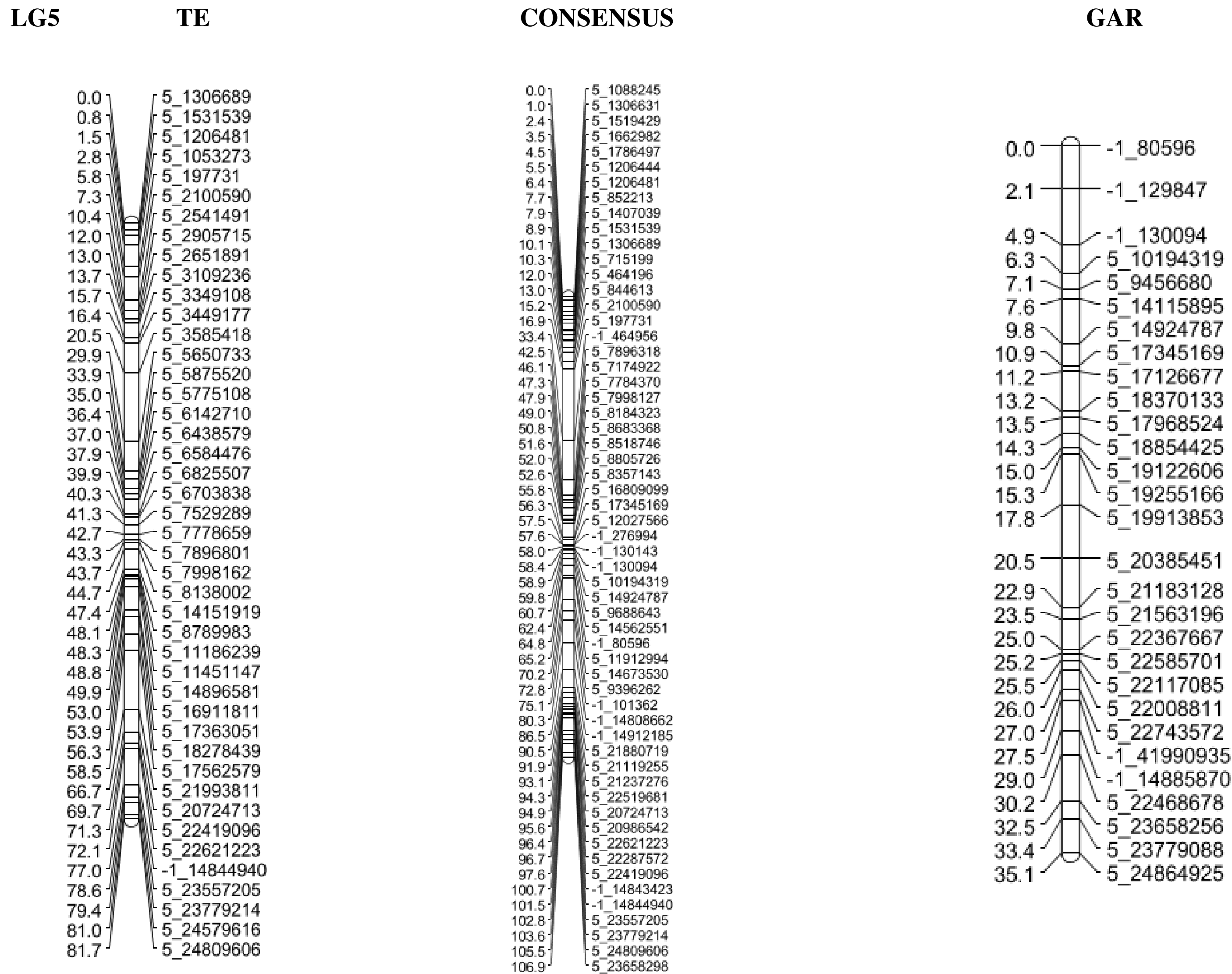


GAR



LG4 **TE** **CONSENSUS** **GAR**




LG5
TE
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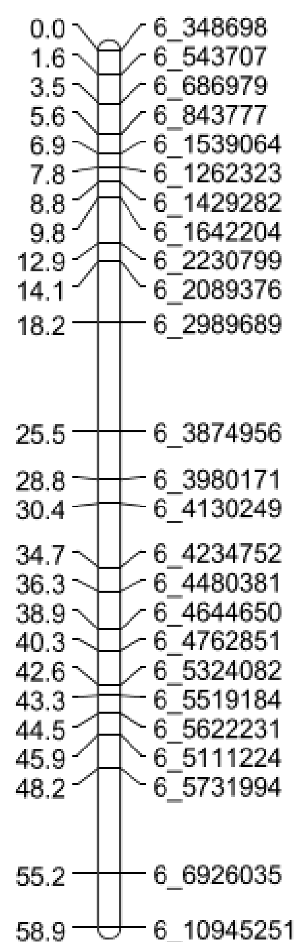
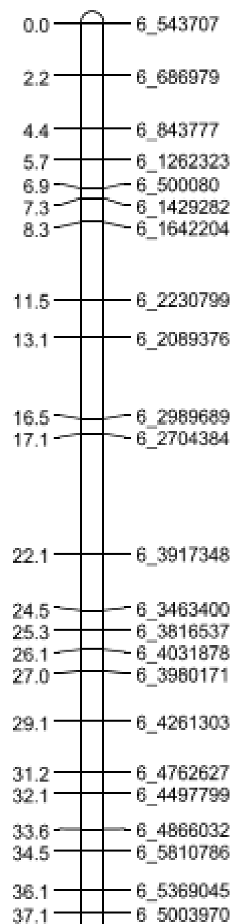
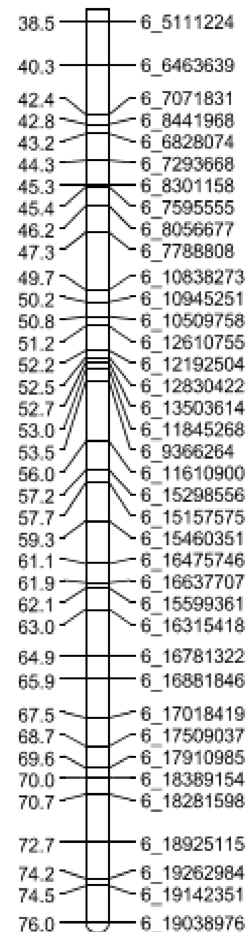
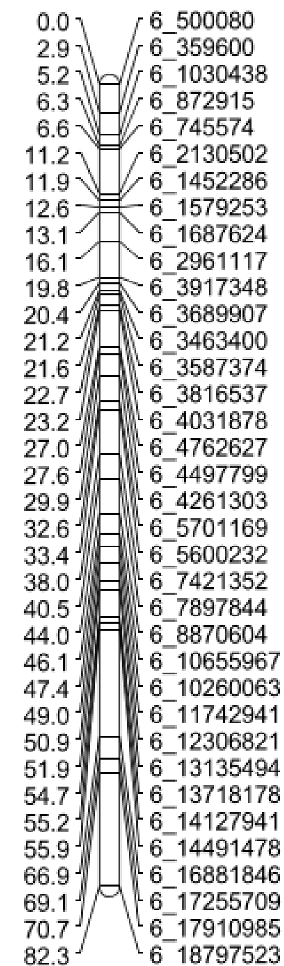
0.8 5_1531539
1.5 5_1206481
2.8 5_1053273
5.8 5_197731
7.3 5_2100590
10.4 5_2541491
12.0 5_2905715
13.0 5_2651891
13.7 5_3109236
15.7 5_3349108
16.4 5_3449177
20.5 5_3585418
29.9 5_5650733
33.9 5_5875520
35.0 5_5775108
36.4 5_6142710
37.0 5_6438579
37.9 5_6584476
39.9 5_6825507
40.3 5_6703838
41.3 5_7529289
42.7 5_7778659
43.3 5_7896801
43.7 5_7998162
44.7 5_8138002
47.4 5_14151919
48.1 5_8789983
48.3 5_11186239
48.8 5_11451147
49.9 5_14896581
53.0 5_16911811
53.9 5_17363051
56.3 5_18278439
58.5 5_17562579
66.7 5_21993811
69.7 5_20724713
71.3 5_22419096
72.1 5_22621223
77.0 -1_14844940
78.6 5_23557205
79.4 5_23779214
81.0 5_24579616
81.7 5_24809606
CONSENSUS
0.0 5_1088245
1.0 5_1306631
2.4 5_1519429
3.5 5_1662982
4.5 5_1786497
5.5 5_1206444
6.4 5_1206481
7.7 5_852213
7.9 5_1407039
8.9 5_1531539
10.1 5_1306689
10.3 5_715199
12.0 5_464196
13.0 5_844613
15.2 5_2100590
16.9 5_197731
33.4 -1_464956
42.5 5_7896318
46.1 5_7174922
47.3 5_7784370
47.9 5_7998127
49.0 5_8184323
50.8 5_8683368
51.6 5_8518746
52.0 5_8805726
52.6 5_8357143
55.8 5_16809099
56.3 5_17345169
57.5 5_12027566
57.6 -1_276994
58.0 -1_130143
58.4 -1_130094
58.9 5_10194319
59.8 5_14924787
60.7 5_9688643
62.4 5_14562551
64.8 -1_80596
65.2 5_11912994
70.2 5_14673530
72.8 5_9396262
75.1 -1_101362
80.3 -1_14808662
86.5 -1_14912185
90.5 5_21880719
91.9 5_21119255
93.1 5_21237276
94.3 5_22519681
94.9 5_20724713
95.6 5_20986542
96.4 5_22621223
96.7 5_22287572
97.6 5_22419096
100.7 -1_14843423
101.5 -1_14844940
102.8 5_23557205
103.6 5_23779214
105.5 5_24809606
106.9 5_23658298
GAR
0.0 -1_80596
2.1 -1_129847
4.9 -1_130094
6.3 5_10194319
7.1 5_9456680
7.6 5_14115895
9.8 5_14924787
10.9 5_17345169
11.2 5_17126677
13.2 5_18370133
13.5 5_17968524
14.3 5_18854425
15.0 5_19122606
15.3 5_19255166
17.8 5_19913853
20.5 5_20385451
22.9 5_21183128
23.5 5_21563196
25.0 5_22367667
25.2 5_22585701
25.5 5_22117085
26.0 5_22008811
27.0 5_22743572
27.5 -1_41990935
29.0 -1_14885870
30.2 5_22468678
32.5 5_23658256
33.4 5_23779088
35.1 5_24864925

LG6 **TE** **CONSENSUS** **GAR**

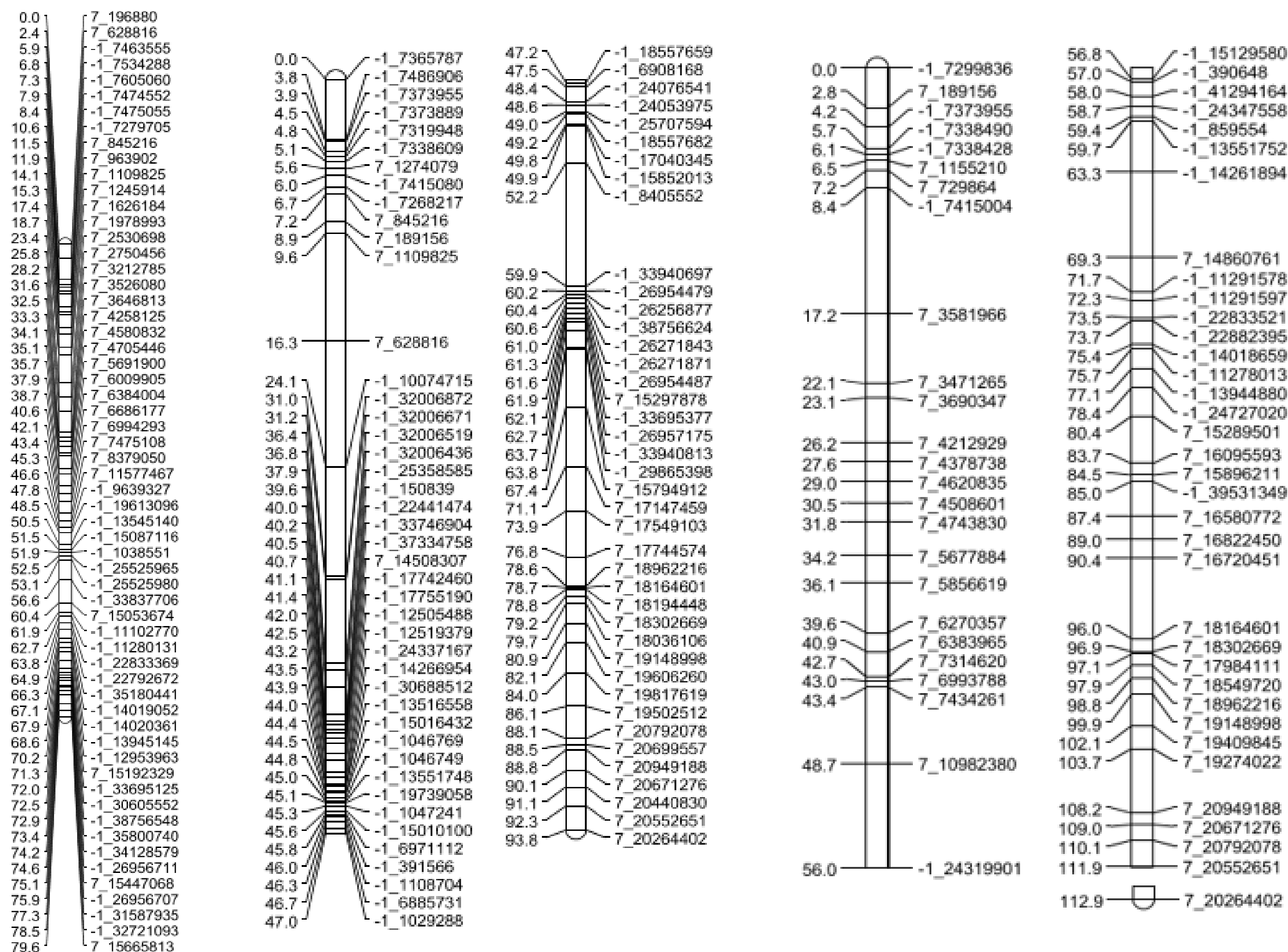


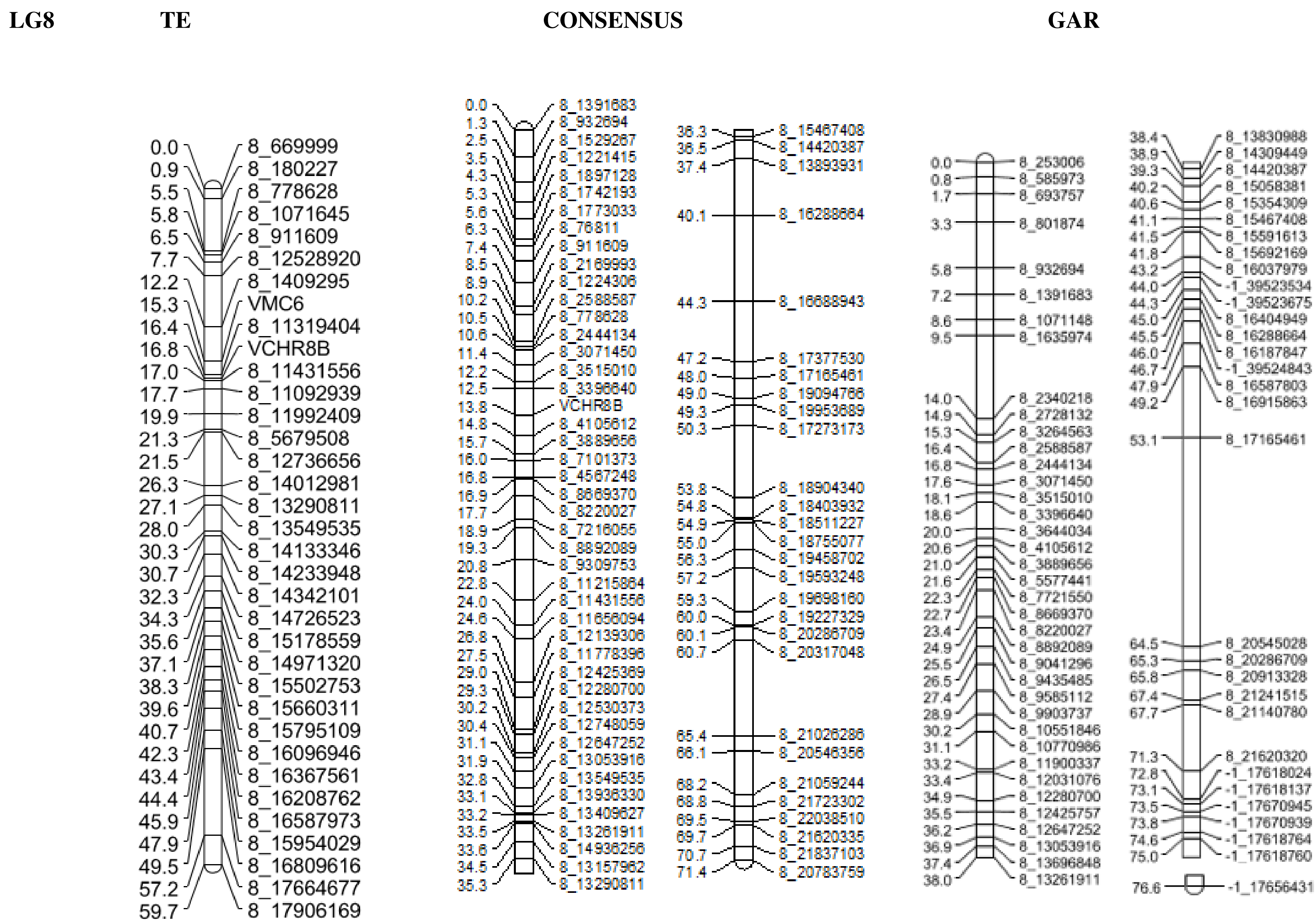






LG7 **TE** **CONSENSUS** **GAR**




LG8
TE
CONSENSUS
GAR
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0.9 8_180227
5.5 8_778628
5.8 8_1071645
6.5 8_911609
7.7 8_12528920
12.2 8_1409295
15.3 VMC6
16.4 8_11319404
16.8 VCHR8B
17.0 8_11431556
17.7 8_11092939
19.9 8_11992409
21.3 8_5679508
21.5 8_12736656
26.3 8_14012981
27.1 8_13290811
28.0 8_13549535
30.3 8_14133346
30.7 8_14233948
32.3 8_14342101
34.3 8_14726523
35.6 8_15178559
37.1 8_14971320
38.3 8_15502753
39.6 8_15660311
40.7 8_15795109
42.3 8_16096946
43.4 8_16367561
44.4 8_16208762
45.9 8_16587973
47.9 8_15954029
49.5 8_16809616
57.2 8_17664677
59.7 8_17906169
0.0 8_1391683
1.3 8_932694
2.5 8_1529267
3.5 8_1221415
4.3 8_1897128
5.3 8_1742193
5.6 8_1773033
6.3 8_76811
7.4 8_911609
8.5 8_2169993
8.9 8_1224306
10.2 8_2588587
10.5 8_778628
10.6 8_2444134
11.4 8_3071450
12.2 8_3515010
12.5 8_3396640
13.8 VCHR8B
14.8 8_4105612
15.7 8_3889656
16.0 8_7101373
16.8 8_4567248
16.9 8_8669370
17.7 8_8220027
18.9 8_7216055
19.3 8_8892089
20.8 8_9309753
22.8 8_11215864
24.0 8_11431556
24.6 8_11656094
26.8 8_12139306
27.5 8_11778396
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33.1 8_13938330
33.2 8_13409627
33.5 8_13261911
33.6 8_14936256
34.5 8_13157962
35.3 8_13290811
36.3 8_15467408
36.5 8_14420387
37.4 8_13893931
40.1 8_16288664
44.3 8_16688943
47.2 8_17377530
48.0 8_17165461
49.0 8_19094766
49.3 8_19953689
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54.8 8_18403932
54.9 8_18511227
55.0 8_18755077
56.3 8_19458702
57.2 8_19593248
59.3 8_19698160
60.0 8_19227329
60.1 8_20286709
60.7 8_20317048
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66.1 8_20546356
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68.8 8_21723302
69.5 8_22038510
69.7 8_21620335
70.7 8_21837103
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1.7 8_693757
3.3 8_801874
5.8 8_932694
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8.6 8_1071148
9.5 8_1635974
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26.5 8_9435485
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44.3 -1_39523675
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45.5 8_16288664
46.0 8_16187847
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47.9 8_16587803
49.2 8_16915863
53.1 8_17165461
64.5 8_20545028
65.3 8_20286709
65.8 8_20913328
67.4 8_21241515
67.7 8_21140780
71.3 8_21620320
72.8 -1_17618024
73.1 -1_17618137
73.5 -1_17670945
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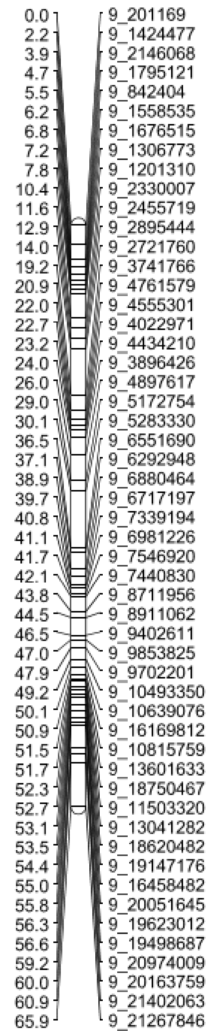
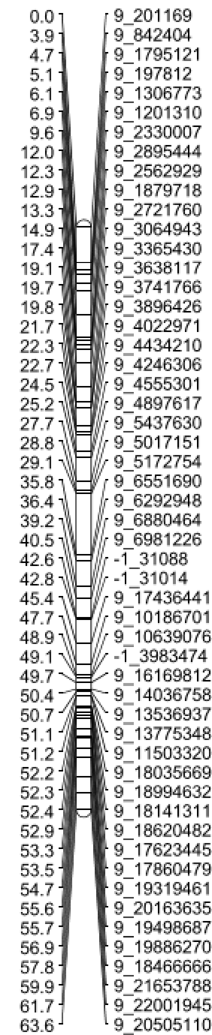
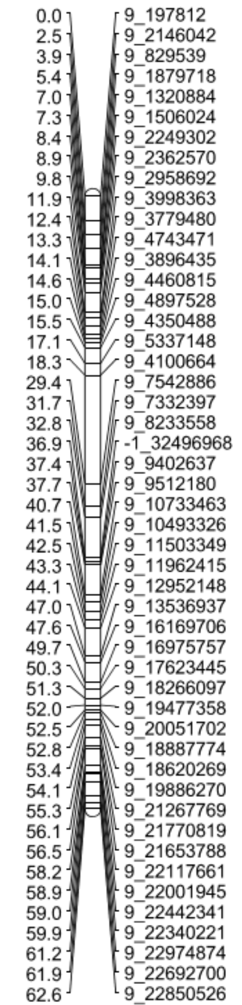
76.6 -1_17656431

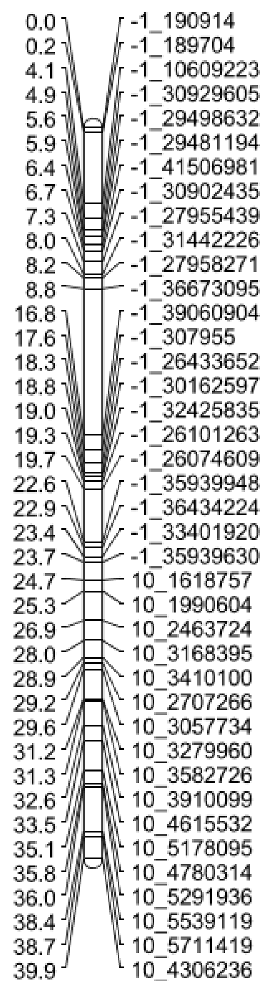
LG9 **TE** **CONSENSUS** **GAR**

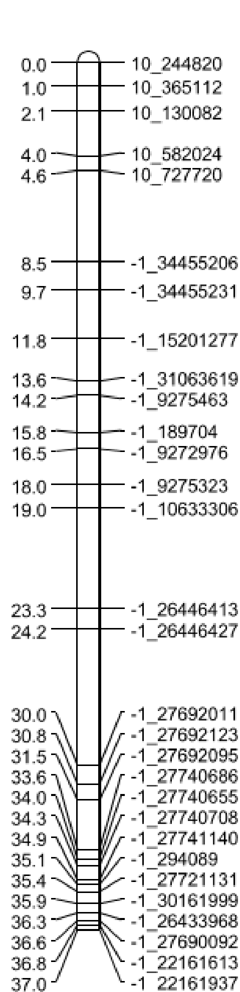


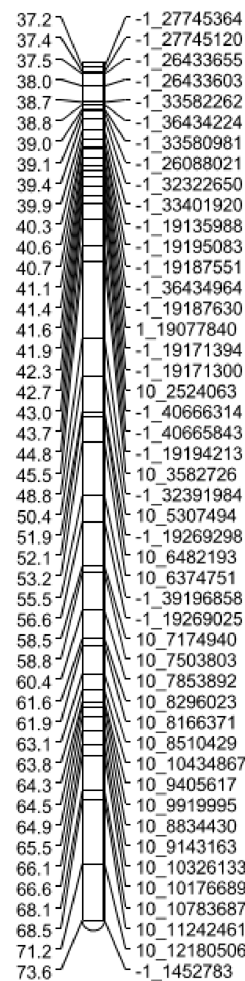


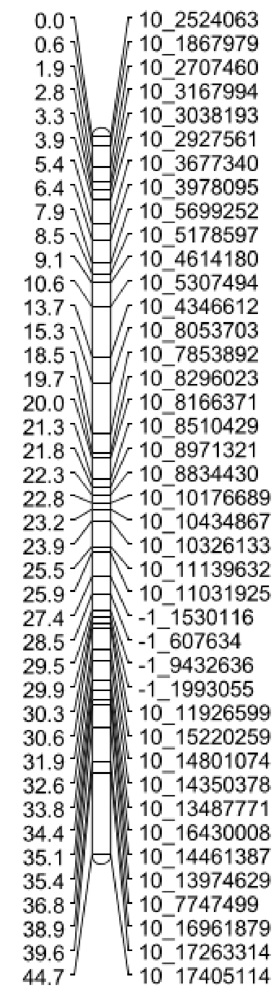


LG10 **TE** **CONSENSUS** **GAR**

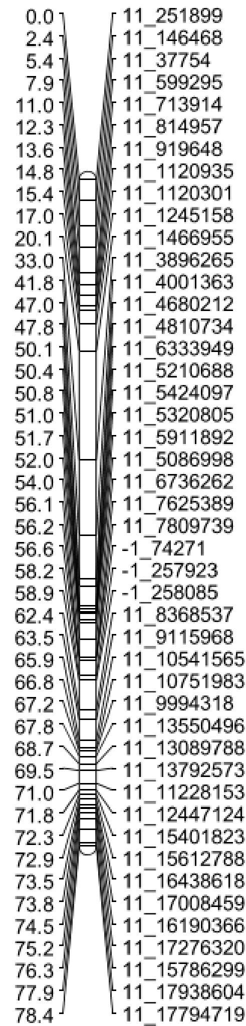


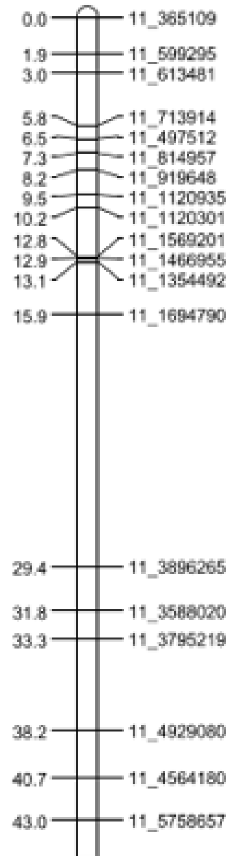


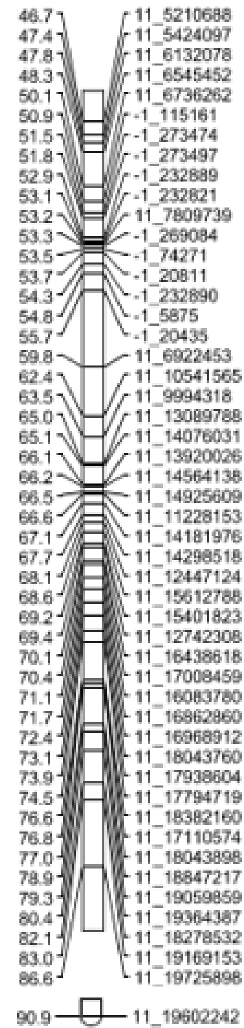


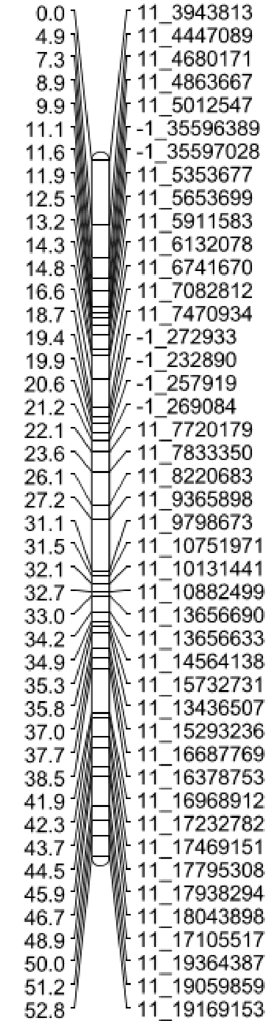


LG11 **TE** **CONSENSUS** **GAR**









LG12

TE

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7.8 12_2100009
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39.0 12_18208791
40.7 12_13007680
41.3 12_14070020
42.1 12_13832828
42.7 12_15529953
43.0 12_16822607
43.2 12_13344054
44.6 12_18412179
44.7 12_18523106
45.2 12_18023320
46.0 12_18842612
47.0 12_19449866
47.4 12_19020782
48.9 12_19182259
51.6 12_20018808
52.1 12_20268404
53.1 12_19752488
54.1 12_21251492
54.7 12_20808190
55.5 12_20575575
57.6 12_22529139
61.4 12_20940864

CONSENSUS

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0.5 12_531228
2.0 12_238801
3.8 12_724286
4.8 12_845353
5.5 12_1418787
5.6 12_22168
6.2 12_741746
7.1 12_1150529
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9.8 12_1648023
10.9 12_1813344
13.8 12_2100009
15.1 12_2277760
17.2 12_1982474
18.1 12_2378611
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23.9 12_2769416
34.7 12_6727662
42.5 12_8261721
44.3 12_9713704
46.1 12_9146347
46.9 12_8142392
47.9 12_8779916
49.3 12_9707347
49.6 12_9295118
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53.1 -1_1169509
53.6 -1_829581
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57.9 12_18523106
58.7 12_14753036
62.4 12_15529865
63.5 12_16877750
64.4 12_17053652
67.1 12_18499254
67.8 12_17724169
68.7 12_18023492
69.2 12_18391636
69.9 12_18391090
70.3 12_18668413
71.0 12_21742665
72.8 12_18793672
73.3 12_20150176
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76.8 12_20633522
79.3 12_19583519
83.3 12_21105311

GAR

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5.8 12_3758134
6.1 12_3626130
8.0 12_4977226
8.4 12_3909227
8.7 12_4306929
9.1 12_4426661
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12.1 12_4833792
13.4 12_5099831
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15.0 12_5569878
15.8 12_5779783
16.4 12_5679346
17.0 12_6052899
17.4 12_6173171
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19.9 12_6588733
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21.8 12_6727662
24.1 12_7174040
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29.9 12_8261721
31.4 12_8779916

32.6 12_9146347
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38.2 12_8366184
40.4 12_8678603
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43.7 12_13981191
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50.9 12_17724169
51.3 12_18023492
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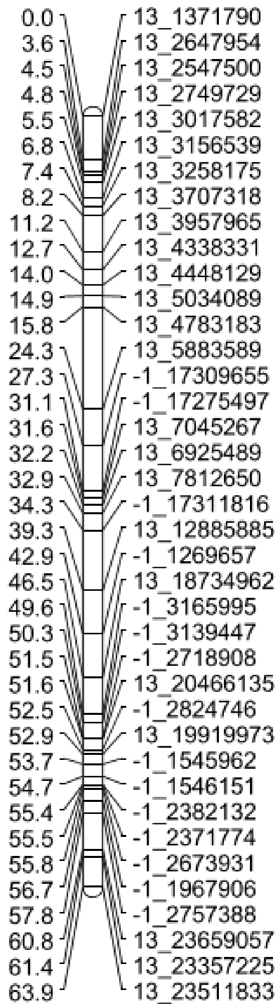
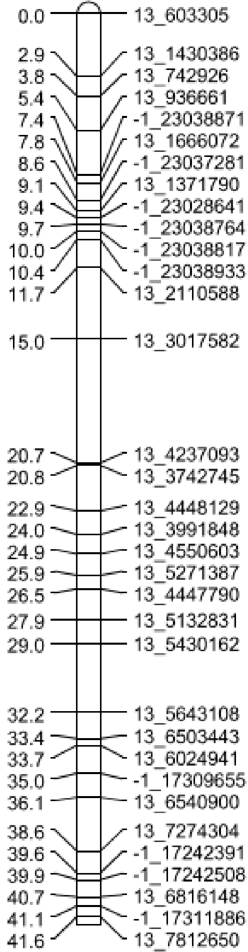
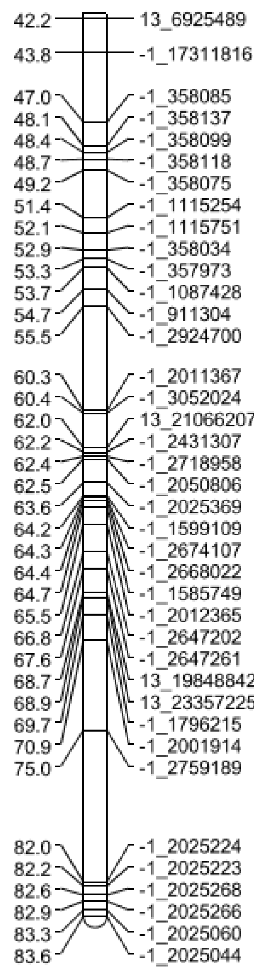
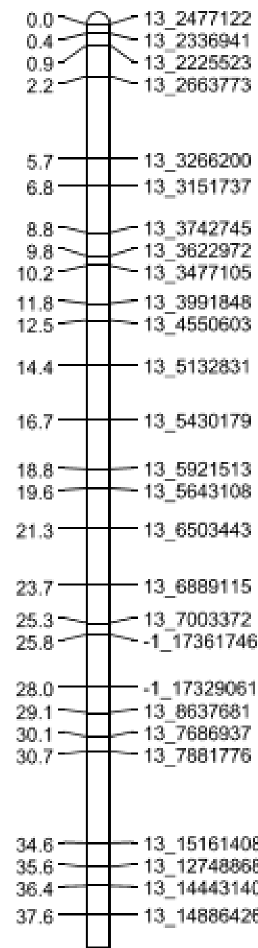
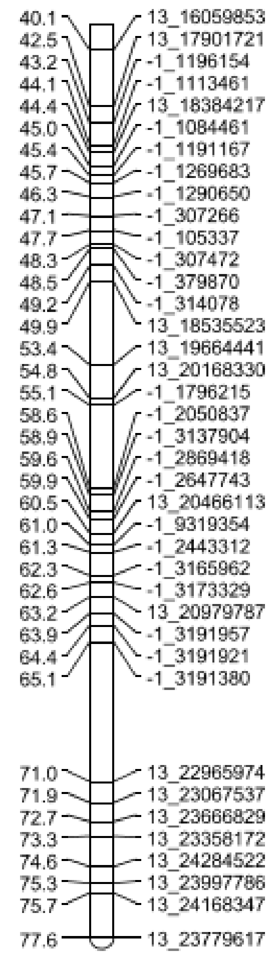
LG13 **TE** **CONSENSUS** **GAR**



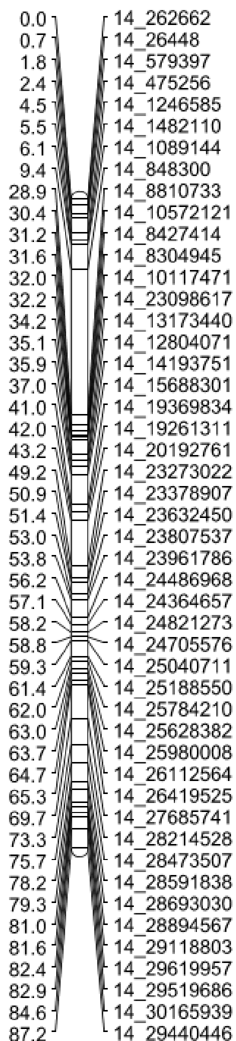


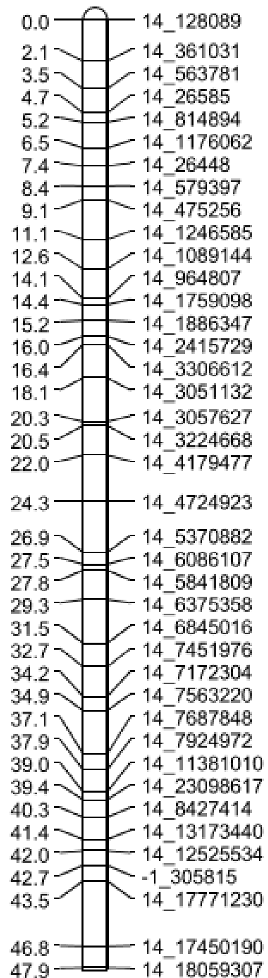


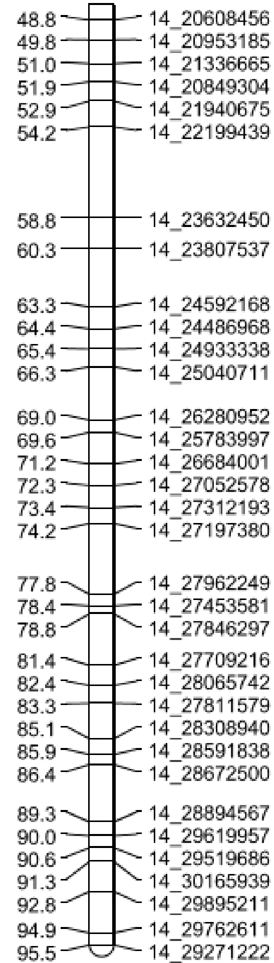


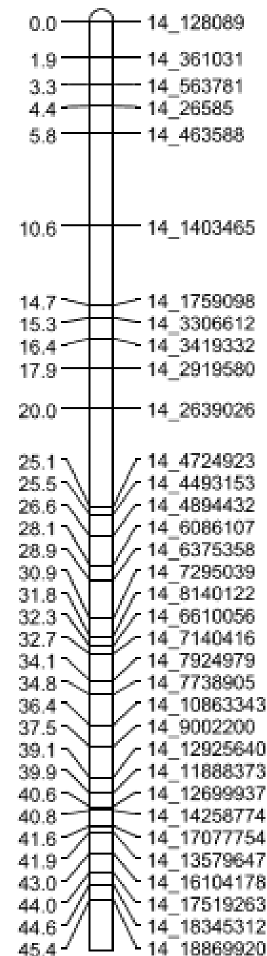


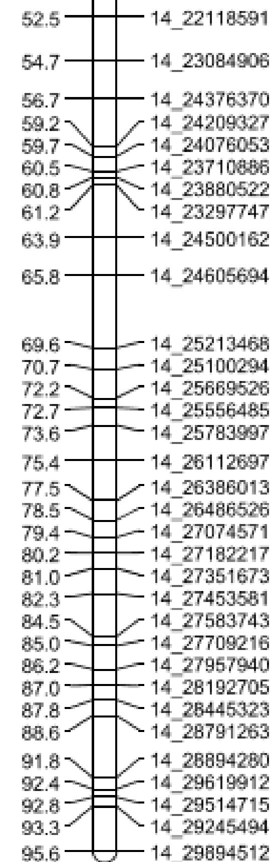
LG14 **TE** **CONSENSUS** **GAR**



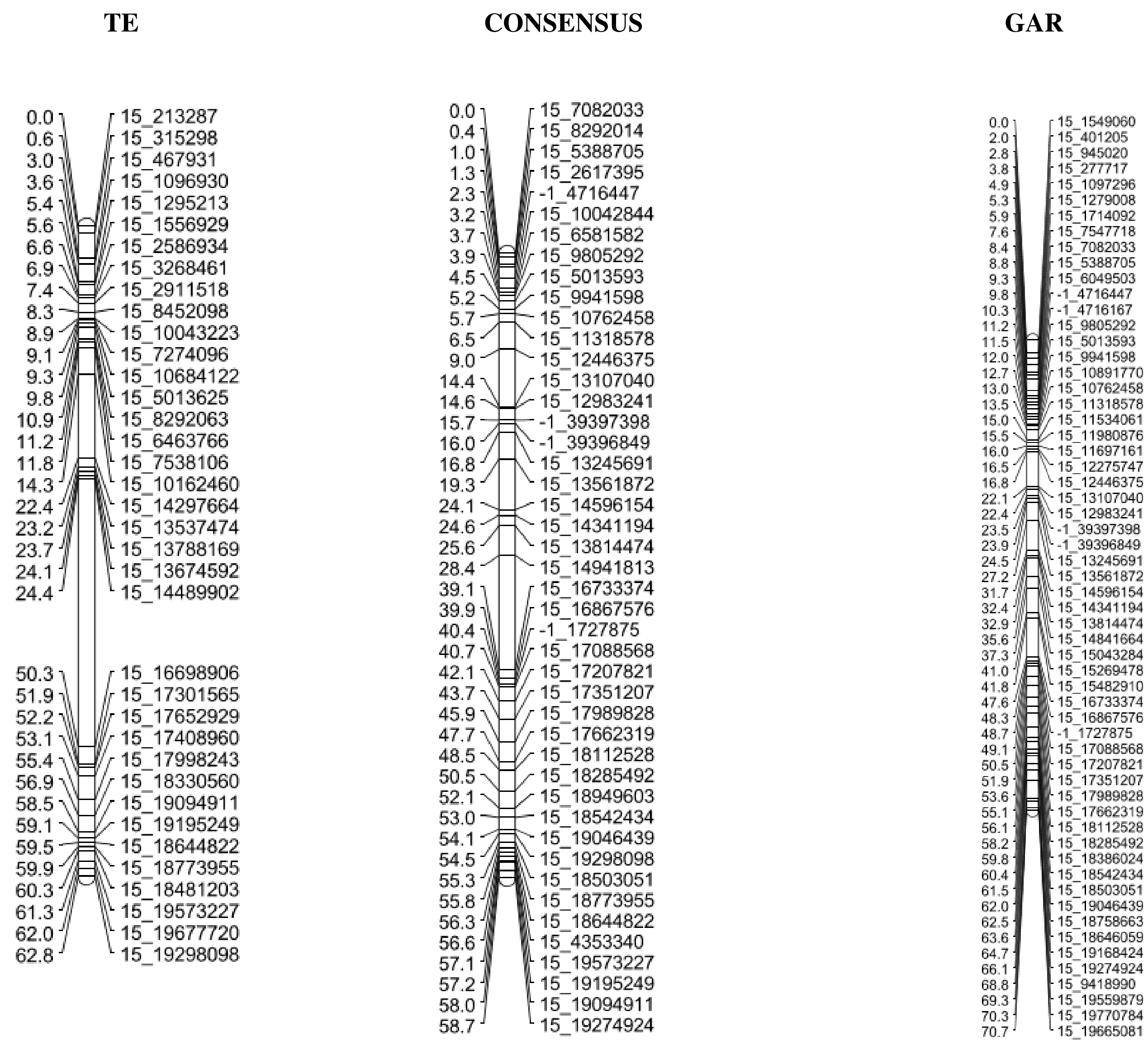




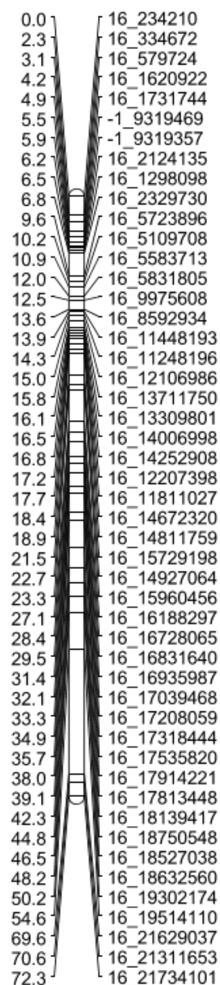


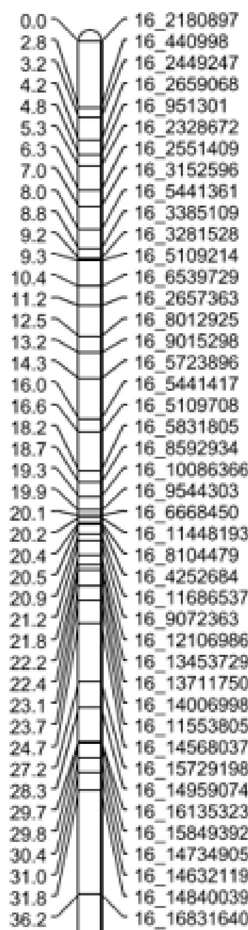


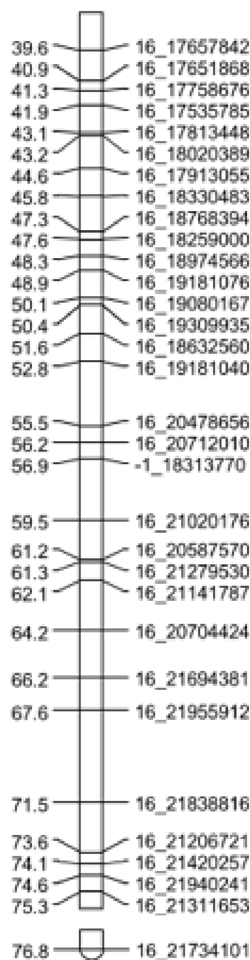
LG15

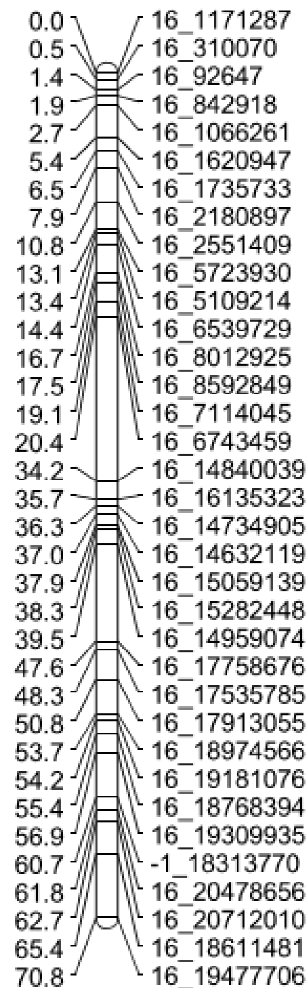


LG16 **TE** **CONSENSUS** **GAR**

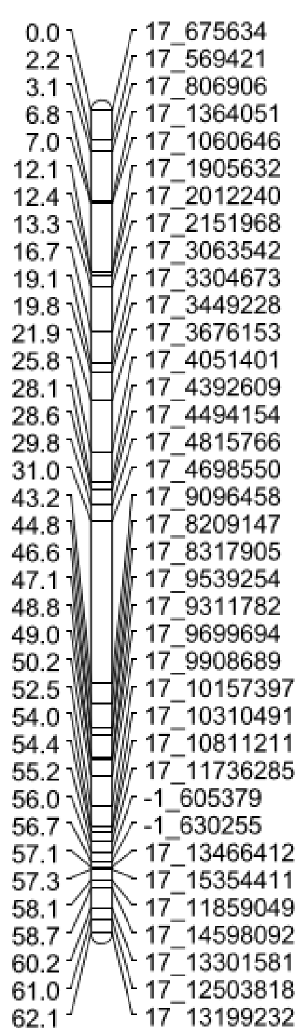


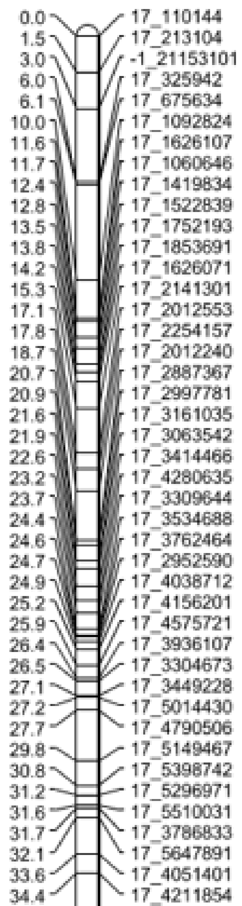


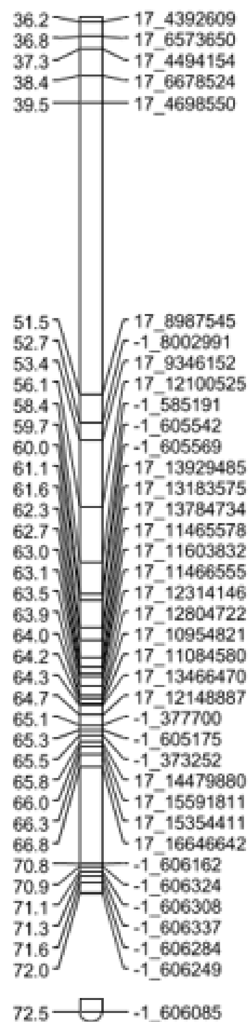


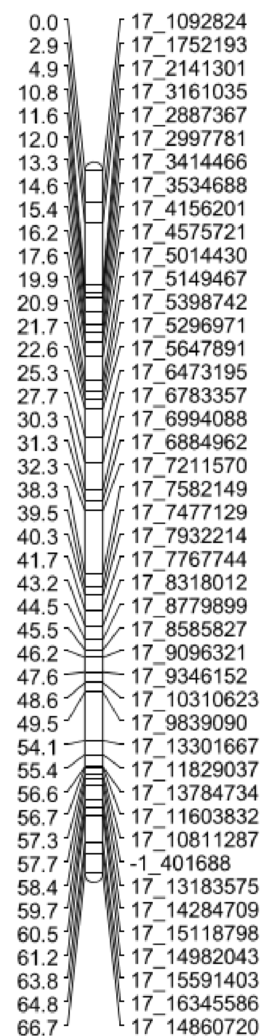


LG17 **TE** **CONSENSUS** **GAR**

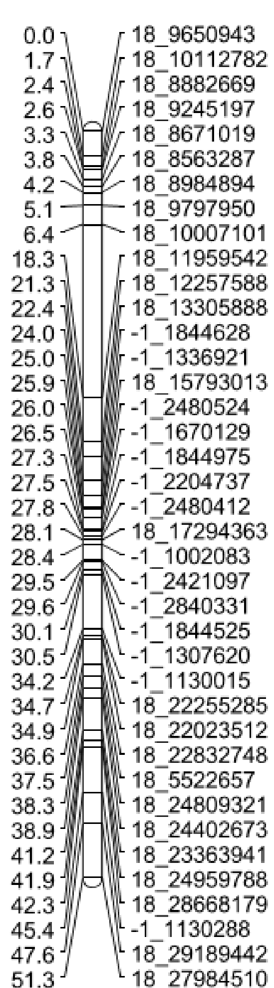


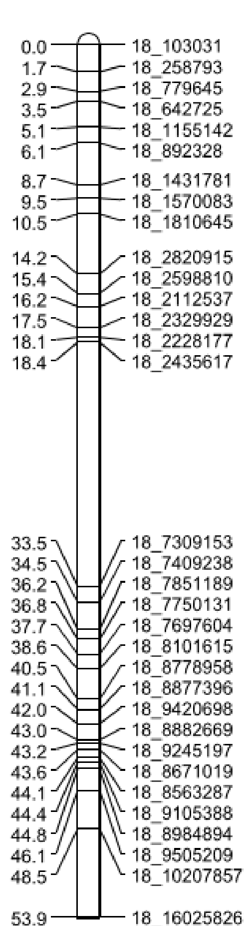


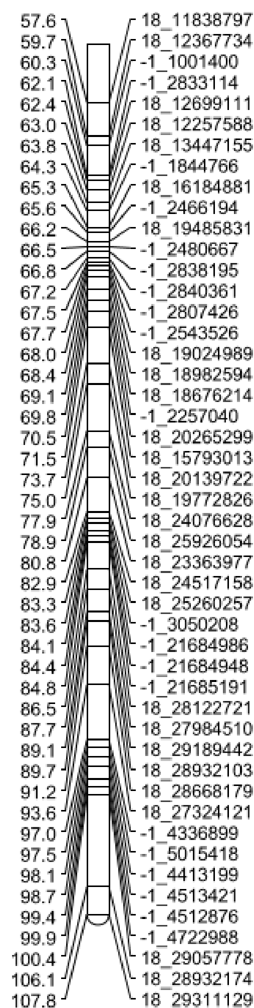


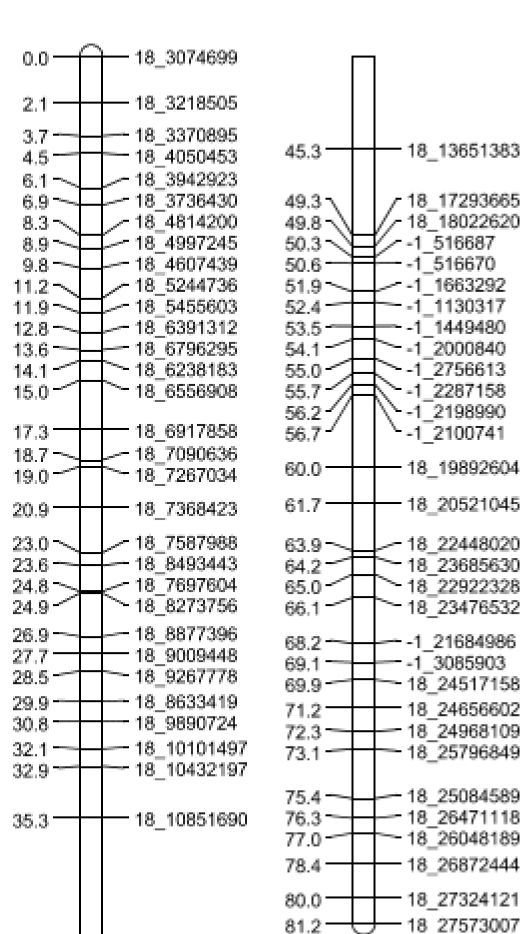


LG18 **TE** **CONSENSUS** **GAR**

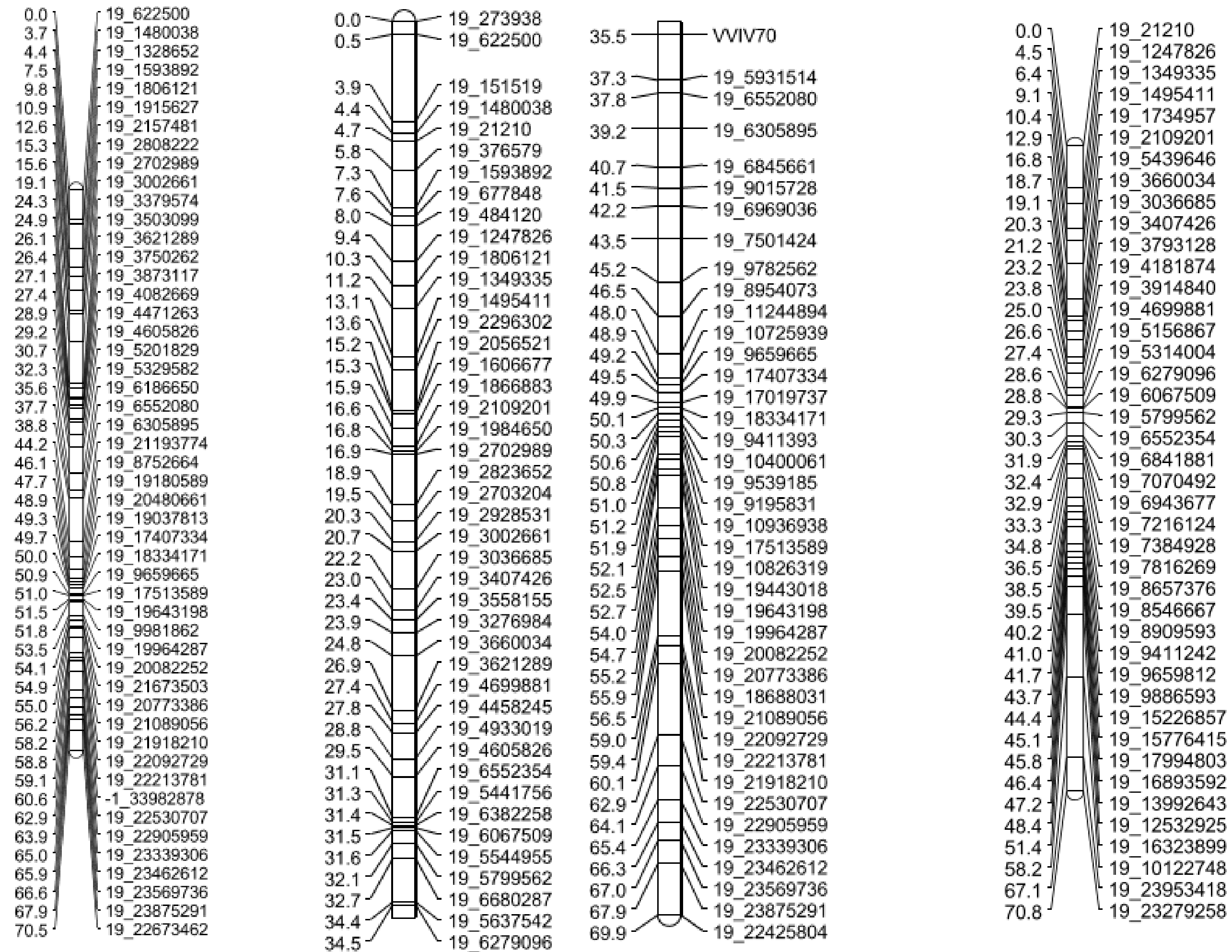








LG19 **TE** **CONSENSUS** **GAR**



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3.3. QTL analysis of berry flower and seed traits in two Tempranillo segregating populations. Influence of the *Sex* locus

Abstract

Berry weight, seed content and ripening date are relevant traits to viticulturists in order to establish the quality and consumer acceptance of grapes. The aim of this study was to perform a genetic analysis of 12 berry, flower and seed traits in order to elucidate their genetic control. QTL analysis was conducted with two different wine-grape segregant populations during a minimum of two consecutive vintages in different locations. Significant QTL were detected for berry weight and berry diameter in LG3 and LG18 and for berry shape in LG1 and LG9 in both genetic backgrounds. QTL were detected in LG11 and LG14 for flower diameter (FD), in LG8 and LG11 for ovary length (OL) and in LG11 for pistil shape (PS) and in LG18 related to seed traits in both populations. Besides, for ovary shape (OS), QTL on LG2, LG7, LG8 and LG11 were detected in both progenies, with LG11 having the strongest and most stable effect over the two years. For seed traits, QTL on LG3, LG5, LG6, LG10, LG18 and LG19 were found stable across years, being only LG18 common to both genetic populations.

Sex locus was mapped close to markers VVIB23 and VMD34 in LG2, in a region where QTL for flower-morphology (PS, OS), seed traits (SN, SW), and flowering date were mapped. *Sex* locus strongly influenced flower morphology traits such as ovary shape in both progenies. In one population (G × T), co-localization of QTL for flower morphology traits, and flowering date were found in LG7 and LG13. A region in LG17 was found significantly associated to berry morphology and seed parameters, suggesting close linkage or pleiotropic effects. In T × G progeny, QTL for flower morphology, seed traits and phenology events were mapped in LG3 and LG11. For that progeny QTL for seed traits in LG18 resulted associated to locus *SDI*, and QTL on LG5 for berry, seed and flower traits co-localized with the *FERONIA* locus. A candidate gene VIT_11s0016g03650 with a function associated to pollen morphology is proposed associated to the highly significant QTL detected in LG11 for flower traits in both progenies.

The present study provides useful information on the genetic basis of natural genetic variation present for berry, seed and phenology traits that would be helpful for the development of breeding programs in wine grape.

Introduction

Berry size and seed content are considered relevant parameters in grape breeding. Berry size constitutes an important quality attribute of both table grapes and wine-grapes. In wine grape, smaller berries result in higher concentration of aromatic and phenolic compounds that are associated with sensory quality (Barbagallo et al. 2011, Roby et al. 2004). It is estimated that berry size is an indicator of yield in wine production, and that small berry size is desirable to increase skin-to-flesh ratio, improving final concentrations of wine anthocyanins (Holt et al. 2008, Barbagallo et al. 2011) and modifying must composition traits such as acidity (Gil et al. 2015). Like in tomato or watermelon, final berry size is reported to be determined before anthesis, being related to flower morphology (Houel et al. 2013). Although the correlation between seed number and weight and berry size has been accepted in the past (May 2000, Walker et al. 2005) these relationships have been challenged (Doligez et al. 2013). Seed abortion determined by the *Seed*

Development Inhibitor (*SDI*) locus is the source for seedlessness. The *SDI* mutation has been recently identified in a 323-kb region on chromosome 18 (Royo et al. 2018), being seed abortion linked to activation of salicylic acid-dependent autoimmunity.

Recent investigations have analyzed berry and seed-related traits (Doligez et al. 2013, Houel et al. 2015, Ban et al. 2016, Guo et al. 2019), flower-morphology parameters and flowering date (Kamal et al. 2018), with the goal of elucidating their genetic control. Most of these events are not independent, hence understanding of flowering and other development stages may help control variation in berry production (Kamal et al. 2018). Length of flowering period and inflorescence size may play a role in generating berry weight variability, both within and between clusters (Poni & Libelli 2008), which may be also affected by cultural practices and environmental factors that occur during floral differentiation and after flowering (Dunn & Martin 2000; Gray & Coombe 2009, Barbagallo et al. 2011).

Flower sex is expected to influence seed number (Constantini et al., 2008), flower morphology (Margueritt et al. 2009) or phenology events (Nunes-Ramos et al. 2016). However, the effect of *Sex* locus on seed, berry and flower morphology traits has not been deeply investigated. Little insights lead to understand its influence since seed number in female plants is expected to be lower due to a higher rate of parthenocarpic berry set (Appazzova et al. 1977) and female table varieties usually have rather large berries (Boursiquot et al. 1995). Interestingly, Constantini et al. (2008), reported QTL for berry weight and seed number associated to LG2 in a Syrah × Grenache progeny, most probably explained by the *Sex* locus that segregated in that mapping population. Doligez et al. (2013), decided to include flower sex (females vs hermaphrodites) as a covariate into the QTL analysis but no QTL were found significative on LG2 for berry weight and seed traits. In this research we have focused on the influence of the *Sex* locus in berry and flower morphology and seed traits.

The genetic control of major traits in grape, such as berry size, phenology stages, must composition, has been explored via simple sequence repeat (SSR) markers in biparental populations (Constantini et al. 2008, Fechter et al. 2014, Ban et al. 2016, Bayo-Canha et al. 2019). The low chromosome coverage by molecular markers and the lack of phenotypic data over multiple seasons difficult the understanding of the genetic basis of traits. Besides, the results of QTL mapping usually vary greatly among populations (Dai et al. 2011), being specific to the bi-parental populations studied and less applicable against wider genetic backgrounds (Tello et al. 2019). Nowadays, thousands to millions of markers are developed by next generation sequencing technologies that allow improving mapping coverage and resolution (Deschamps et al. 2012). In order to take advantage of NGS tools and reduce mapping limitations, genotyping by sequencing (GBS) analysis was used to develop a dense linkage map for the F_1 population derived from crossing Grenache × Tempranillo. Genetic maps were combined with phenotypic data collected over four seasons to identify stable QTL related to flower morphology, flowering time, berry size and seed traits. Furthermore, QTL analysis was conducted for the same traits in a Graciano × Tempranillo population, previously mapped, allowing the comparison among genetic backgrounds with Tempranillo cv. as a common parent.

Materials and Methods

<u>Plant material</u>

Two segregating populations obtained from controlled crosses between the wine grape cultivar Tempranillo (male parent) and Grenache (130 genotypes, G × T) and Graciano (151

plants, T × G) as female parents respectively, were used for this study. The individual hybrids (one plant of each genotype) have been grown on their own roots since 2004, in a sandy-loam soil with East–West orientation (3m x 1m) in double Royat cordon in Varea, La Rioja. The G × T population was duplicated in an additional plot at the University of la Rioja Experimental field in Logroño in 2012. Standard irrigation, fertilization and plant protection practices for La Rioja region were performed. The plants first flowered and fruited in 2007 in Varea. The G × T population was genotyped for 5 SSRs markers: VMC6 VChr3a, VChr8b, VVIB23, VVIV70 (M&M chapter 3.2.) in order to discard individuals resulting from self-pollinations and foreign pollen sources, resulting in a final population of 130 plants, only 4 plants were removed. The T × G population had been previously genotyped (Song et al. 2014).

Phenotypic evaluation

Twelve traits related to berry and flower morphology, seed number and weight and flowering time, were evaluated in the two populations in at least three years during the 2012 - 2017 period. Weather data from April to October for the vintages studied are showed in Figure 3.3.1. Flower and seed traits were only evaluated in 2015 and 2016 vintages (Figure 3.3.1). The number of genotypes that bore fruit varied each year due to hail, disease incidence and bird attack during flowering or veraison-ripening stages, respectively. Thus, for G × T population 127, 111, 117 and 120 genotypes were harvested in 2014, 2015, 2016 and 2017, respectively. For T × G progeny, 102, 114 and 102 genotypes were analyzed in 2012, 2016 and 2017 respectively.

The two experimental plots were located at Viveros Provedo (Varea, La Rioja, Spain), and at the University of La Rioja (UR), both belonging to D.O.Ca. Rioja Alta Varea soil is sandy to sandy-loam (50 %-33 %) with 12.7 % clay, and 19 % carbonate, pH 7.8, and 1.8 % organic matter. The UR plot was characterised as sandy-loam (49 % - 38 %) 16.6 % clay and 16 % carbonate, pH 7.9, and 1.2 % organic matter.

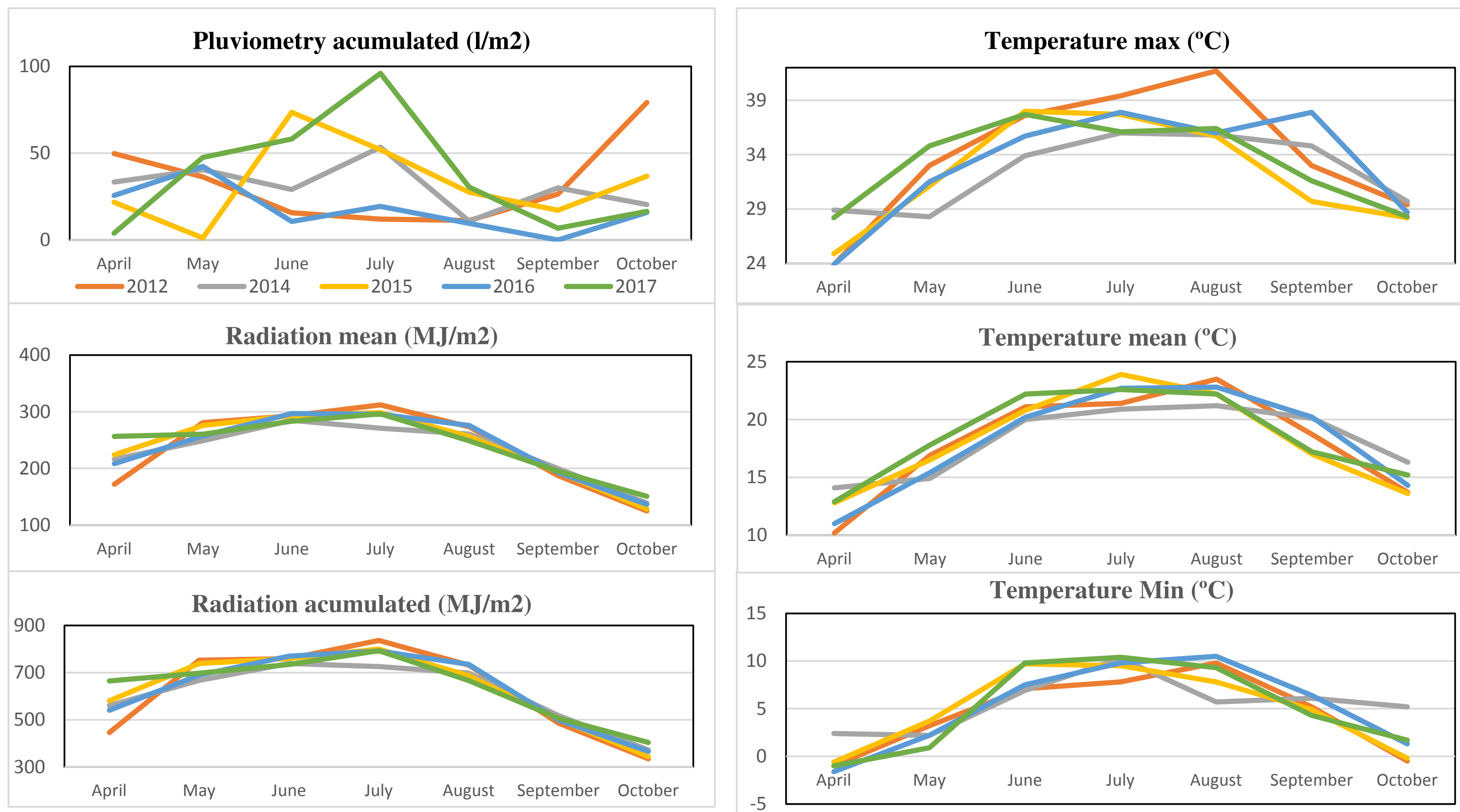
Berry traits

Berry data were collected in three consecutive years (2014 - 2016) for G × T and two years (2012 and 2016) for T × G progeny. Ripening stage was stablished as the date when random grapes picked from the top, medium and bottom of the clusters reached technological maturity (23.4 ºBrix). At harvest date, 200 whole berries from each genotype were sampled from representative clusters to calculate mean berry weight and 90 berries were frozen at – 20 ºC to measure berry shape parameters. In 30 berries per plant, length and diameter were measured with a Mitutuyo digital calibre and shape coefficient was calculated as the ratio between length and diameter (Houel et al. 2013) (Chapter 3.1).

Flower traits

Between 9 and 30 inflorescences per individual were harvested, photographed with a digital camera and measured with the image analysis software ImageJ, in each population in two different years. At anthesis, considered when 50 % flowers were in Baggiolini stage I (Baggiolini 1952), flowering date was noted. Inflorescence morphology was inferred based on measurements of ovary and pistil length, and flower diameter, and on shape coefficients: ovary shape being the ratio between ovary length and flower diameter, and pistil shape the ratio between pistil length and flower diameter as described in Chapter 3.1

Figure 3.3.1. Climatic characteristics in the growing season (April-October) in all the years of the study. Source: SIAR La Rioja.



Seed traits

Mean seed number per berry (SN) and mean seed fresh weight (SW) were measured by triplicate from a sample of 20 berries per genotype randomly selected. Seeds were taken out from grape berries carefully, cleaned with absorbent paper, dried at room temperature for 24 hours, and weighted with an analytical balance.

Berry and flower morphology traits were measured in both populations, Grenache × Tempranillo (G × T) and Graciano × Tempranillo (T × G) as shown in Figure 3.3.2.

Figure 3.3.2. Summary of the traits analyzed in both progenies (years and environments).

	Trait	Abr.	G × T progeny Year / Environment	T × G progeny Year / Environment
Berry morphology	**Berry length**	**BL**	3 years, 2 Plots	2 years, 1 Plot
	Berry diameter	**BD**	2014 & 2015 (UR)	2016(Varea)
	Berry shape	**BS**	2016 (Varea)	2012 (Varea)
	Berry weight	**BW**	*BW also in 2017 (UR)	
Flower morphology	**Pistil length**	**PL**		
	Flower diameter	**FD**	2 years, 2 Plots	2 years, 1 Plot
	Ovary length	**OL**	2015 (UR)	2016(Varea)
	Pistil shape	**PS**	2016 (Varea)	2017(Varea)
	Ovary shape	**OS**		
Flowering time	**Flowering date**	**F**	3 years, 2 Plots 2014-2016 (UR) 2016 (Varea)	2 years, 1 Plot 2016(Varea) 2017(Varea)
Seed traits	**Seed number**	**SN**	2 years, 2 Plots	1 year, 1 Plot
	Seed weight	**SW**	2015 (UR)	2016 (Varea)
			2016 (Varea)	

Statistical analysis

Differences between parental values were evaluated with t-test. Analysis of variance followed by LSD test was used to evaluate population mean value differences among years. The normality of each trait distribution was checked by the Kolmogorov-Smirnov test. Data that significantly deviated from normality were analyzed by non-parametrical tests. Phenotypic correlations between traits were determined in each year with the Spearman rank-correlation coefficient ($p < 0.05$). Correlation analysis between years was used to evaluate the genotype stability across years for each trait. Year effect was tested with analysis of variance and non-parametric Kruskal-Wallis test.

Genotyping

DNA extraction and map construction protocols are fully described in Chapter 3.2**.**

Mapping of the *Sex* locus and QTL Analysis

Sex determinism was analyzed by genotyping both populations with VVIB23 and VMD34 SSR marker using Joinmap 4 and MapQTL6. In the first analysis only two phenotypic classes were considered: female (ff) and hermaphrodite (H-) plants. Then, a second analysis was run with the three genotypes (*ff, Hf, HH*).

QTL analysis was carried out on the parental and Consensus maps separately using Map QTL 6.0 software and the phenotypic data from each year (Van Ooijen 2009). Logarithm of odds (LOD) thresholds corresponding to $\alpha = 0.05$ genome-wide and chromosome wide were determined using 1000 permutations (Churchill & Doerge 1994) of the phenotypic data.

Non-parametric Kruskal-Wallis (KW) rank sum test was applied to the data using a stringency significant level of $p = 0.005$ (****). Interval mapping was then conducted to detect significant QTL regions. Maximum LOD values were used to estimate QTL peak position. Confidence intervals (1-LOD) were estimated in cM and corresponded to a LOD score drop of one on either side of the likelihood peak. QTL analysis were performed first considering the whole population and then only with the hermaphrodite plants in both genetic backgrounds in order to assess sex influence. Besides, MQM analysis using *Sex* locus as cofactor was also performed with the same goal. A QTL was considered significant when the maximum LOD exceeded the genome-wide (GW) and putative when it exceeded the chromosome-wide (CW) threshold. A QTL was considered stable when detected in at least two seasons or two environments. Putative QTL were also retained since several relevant agronomical traits are controlled by multiple genes each making a small contribution to the genetic determinism of the character.

Candidate genes

Genes within and overlapping each QTL were identified using Ensembl (http://ensemble.org) in the BioMart community portal (Smedley et al. 2015) and the annotated molecular function available at NCBI (http://www.ncbi.nlm.nih.gov). Candidate genes for the traits reviewed were searched for within each confidence interval of the QTL detected in more than one year and that resulted highly significative. The most proximal marker (SNP) was selected to delimit the confidence interval, and the physical position of the marker was identified in the NCBI database.

Results

Phenotypic segregation

Wine grape varieties showing complementary agronomical and oenological attributes were chosen as parents for this study. A t-test (Chapter 3.1) detected significant differences between Grenache and Tempranillo in berry length and shape, flower diameter and pistil shape, with Grenache showing higher values than Tempranillo in all the traits. On the other hand, Graciano presented lower values for berry length, diameter and weight, pistil length, flower diameter, seed number and seed weight compared with Tempranillo, but a longer pistil shape.

Phenotypic data distributions for both progenies were similar for all the years studied and Figures 3.3.3.a and b display data for only one year. Transgressive segregation, indicative of high genetic variability, was observed for all characters evaluated. For seed weight in T × G progeny the population presented only lower values than both parents (Fig. 3.3.3. b). The Kolmogorov–Smirnov test indicated that pistil shape and flowering date were the only traits that deviated significantly from a normal distribution in both populations.

Mean G × T population values for berry length and berry diameter were lower than parental values. However, berry shape, berry weight, seed traits, and flower-related traits showed intermediate values between the parents (Fig. 3.3.3.a). For T × G progeny (Fig. 3.3.3.b), intermediate values for the population were observed for berry, pistil and ovary length and berry and flower diameter whereas population mean was lower than the mean parental values for all traits except seed number. It is remarkable that berry size is supposed to be dominant in the segregation (Doligez et al. 2013), but this effect has not been confirmed in this study. Table 3.3.1. shows the mean progeny values for the years studied, while parent data are displayed in Chapter 3.1 Figure 3.1.1.

ANOVA was conducted to assess vintage effect in both progenies and also plot influence in G × T progeny (Chapter 3.1, Figure 3.3.1.). As mentioned, in G × T progeny, vintage resulted significant for all the traits sudied ($p < 0.01$) except for berry diameter, berry weight, flower diameter and seed weight, being plot also significant in berry length, berry shape and flowering date. In T × G progeny, vintage resulted significant for all the traits studied ($p < 0.001$) but for berry length, diameter and shape, flower diameter, ovary length and seed weight.

Significant correlations ($p < 0.01$) between years (Table 3.3.2) were detected for most traits scored in the same plot except for flowering date and seed number in T x G progeny and seed number and berry weight in G x T population. However, plot effect was higher than year effect in G × T progeny for berry traits, presumably because. geography and soils are very different, even though plots are only 1 km apart. Varea is located in a depression basin, the valley effect and soil characteristics make vines in Varea plot more productive and earlier in phenology compared to UR plot. Besides, years with abnormally high pluviometry, 2016, or higher temperatures, 2017, resulted in greater differences between both environments. The fact that T × G progeny presented higher correlation values in flower related traits than G × T confirms the lower effect of year compared with plot, given that T × G population was evaluated only in Varea plot. Seed traits and flowering date resulted the most influenced parameters by vintage in both progenies and also by plot differences in G × T population.

Fig. 3.3.3.a. Distribution of berry, flower and seed traits in G × T population in 2015. Parental data are indicated: Grenache (GAR) and Tempranillo (TE).

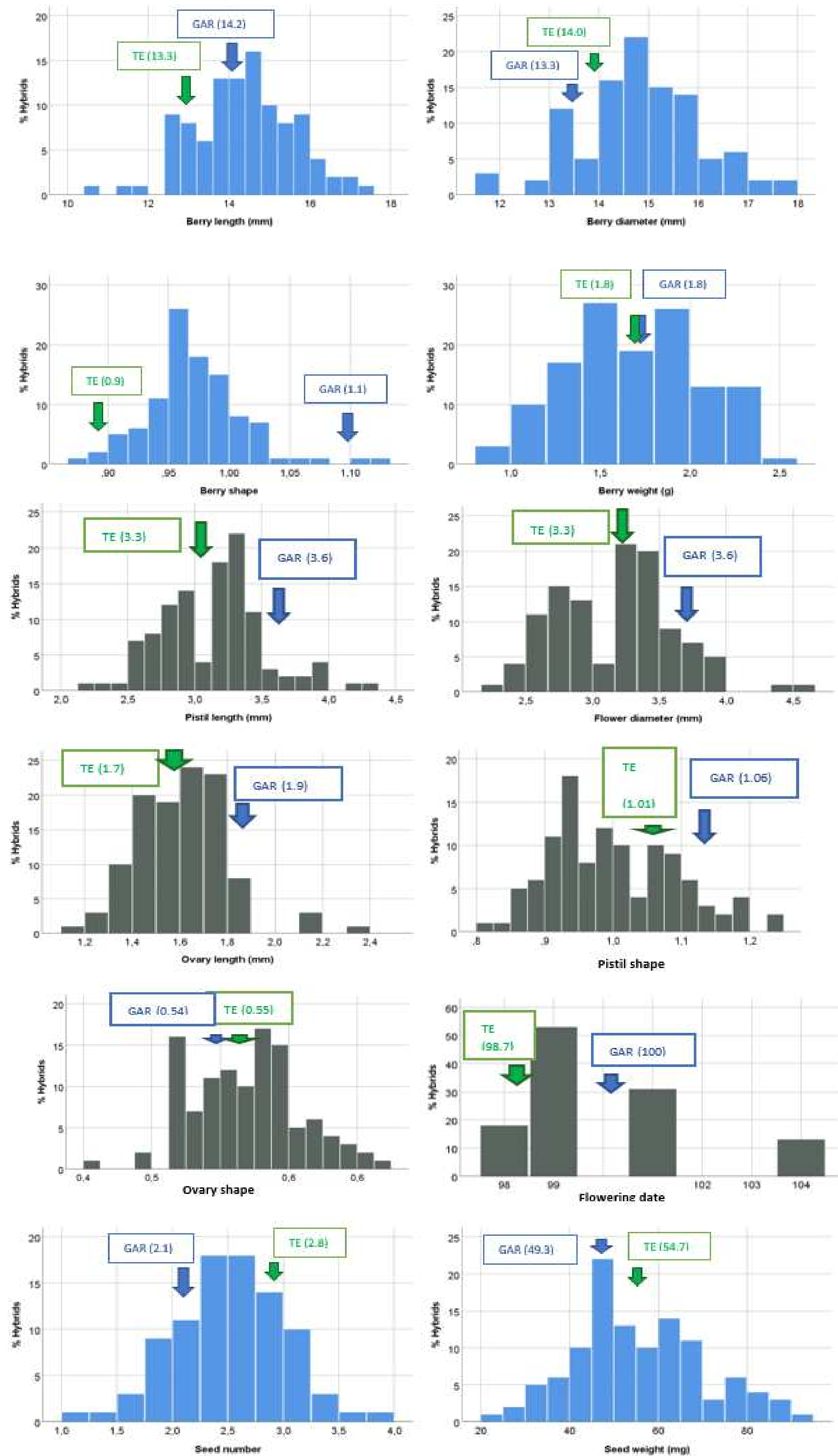


Fig. 3.3.3.b. Distribution of berry, flower and seed traits in T × G population in 2016. Parental data are indicated: Graciano (GRA) and Tempranillo (TE).

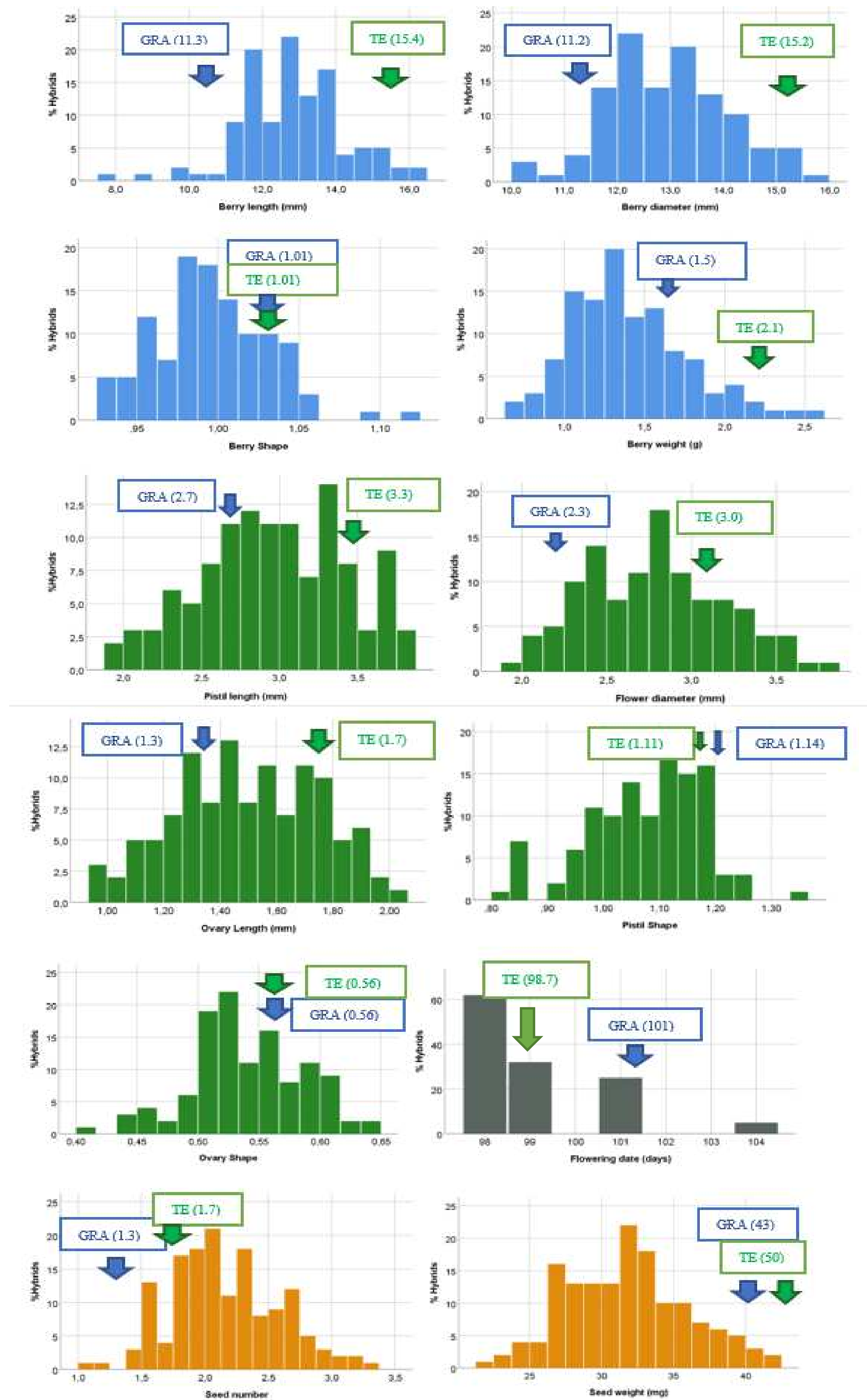


Table 3.3.1. Mean values of traits studied in Grenache x Tempranillo (G × T) and Graciano × Tempranillo (T × G) progenies.

G × T population					
		N	Mean±SD	Min	Max
BL (mm)	**2014**	116	14.32±1.1	11.91	18.7
	2015	114	14.36±1.26	10.5	17.4
	2016	116	14.65±1.58	11.41	17.02
	Mean	114	14.44±1.18	10.5	18.7
BD (mm)	**2014**	116	14.42±1.11	11.9	17.87
	2015	114	14.81±1.21	11.6	17.6
	2016	116	14.64±1.48	10.68	17.3
	Mean	114	14.62±1.2	10.68	17.87
BS	**2014**	116	0.99±0.03	0.94	1.07
	2015	114	0.97±0.04	0.88	1.11
	2016	116	1.0±0.03	0.9	1.09
	Mean	114	0.99±0.02	0.88	1.11
BW (g)	**2014**	127	1.54±0.54	0.64	2.24
	2015	129	1.68±0.36	0.81	2.52
	2016	117	1.70±0.44	0.78	2.36
	Mean	123	1.62±0.36	0.79	2.52
PL (mm)	**2015**	127	0.25±0.05	0.15	0.49
	2016	111	3.1±0.0.3	2.2	4.3
	Mean	111	2.8±0.4	1.5	4.9
FD (mm)	**2015**	127	2.7±0.5	1.6	4.4
	2016	111	3.1±0.4	2.1	4.6
	Mean	111	2.9±0.3	1.6	4.6
OL (mm)	**2015**	127	1.3±0.3	0.7	2.1
	2016	111	1.6±0.2	1.1	2.2
	Mean	111	1.45±0.2	0.7	2.2
PS	**2015**	127	0.92±0.08	0.71	1.3
	2016	111	1.0±0.09	0.84	1.18
	Mean	111	0.96±0.06	0.71	1.3
OS	**2015**	127	0.49±0.05	0.33	0.6
	2016	111	0.52±0.04	0.4	0.62
	Mean	111	2.34±0.24	1.15	3.75
F	**2014**	86	**102.9±2.7a**	97	108
	2015	127	**93.8±2.1b**	92	97
	2016	115	**99.9±1.8b**	98	104
	Mean	115	101.8±1.6	92	108
SN	**2015**	84	**2.51±0.47a**	1.3	3.75
	2016	99	**2.17±0.58b**	1.15	3.55
	Mean	84	2.34±0.24	1.15	3.75

T × G population					
		N	Mean±SD	Min	Max
BL (mm)	**2012**	102	12.9±1.1	10.58	16.23
	2016	114	12.8±1.4	9.80	16.10
	Mean	114	12.8±1.2	9.80	16.10
BD (mm)	**2012**	102	12.9±1.1	10.39	15.80
	2016	114	12.9±1.3	8.90	15.70
	Mean	114	12.9±1.2	8.90	15.70
BS (mm)	**2012**	102	1.0±0.01	0.95	1.07
	2016	114	1.0±0.03	0.90	1.10
	Mean	114	1.0±0.03	0.90	1.10
BW (g)	**2012**	102	1.7±0.3	1.05	2.65
	2016	115	1.49±0.38	1.10	2.60
	Mean	115	1.55±0.38	1.05	2.65
PL (mm)	**2016**	116	2.97±0.46	1.96	3.87
	2017	102	3.2±0.4	1.46	3.98
	Mean	116	3.1±0.4	1.46	3.98
FD (mm)	**2016**	116	2.78±0.41	1.96	3.78
	2017	102	2.8±0.4	1.20	3.75
	Mean	116	2.79±0.41	1.20	3.75
OL (mm)	**2016**	116	1.5±0.25	0.97	2.00
	2017	102	1.5±0.2	0.69	2.04
	Mean	116	1.5±0.2	0.69	2.04
PS	**2016**	116	1.07±0.1	0.83	1.36
	2017	102	1.2±0.1	0.93	1.53
	Mean	116	1.1±0.1	0.83	1.53
OS	**2016**	116	0.54±0.05	0.41	0.64
	2017	102	0.6±0.1	0.43	0.67
	Mean	116	0.57±0.08	0.41	0.67
F	**2016**	124	**99.1±1.5a**	98	104
	2017	102	**85.6±0.5b**	85	87
	Mean	114	92.6±7.5	85	104
SN	**2012**	102	**1.8±0.4a**	1.00	2.9
	2016	114	**2.2±0.4b**	1.10	3.30
	Mean	114	1.9±0.5	1.00	3.30
SW (mg)	**2012**	102	31.7±4.3	21.80	42.30
	2016	114	34.3±5.2	20.2	45.50
	Mean	114	32.3±6.7	20.2	45.50

Continue

	G × T population				
	Year	**N**	**Mean±SD**	**Min**	**Max**
SW (mg)	**2015**	104	56.05±14.88	26.66	73.67
	2016	99	55.85±15.97	28.18	82.42
	Mean	99	55.95±0.77	26.66	82.4
SW (mg)	**2015**	104	56.05±14.88	26.66	73.67
	2016	99	55.85±15.97	28.18	82.42
	Mean	99	55.95±0.77	26.66	82.4

BL berry length, BD berry diameter, BS berry shape, BW berry weight, PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, F Flowering date, SN seed number, SW seed weight

Table 3.3.2. Phenotypic correlations (Spearman) between years in G × T and T × G progenies

	Berry/seed	**BL**	**BD**	**BS**	**BW**	**SN**	**SW**
G × T progeny	**2014-2015**	0.5 **	0.5 **	0.5 **	0.2 **		
	2014-2016	0.6 **	0.6 **	0.6 **	ns	-	
	2014-2016V	ns	ns	0.4 **	ns		
	2015-2016	0.7 **	0.7 **	0.8 **	0.8 **	0.3 **	ns
	2015-2016V	0.3 **	0.3 **	0.7 **	0.7 **	-	
	2016-2016V	ns	ns	0.7 **	0.7 **		
	Flower	**PL**	**FD**	**OL**	**PS**	**OS**	**F**
	2015-2016V	0.4 **	0.5 **	0.3 **	0.4 **	ns	ns
	Berry/seed	**BL**	**BD**	**BS**	**BW**	**SN**	**SW**
T × G progeny	**2012-2016**	0.5 **	0.6 **	0.5 **	0.6 **	ns	0.3**
	Flower	**PL**	**FD**	**OL**	**PS**	**OS**	**F**
	2016-2017	0.8 **	0.8 **	0.8 **	0.7 **	0.5 **	ns

BL berry length, BD berry diameter, BS berry shape, BW berry weight, PL pistil length, FD flower diameter, LO ovary length, OS ovary shape, PS pistil shape, F Flowering date, SN seed number, SW seed weight. Correlations significant at $p < 0.01$ (**); and not significant (ns). Missing data (-). V Varea plot.

Estimation of correlations between traits is a key factor in breeding programs especially if traits present negative correlations, low heritability or are difficult to quantify (Viana et al. 2011). The correlation matrix between traits according to sex shows that he highest correlation indexes were obtained between traits of the same category in both genetic backgrounds (Table 3.3.3.). Thus, berry length, diameter and weight were highly correlated, and the same happened with flower diameter, pistil length and ovary length ($r = 0.9$, $p < 0.01$). In both progenies, berry weight, seed weight and seed number resulted positive and moderate correlated ($r = 0.2 - 0.5$, $p < 0.01$) as was previously found (Walker et al. 2005, Constantini et al. 2008).

Seed traits were also found significantly correlated with flower morphology traits as flower diameter ($r = 0.2$, $p < 0.01$) in both progenies. Besides, berry length is significantly correlated with berry shape ($r = 0.4$, $p < 0.01$) in both genetic backgrounds, being berry length, the shape-driving factor as previously found for Prieto Picudo cv. by Pereira (2014). A positive correlation between pistil shape and berry shape was found in both progenies ($r = 0.3 - 0.4$, $p < 0.05$) what suggests that berry and flower parameters are interconnected. The correlation between flower and berry morphology has been attributed to processes of cell division around flowering

time and cell expansion after anthesis (Gray & Coombe 2009, Fernandez et al. 2013, Nicolas et al. 2013) as it happens in other fruits as watermelon, tomato and pepper where final fruit shape is related to pistil shape (Liu et al. 2002, Périn et al. 2002, Chaim et al. 2003, Borovsky & Paran 2011, Tsaballa et al. 2011, Chusreeaeom et al. 2014). Besides, flowering date resulted moderately correlated with flower morphology traits as pistil and ovary shape, being this relationship positive in G × T progeny ($r = 0.5$, $p < 0.01$) and negative in T × G ($r = -0.4$, $p < 0.05$), suggesting that these traits could be genetically controlled by the same gene family (Kamal et al. 2018). Inconsistent results in both populations may be attributed to differences in flower morphology among progenies, like in seed traits, (negatively associated with berry size in T x G progeny and positive correlated in G x T population).

Table 3.3.3. Correlation matrix for the different traits studied in both progenies.

		T x G progeny											
		BL	**BD**	**BS**	**BW**	**PL**	**FD**	**OL**	**PS**	**OS**	**F**	**SN**	**SW**
G x T progeny	**BL**	**1**	0.9**	0.4**	0.9**	0.5**	0.3**	0.5**	0.3**	0.4**	-0.2*		
	BD	0.9**	**1**		0.9**	0.5**	0.4**	0.5**	0.2**	0.3**	-0.2*		
	BS	0.4**		**1**	0.2*				0.3**	0.2**		-0.2 **	
	BW	0.9**	0.9**	0.1*	**1**	0.6**	0.5**	0.6**	0.2**	0.3**	-0.3**	0.3**	0.3**
	PL	0.4**	0.4*	0.4**		**1**	0.8**	0.9**	0.4**	0.5**	-0.4**	0.2**	0.2*
	FD	0.2**	0.2**	-0.2**		0.8**	**1**	0.8**	- 0.3**	- 0.2*		-0.2**	0.2*
	OL	0.4**	0.4**	0.2**	0.4**	0.9**	0.9**	**1**		0.3**	-0.2**		
	PS	0.2*		0.4**		0.4*	-0.3 **	0.2**	**1**	0.7**	-0.5**	0.3**	0.3**
	OS	0.2*	0.2*	0.3**	0.2*	0.3**	-0.4**	0.4*	0.8**	**1**	-0.4 *		
	F	0.2*		0.4**		0.5**	0.5**	0.5**	0.4**	0.4*	**1**	-0.4**	
	SN		0.3**	- 0.3**	0.2**	- 0.2**	- 0.2*					**1**	-0.5**
	SW	0.5**	0.5**		0.5**							0.7**	**1**

PL, pistil length, FD, flower diameter, LO, ovary length, OS, ovary shape, PS, pistil shape, BL, berry length, BD, berry diameter, BS, berry shape, BW, berry weight, SN, seed number, SW, seed weight, F, Flowering date. Correlations significant at $p < 0.01$ (**); and $p < 0.05$(*).

Mapping of the *Sex* and *Colour* loci

Sex locus was mapped on LG2, at genetic position 21 - 25 cM approximately on Grenache, Tempranillo and G × T Consensus genetic linkage maps (Table 3.3.4). Marker VVIB23 was placed at 0.3 cM, 1.4 cM and 0.4 cM from the *Sex* locus peak in Grenache, Tempranillo and Consensus maps, respectively (Figure 3.3.4). Peak LOD scores of 42.2 and 31.9 (GW = 3.4), were identified on LG2 on Consensus map explaining up to 80 % of the phenotypic variance for *Sex* locus in G × T and T × G populations, respectively. In T × G progeny, VVIB23 marker was not polymorphic for Tempranillo and Graciano (Chapter 3.1), and therefore could not be mapped. Regarding colour, a major QTL with a peak LOD score of 63.3 (GW = 3.4), was identified on LG2, explaining up to 90 % of the phenotypic variance for berry skin colour in G × T progeny. The 1 - LOD interval expanded the genetic map at 30 - 62 cM as shown on Table 3.3.4.

Table 3.3.4. Summary of QTL detected for *Sex* and *Colour* locus in G × T and T × G progenies

	Traits	LG	Map	Pos (cM)	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var. Exp
								CW	GW	
G × T progeny	Sex (ff/H-)	2	GAR	24.994	SEX	24.58	12.9-29.6	2.0	3.2	47.6
			TE	37.660	SEX	8.13	12.5-44.8	3.0	3.4	25.0
			CON	24.257	SEX	50.47	10.7-40.6	3.5	5	83.3
	Sex (ff/Hf/HH)	2	GAR	24.994	SEX	57.63	10.1-41.3	1.9	3.2	87.2
			TE	37.660	SEX	15.92	9.2-48	1.9	3.4	42.5
			CON	24.257	SEX	19.54	15.4-45.8	3.2	4.8	100
	Colour	2	GAR	51.765	2_14902974	13.01	30.5-61.7	1.9	3.2	36.9
			TE	52 .100	2_14565191	10.65	30.5-57	1.8	3.4	31.4
			CON	53.607	2_12678951	63.27	30.4-60	3.4	4.7	89.4
T × G progeny	Sex (ff/H-)	2	CON	23.552	chr2_5236271_G_T	30.53	0-32.6	3.1	4.8	72.1
			TE	25.868	chr2_4137690_A_G	8.13	0-41.6	1.7	3.0	22.8
			GRA	18.356	chr2_4166541_A_G	12.39	0-41.4	1.8	3.1	32.5
	Sex (ff/Hf/HH)	2	CON	23.552	chr2_5236271_G_T	31.87	0-32.6	3.0	4.7	77.4
			TE	25.868	chr2_4137690_A_G	11.13	0-41.6	1.8	3.0	32.4
			GRA	26.576	chr2_5236271_G_T	15.80	0-41.4	1.8	3.2	42.6

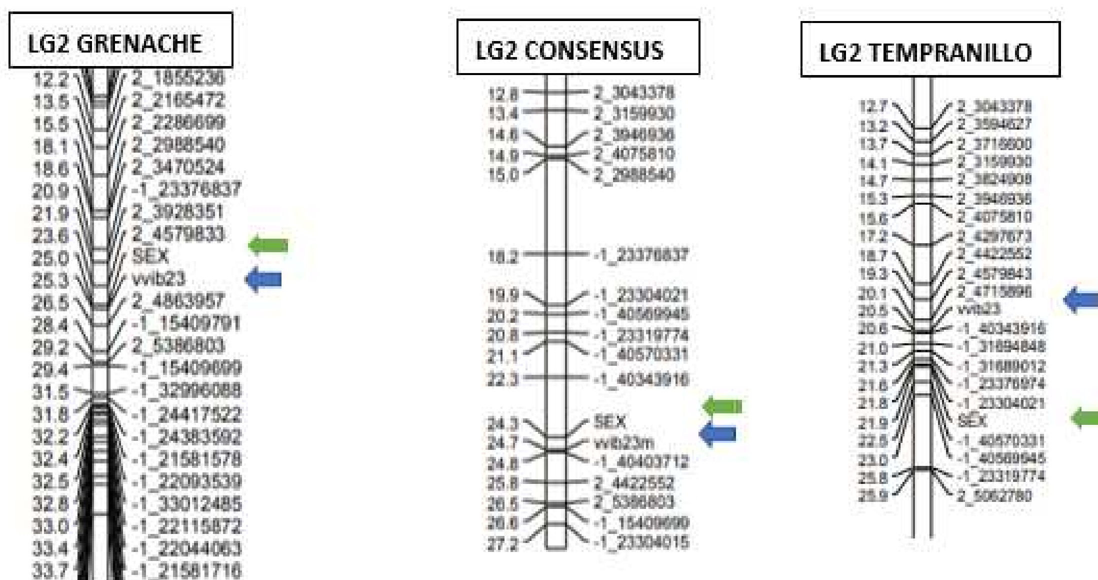
LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome-wide (CW) and genome-wide (GW) LOD threshold ($p < 0.05$), % Var Expl. Proportion of the total phenotypic variance explained by the QTL, f: female, H: hermaphrodite.

Colour did not segregate in T × G population, because Graciano is homozygous for the red allele. However, the CAP marker 20D18CB9 (Cleaved Amplified Polymorphic Sequence) (Walker et al. 2007) segregated 1:1 in this progeny (Song et al. 2014) and was mapped closely linked to a major QTL (Variance explained up to 67.5 %) on LG2 in Consensus (position 37.5 cM) and Tempranillo maps (position 53.4 cM). It corresponds to the locus identified on chromosome 2 responsible for berry colour, associated with VvMybA1 and VvMybA2 genes involved in the regulation of anthocyanin biosynthesis (Fournier-Level et al. 2010).

QTL analysis

All QTL were detected at p = 0.05 Genome Wide (GW) and Chromosome Wide (CW) applying interval mapping method for berry, flower and seed phenology data in both genetic backgrounds. In a preliminary QTL analysis, the presence of a QTL on LG2 for traits such as seed number, flowering date, ovary and pistil shape, supported the hypothesis formulated in Chapter 3.1., that the *Sex* locus had a broader influence than just flower sex determination. Two alternative approaches were considered to assess sex influence in the traits studied: either setting VVIB23 and SEX marker as cofactors or do a separate analysis with data for only hermaphrodite plants. Due to the limited number of female plants, 28 and 31 in G × T and T × G progenies, respectively, it was not possible an independent study of QTL in separate female subpopulations. It was noticed that setting SEX and VVIB23 markers as cofactors allowed the detection of more putative QTL, whilst the strategy of removing female plants reported additional significant QTL, which explained the phenotypic variance previously explained by QTL on LG2. Therefore, performing independent analysis with the whole population and with only hermaphrodite plants seemed the best option in order to assess the influence of *Sex* locus in both genetic backgrounds.

Figure 3.3.4. Position of VVIB23 and SEX markers in LG2 maps for G × T population.



QTL associated to berry traits

Berry traits such as berry length (BL), berry diameter (BD) and berry shape (BS) were analyzed in three consecutive years (2014, 2015 and 2016) in one environment (UR), and in 2016 also at the Varea plot (V), for G × T progeny (Fig. 3.3.2). In addition, berry weight (BW) data for 2017 (UR) were included in the analysis. For T × G population all berry traits were evaluated in two different years, 2012 and 2016, in one environment (V) (Table 3.3.2).

QTL analysis conducted in both progenies with and without female genotypes, revealed that flower sex influenced QTL detection, with a higher number of stable QTL in hermaphrodite subpopulation in T × G progeny, whilst in G × T progeny more stable QTL were detected in the whole population. This difference could be related to the different effective population size. Only significative (LOD > GW) or stable/reproducible (detected in at least two years) QTL will be shown represented in graphs, whilst Supplementary material 3.3.1 a to 3.3.4 b contains all the QTL (significant and putative).

Berry QTL analysis rendered differences between both genetic backgrounds, with a larger number of reproducible QTL identified in T × G compared to G × T population as expected since higher polymorphism was present in T × G population for berry traits. Overall, two stable QTL colocalized in both populations for berry traits: QTL for berry shape in LG1 and LG9 and a QTL for berry weight in LG3, that were reproducible across 2 years.

Berry traits analyses in G × T population

No significant differences between the two analysis (with and without female plants) were detected for berry traits. In general, only few QTL with low reproducibility were identified in this population. The most significant QTL are shown in Figure 3.3.5, and all can be checked in Supplementary material 3.3.1 a - 3.3.1 b.

Significant QTL (LOD > GW) were identified on LG17 (QTL B17_2) that explain up to 22.5 % of the phenotypic variance for berry length, berry diameter and berry weight. This QTL was found for Grenache and Consensus maps in 2016 (Varea plot), being also detected for berry length for Grenache in 2015. One significant stable QTL for berry weight was detected in LG18 (QTL B18) in Grenache (2015 and 2017) and Consensus (2015) maps, explaining up to 17 % of the variance (Supplementary material 3.3.1 a.). This QTL was also found for berry length explaining 12 % of the variance in Grenache (across two years) and Consensus maps. For berry shape, significant effects (LOD > GW) were identified in LG6 and LG10, explaining up to 20 and 14 % of the phenotypic variance in Consensus and Grenache map, respectively. Two different QTL were detected in LG10 in Consensus and Grenache maps for 2015 and 2016 vintages.

Other QTL reproducible in at least two vintages with LOD > CW and explaining more than 10 % of the phenotypic variance are listed in Supplementary material 3.3.1 a. QTL: B17_1 was found in Grenache and Consensus maps explaining up to 18 % and 22 % of the variance respectively for BL (LOD = 3.2) and BW (LOD = 3.6). A QTL on LG15, (QTL BD15) was found associated to BD in two years in Tempranillo and Consensus maps, explaining up to 23 % of the variance. For berry shape, QTL BS15 explained 10 % of the variance in Grenache and Consensus maps. Minor QTL reproducible across two years were detected in LG8 for BL and in LG1 and LG9 for BS (Supplementary material 3.3.1 a.)

When female plants were removed from the analysis, the same stable QTL B17_2 was found associated to BL, BD and BW traits. QTL B18 that was associated to BL and BW, was also detected for BD in this new analysis. QTL on LG10 was also found for BS and QTL on LG1 was detected as significant in this new analysis (Supplementary material material 3.3.2 a – 3.3.2 b).

The major difference between both analyses was that a QTL on LG3 (B3) was found associated to BD and BW in Tempranillo (across 3 years) and Consensus maps, explaining up to 20 % and 25 % of the phenotypic variance, respectively.

Berry traits analysis in T × G population

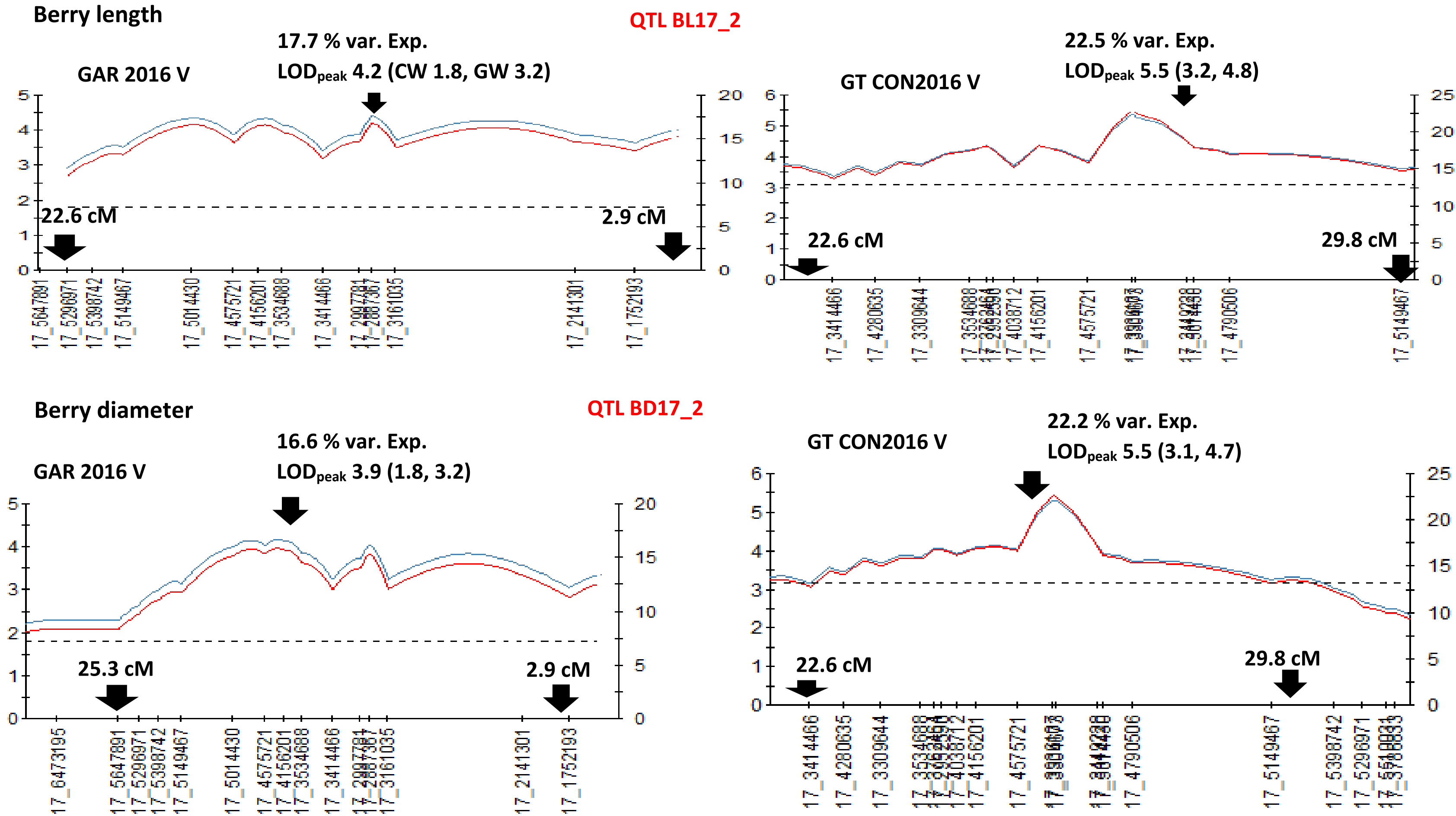
Unlike G × T progeny, most QTL detected for T × G progeny were significant (LOD > GW) as expected due to the larger polymorphism present in this population Figure 3.3.6, Supplementary material 3.3.3 a – 3.3.3 b).

Highly significant QTL for berry length, berry diameter and berry shape were identified on LG3 and LG5 in Consensus and Graciano maps. Although for berry length the effect was only detected in one year, QTL B3 and B5 for BD and BW were detected in both years of the study in both maps explaining up to 25 % and 26 % of the total variance respectively Figure 3.3.6.

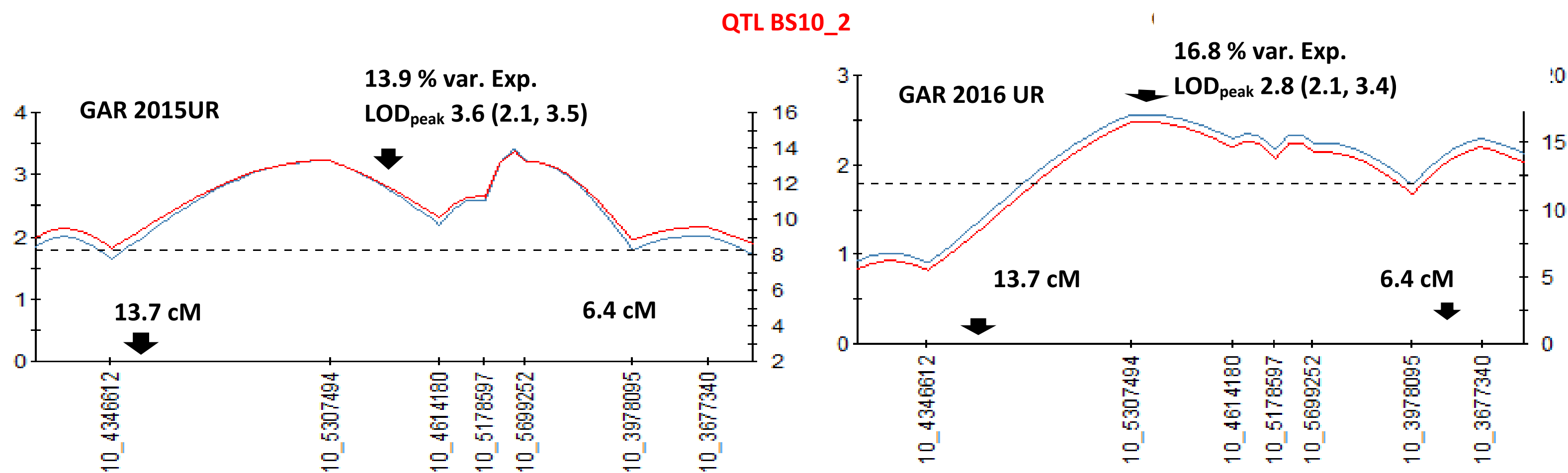
Besides, a significant QTL BL1 was detected for berry length in LG1 in Consensus and Graciano maps, explaining 18 % of the phenotypic variance. On LG1, two additional putative QTL for berry shape were identified, explaining 15 % of the variance each, but they do not colocalize with BL1.

When the hermaphrodite subpopulation was analyzed QTL on LG3 and LG5 were also detected for BL, BD and BS gathering up to 30 % and 26 % of the phenotypic variance in Consensus and Graciano maps, respectively. Besides, for berry shape, a significant effect in LG9 was detected in Tempranillo (across two years) and Consensus maps explaining 10 % and 15 % of the variance, respectively. QTL on LG1 associated to BS resulted stable in Graciano map and Consensus map, explaining 11 % and 18 % of the variance (Supplementary material 3.3.4a- 3.3.4 b).

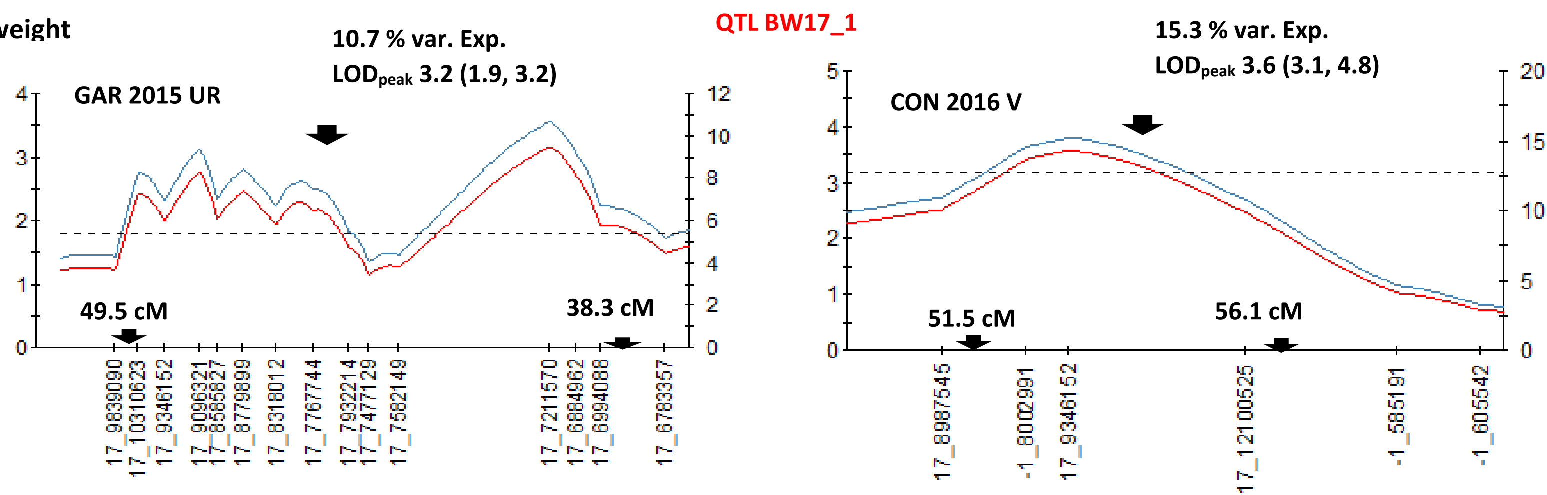
Figure 3.3.5. Summary of significant QTL for berry traits in G × T progeny. LOD (left axis) and % of explained variance (right axis). In parenthesis LOD (CW, GW). Horizontal dash line indicates CW.



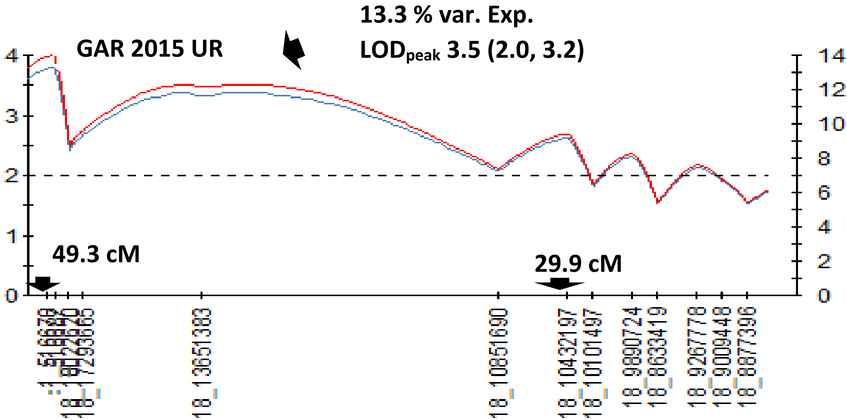
Berry shape



Berry weight



QTL BW18



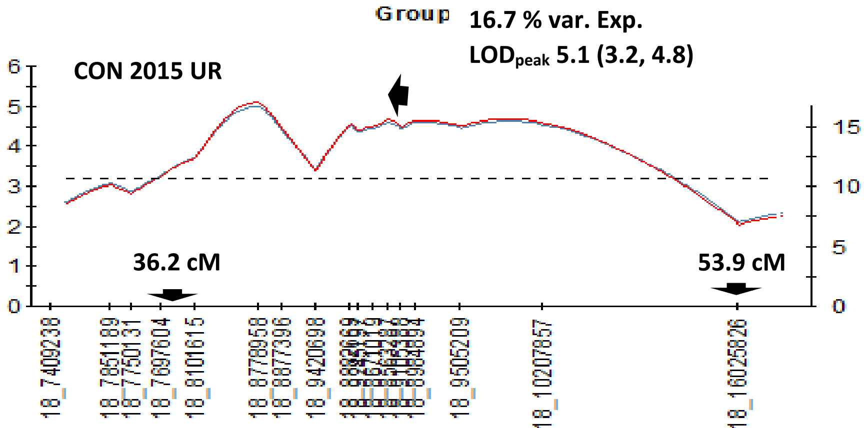
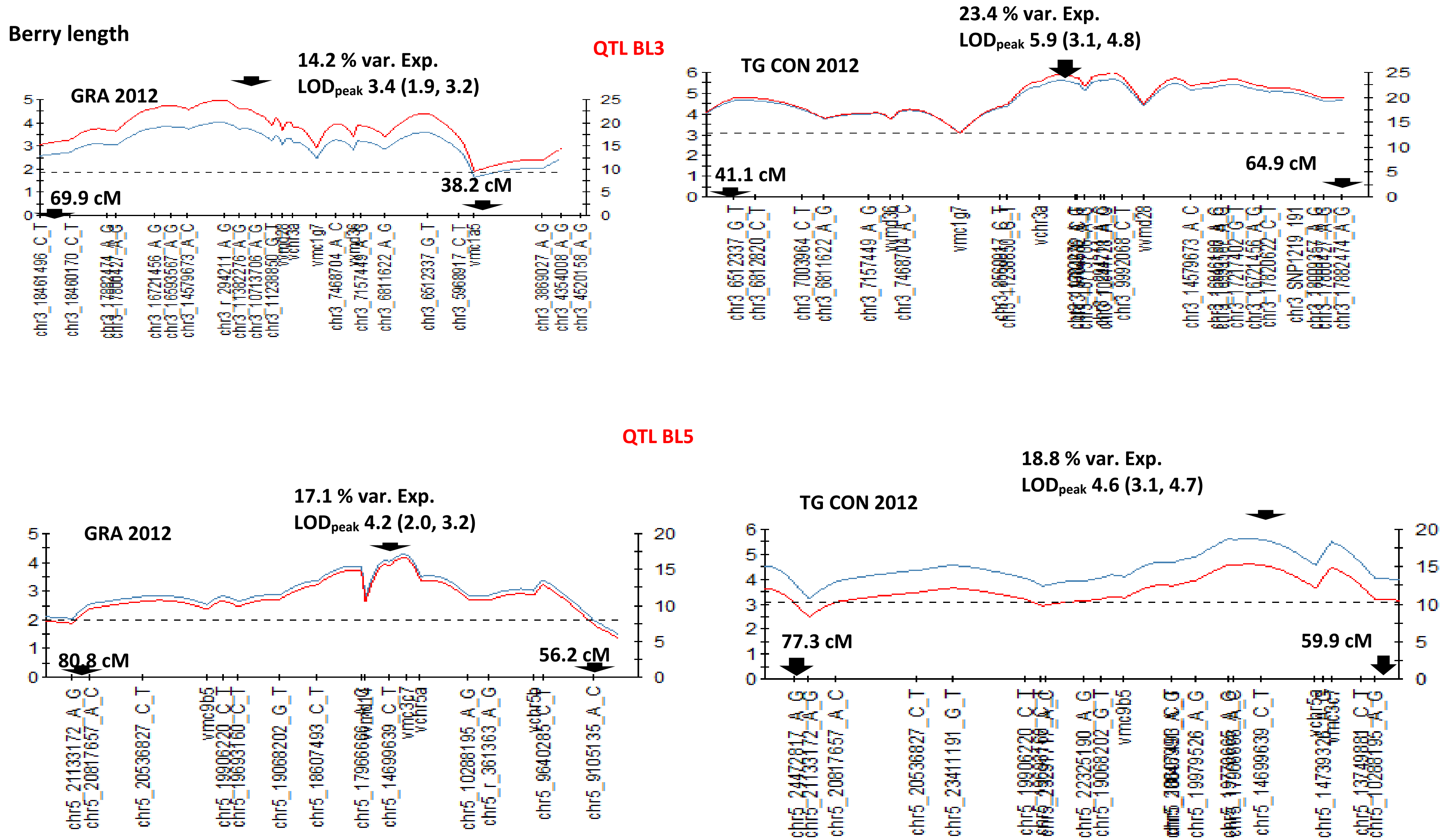
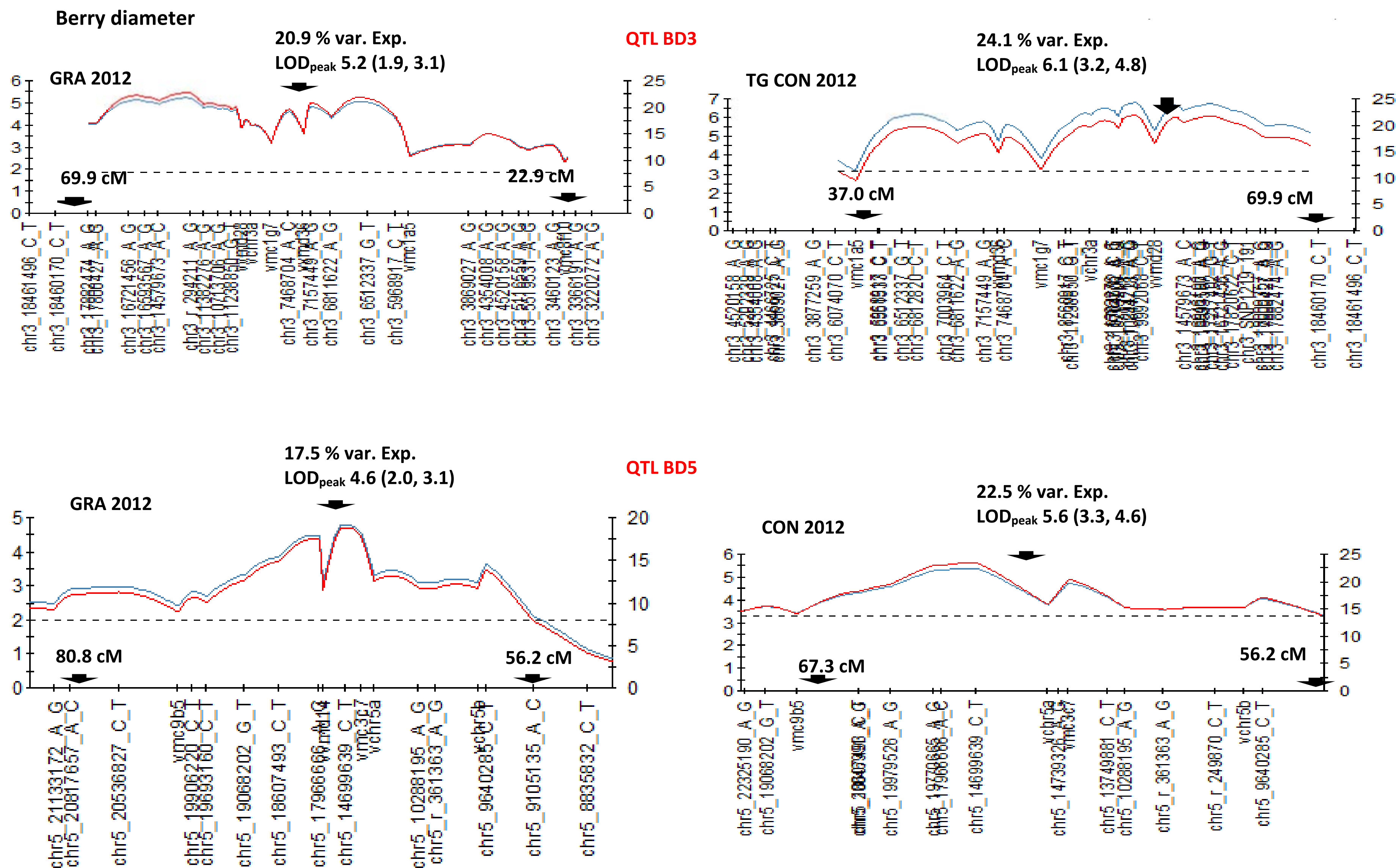


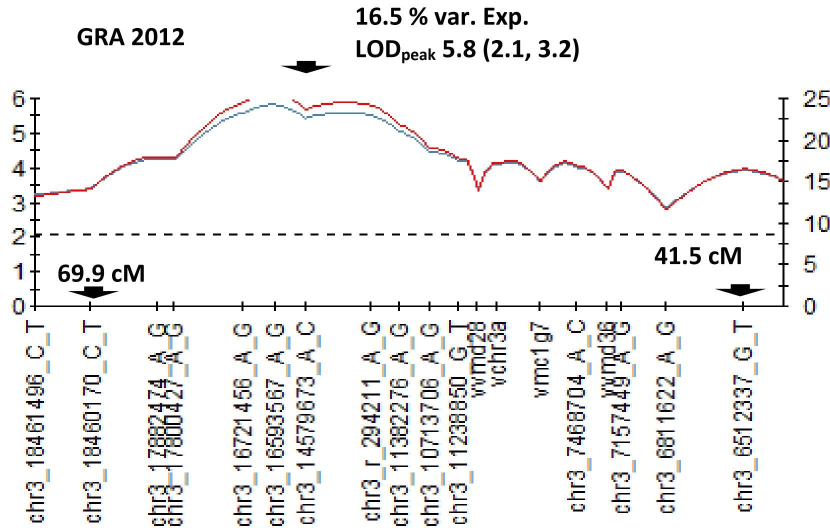
Figure 3.3.6. Summary of significant QTL for berry traits in T × G progeny. LOD (left axis) and % of explained variance (right axis). In parenthesis LOD (CW, GW). Horizontal dash line indicates CW.

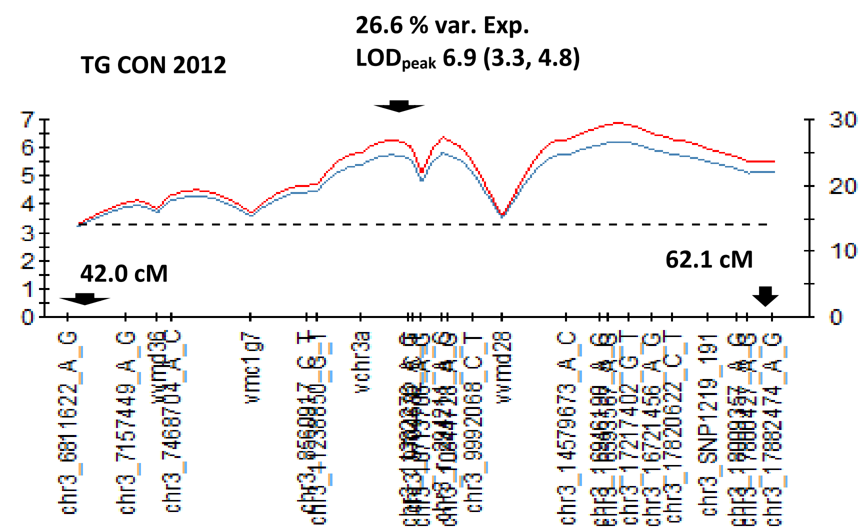



Berry diameter
QTL BD3
GRA 2012
20.9 % var. Exp.
LODpeak 5.2 (1.9, 3.1)
69.9 cM
22.9 cM
TG CON 2012
24.1 % var. Exp.
LODpeak 6.1 (3.2, 4.8)
37.0 cM
69.9 cM
QTL BD5
GRA 2012
17.5 % var. Exp.
LODpeak 4.6 (2.0, 3.1)
80.8 cM
56.2 cM
CON 2012
22.5 % var. Exp.
LODpeak 5.6 (3.3, 4.6)
67.3 cM
56.2 cM

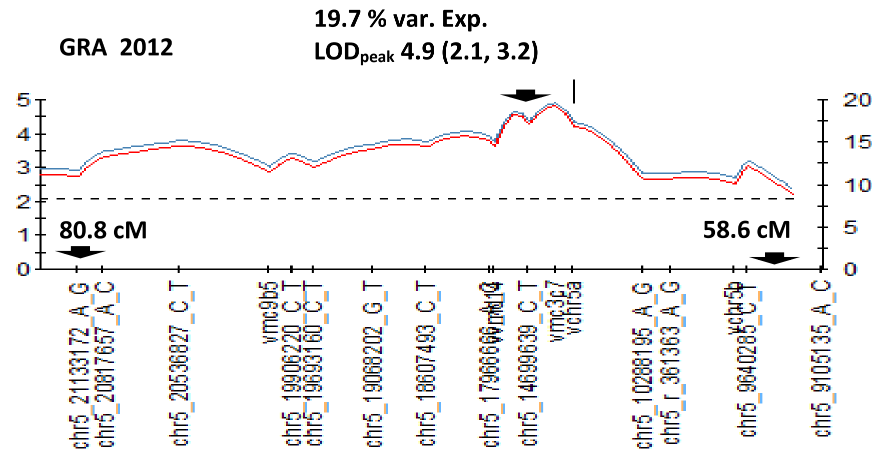
Berry weight

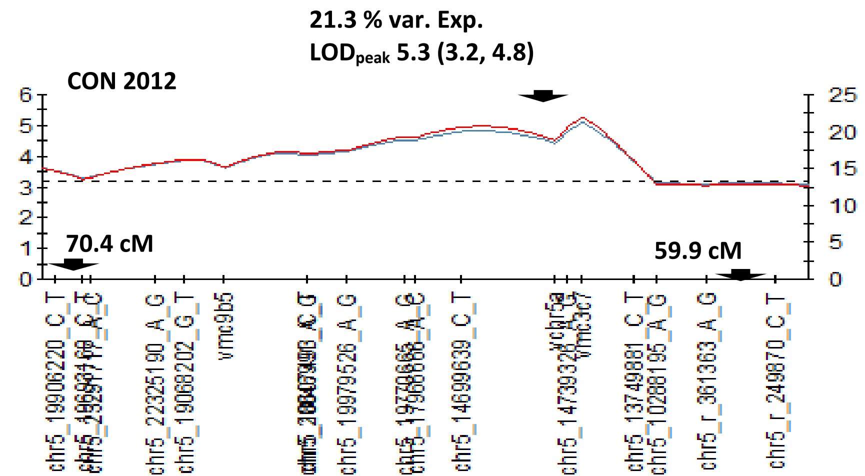
QTL BW3





QTL BW5





QTL associated to flower traits

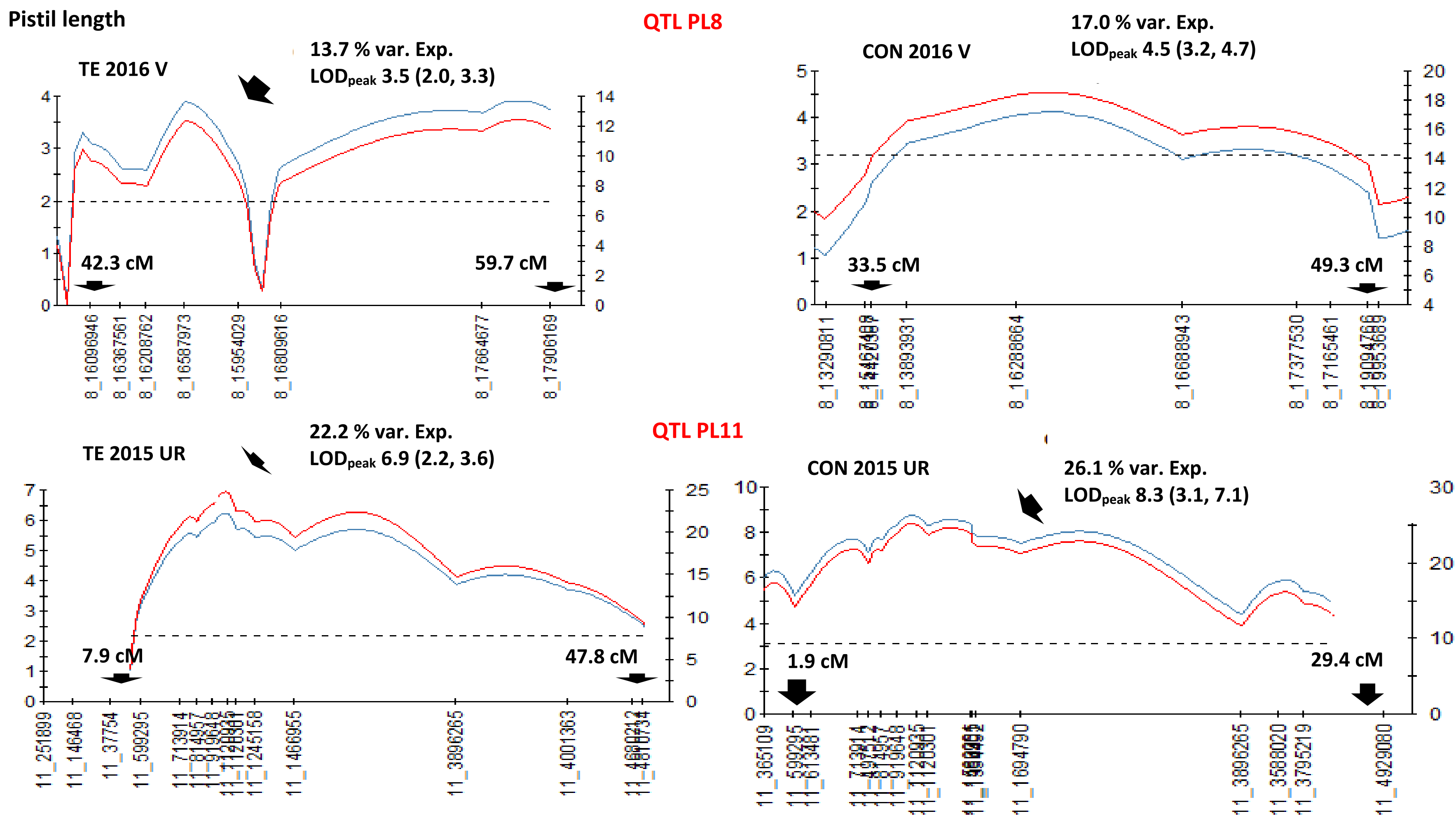
Likewise berry traits, a higher number of stable and/or significant QTL were detected using the whole progeny in both populations compared with the analysis only with hermaphrodite plants, suggesting that in both berry and flower traits, female plants added more variability enhancing the detection of QTL. The larger population size could also play a role in that result. Stable and significative QTL were identified in both genetic backgrounds for flower morphology traits on LG11, as well as on LG8 for G × T progeny and on LG5 and LG14 for T × G progeny.

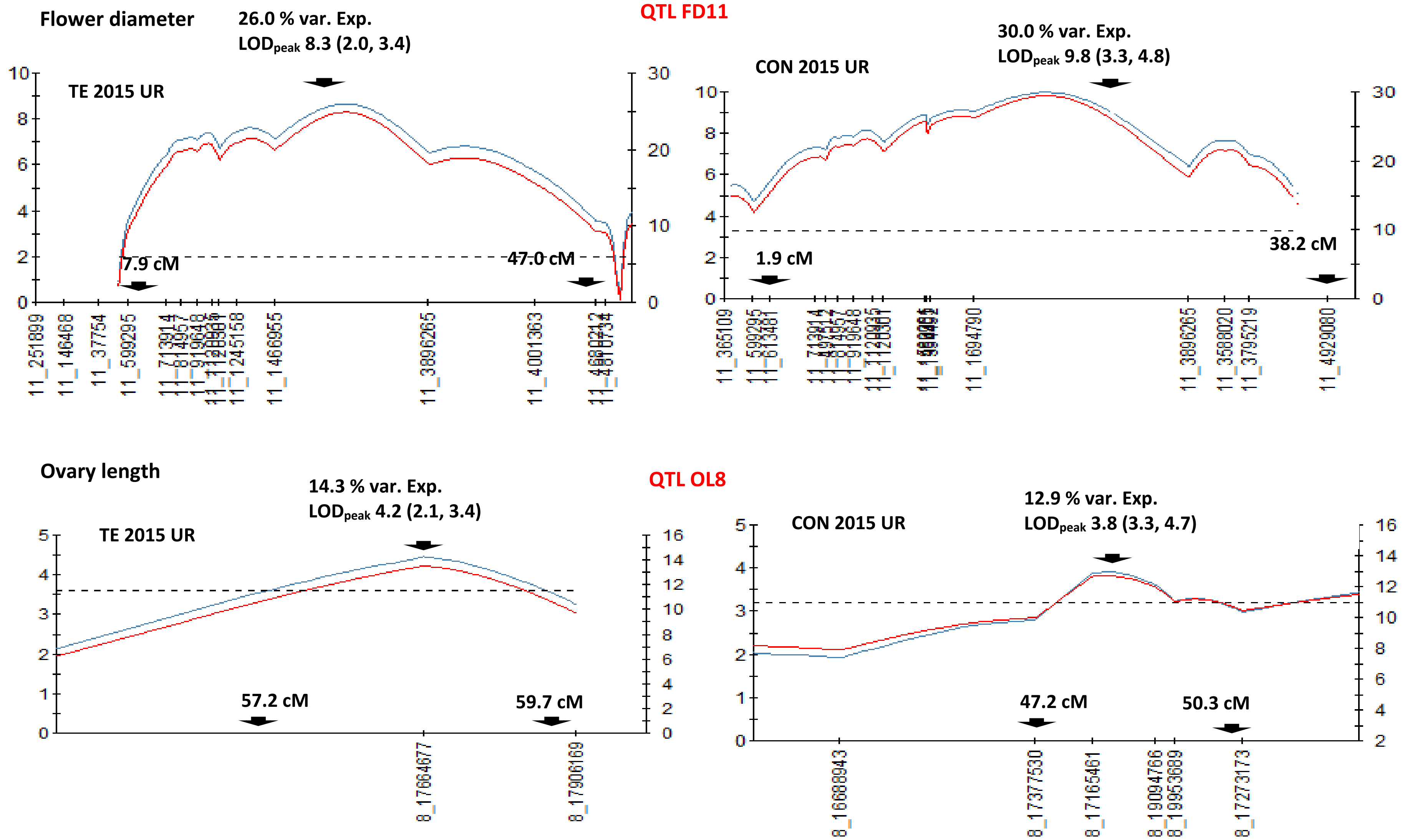
Flower traits analysis in G × T population

QTL analysis conducted on the whole progeny discovered stable and or significant QTL on LG8 (PL8) and LG11(PL11) for pistil length (PL), 26 % of variance explained, pistil shape (PS), 17 % variance, and ovary shape (OS), explaining up to 18 % of the phenotypic variance in the two years of study. For flower diameter (FD), two major QTL: FD11 (LOD = 9) and FD13 (LOD = 5.4) explained up to 50 % of the total variance. Besides, a significant QTL for OS in LG2 was located for 2015 on all maps, suggesting an influence of flower sex in this trait (Figure 3.3.7). Putative QTL were also detected in LG10 for pistil length, LG14 for flower diameter, LG13 and LG17 for ovary length and in LG7 for pistil shape and ovary shape (Supplementary material Tables 3.3.1 b).

Using only the hermaphrodite subpopulation, QTL on LG11 although stable in two years explained only up to 36 % of the variance. A QTL on LG2 was not detected when female plants were excluded from the analysis. Putative QTL were found in LG10 and LG15 for PL; LG13 and LG17 for OL; and LG7and LG11 for PS (Supplementary material Tables 3.3.2 a and 3.3.2 b).

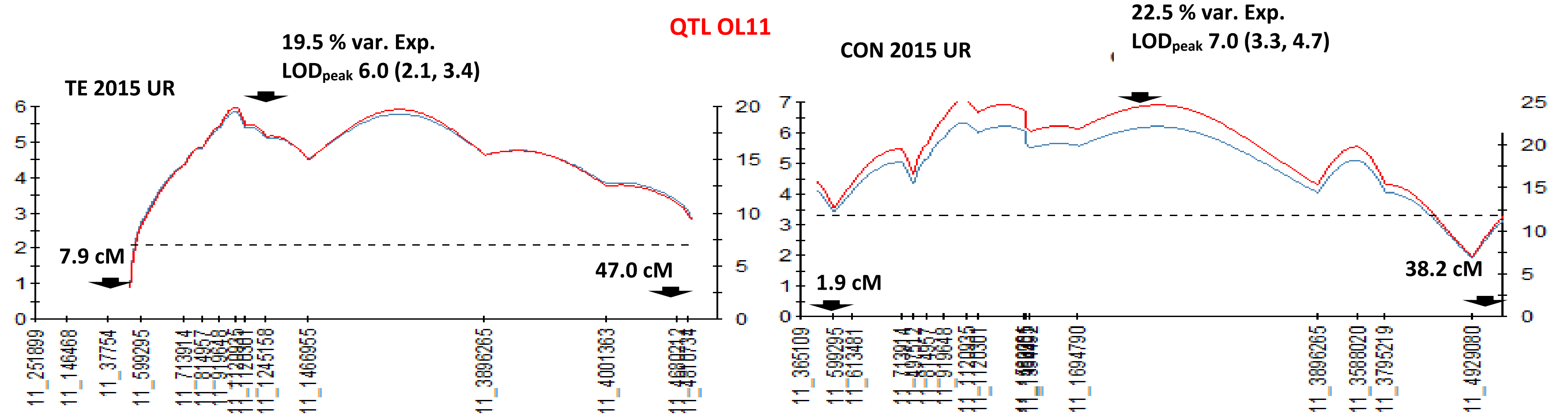
Figure 3.3.7. Summary of significant QTL for flower traits in G × T progeny. LOD (left axis) and % of explained variance (right axis). In parenthesis LOD (CW, GW). Horizontal dash line indicates CW.

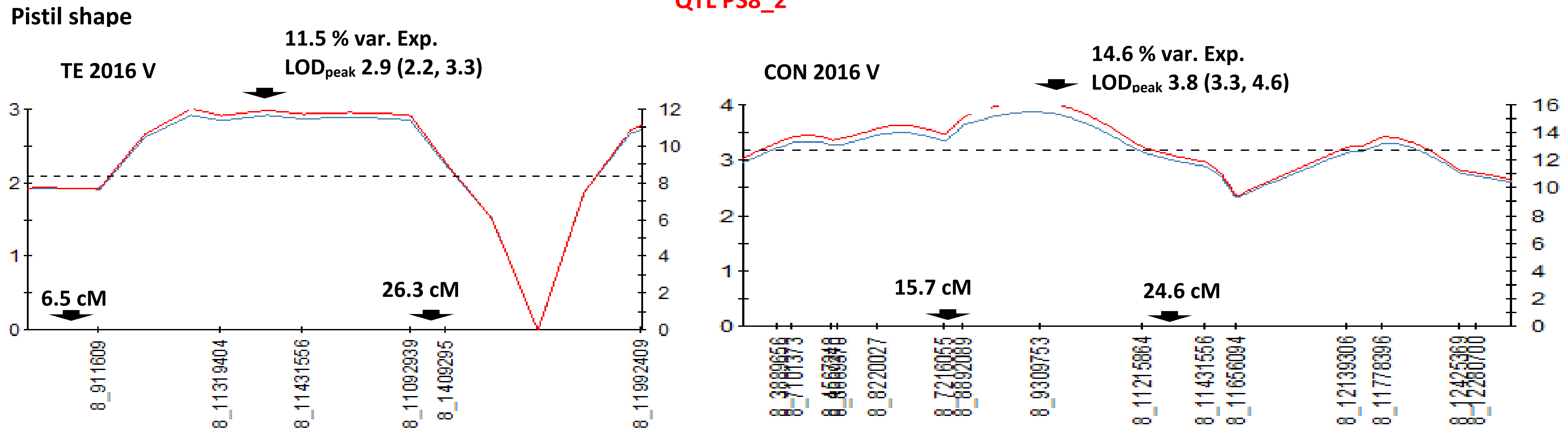


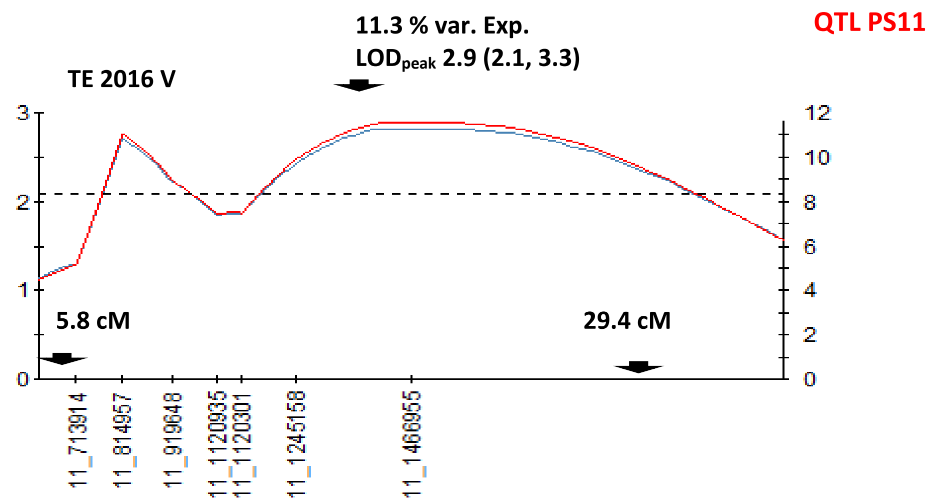


Flower diameter
QTL FD11
TE 2015 UR
26.0 % var. Exp.
LODpeak 8.3 (2.0, 3.4)
7.9 cM
47.0 cM
CON 2015 UR
30.0 % var. Exp.
LODpeak 9.8 (3.3, 4.8)
1.9 cM
38.2 cM
Ovary length
QTL OL8
TE 2015 UR
14.3 % var. Exp.
LODpeak 4.2 (2.1, 3.4)
57.2 cM
59.7 cM
8_17664677
8_17906169
CON 2015 UR
12.9 % var. Exp.
LODpeak 3.8 (3.3, 4.7)
47.2 cM
50.3 cM
8_16688943
8_17377530
8_17165461
8_19094766
8_19953689
8_17273173

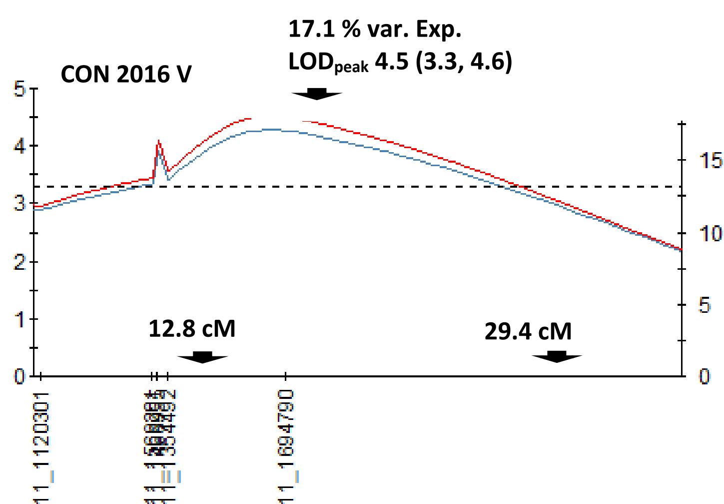
Ovary length



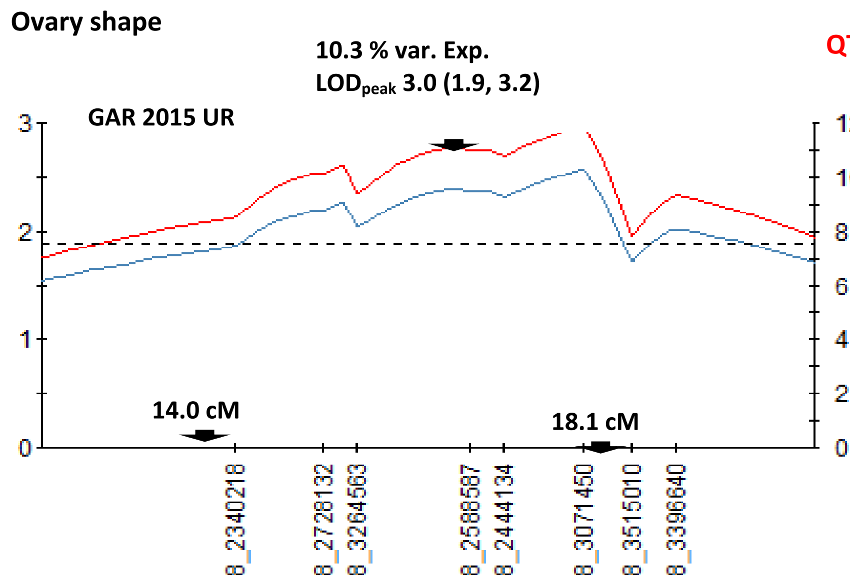




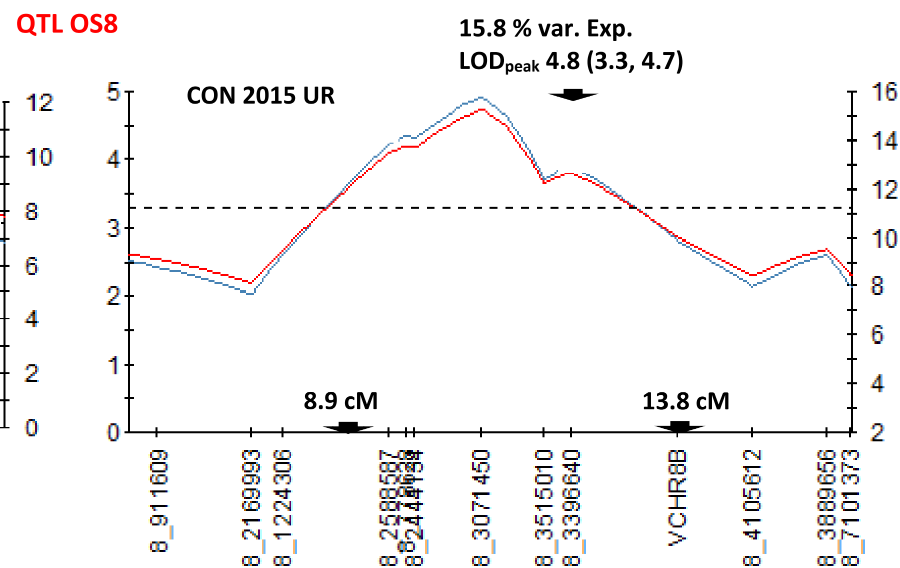
QTL PS11

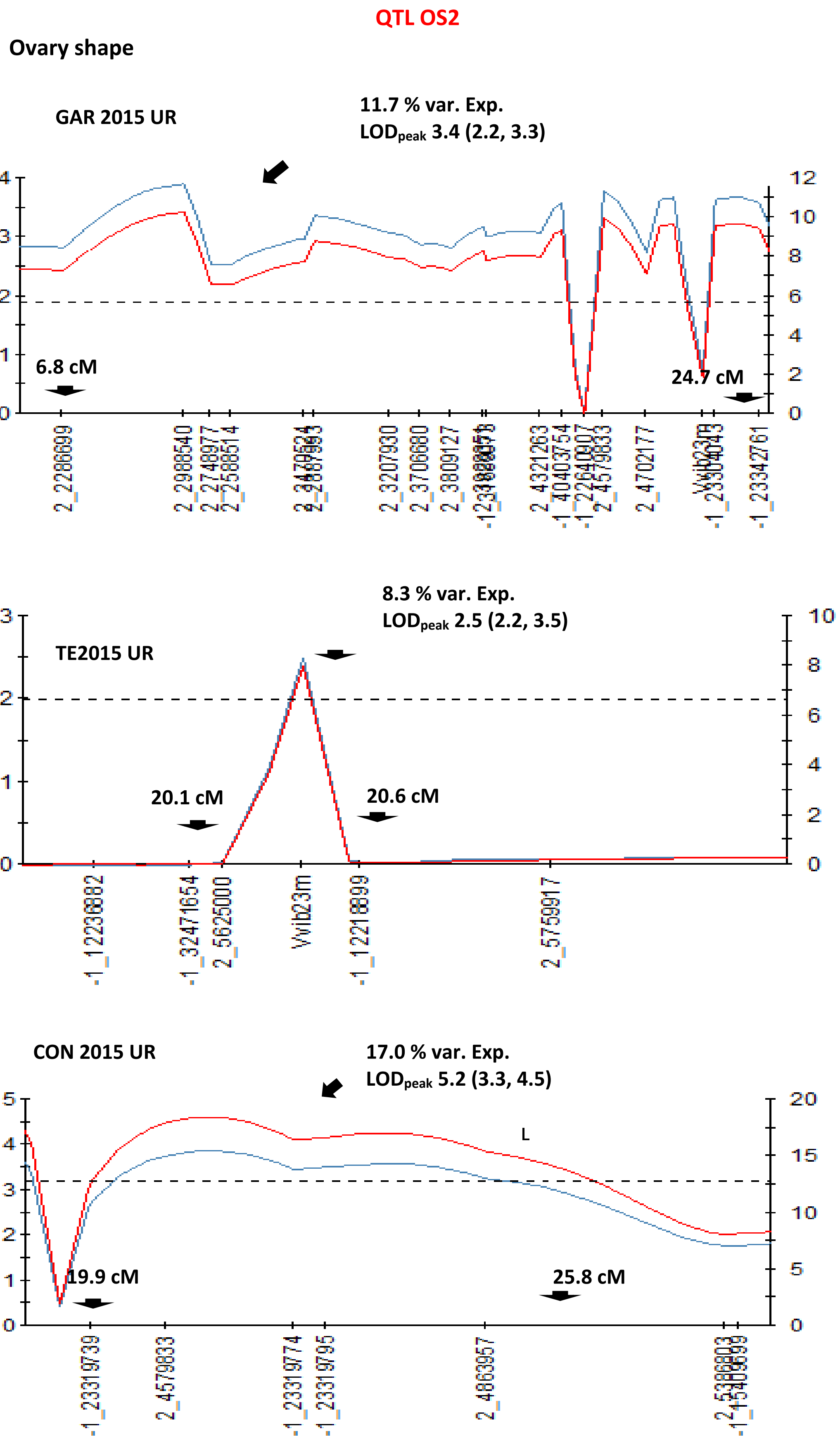


Ovary shape



QTL OS8




QTL OS2
Ovary shape
GAR 2015 UR
11.7 % var. Exp.
LODpeak 3.4 (2.2, 3.3)
6.8 cM
24.7 cM
8.3 % var. Exp.
LODpeak 2.5 (2.2, 3.5)
TE2015 UR
20.1 cM
20.6 cM
CON 2015 UR
17.0 % var. Exp.
LODpeak 5.2 (3.3, 4.5)
19.9 cM
25.8 cM

QTL for flower traits in T × G population

All the flower-related traits were analyzed for T × G population in two consecutive years, 2016 and 2017, in Varea plot. In this progeny, a QTL located close to VVS2 marker in LG11 was found as highly significant and stable for flower diameter, ovary length and pistil shape in Tempranillo and Consensus maps, explaining up to 26 % of the phenotypic variance (Figure 3.3.8, Supplementary material 3.3.3 a). Moreover, regions on LG14, close to VMC5B3 marker, and on LG5 resulted significant for pistil length, flower diameter and ovary length (up to 20 % and 14 % of variance explained), respectively. (Fig. 3.3.8)

Remarkably, a highly significant and stable QTL was located in LG2 for pistil shape (LOD = 10.69) and ovary shape (LOD = 7.39) traits, suggesting the influence of flower sex in the genetic determinism of these parameters, confirming results from phenotypic analysis in Chapter 3.1 (Table 3.1.3). Putative QTL were detected in LG18 for PL, FD and OL, in LG8 for OL and OS, in LG5 for OL and in LG7 for OS in Consensus and Graciano maps (Supplementary material 3.3.3 b).

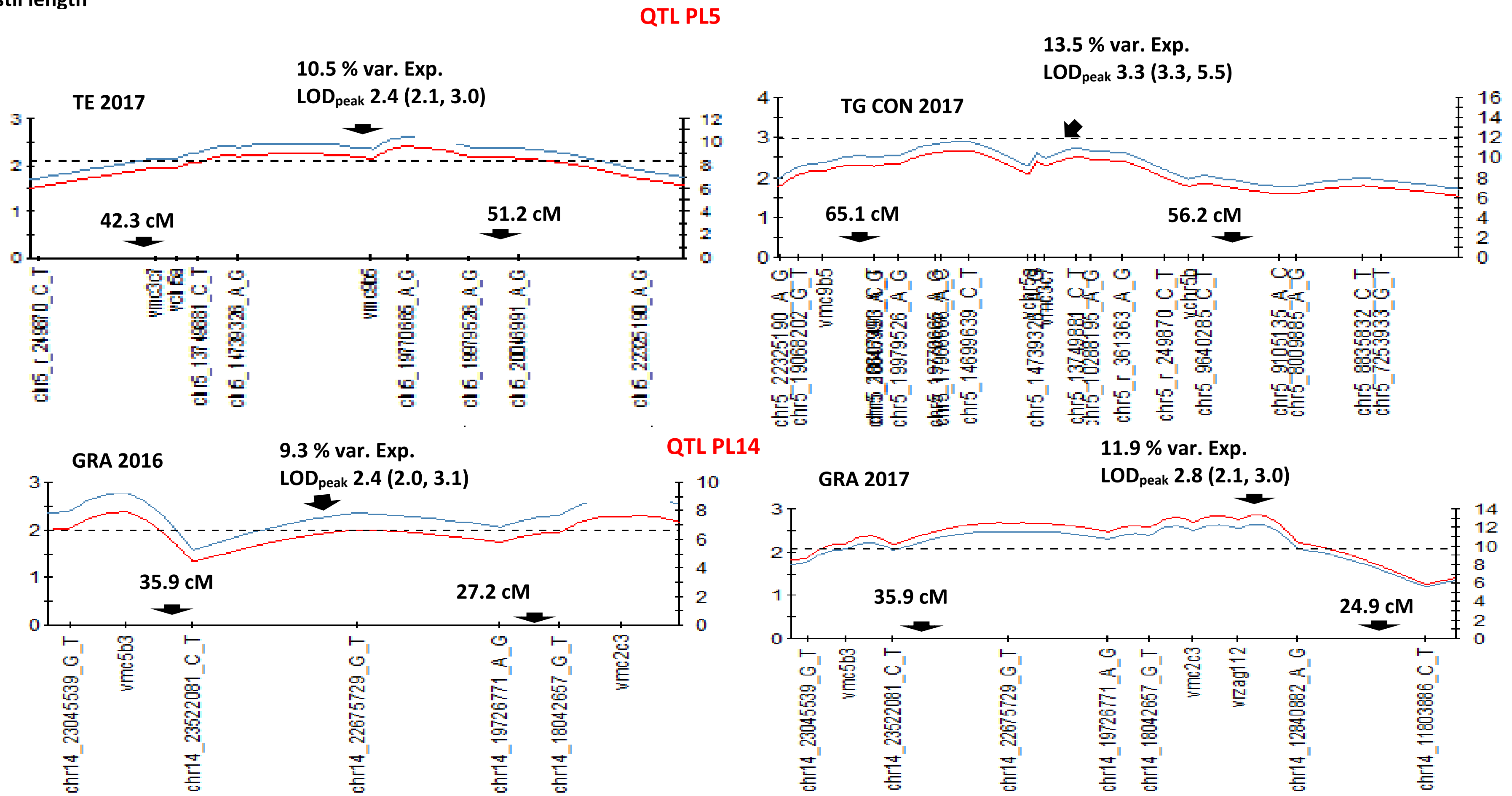
Likewise, T × G population revealed fewer stable QTL when female plants were removed from the analysis of flower traits (Supplementary material 3.3.4 a). In this progeny, in the case of berry traits, fewer stable QTL were found when female plants were considered. Apparently, female plants showed larger differences compared to hermaphrodite in flower traits than in berry traits, in both genetic backgrounds as reported in Chapter 3.1. Besides, the significant QTL on LG2 detected in the whole population analysis was not found in the hermaphrodite progeny, revealing a sex influence in pistil shape and ovary shape, as previously suggested. Putative QTL were detected in LG5 for flower diameter and ovary shape, and in LG15 for ovary length (Supplementary material 3.3.4 b).

QTL associated to flowering date

A delay in flowering date was observed for female genotypes compared with hermaphrodite plants, for both segregating populations (Table 3.3.3) as in Nunes-Ramos et al. (2017) who found differences in blossom date according to flower sex. Therefore, it was expected that *Sex* locus had an influence in the QTL detection for that trait, but it was only significantly associated with LG2 for T x G population in 2016.

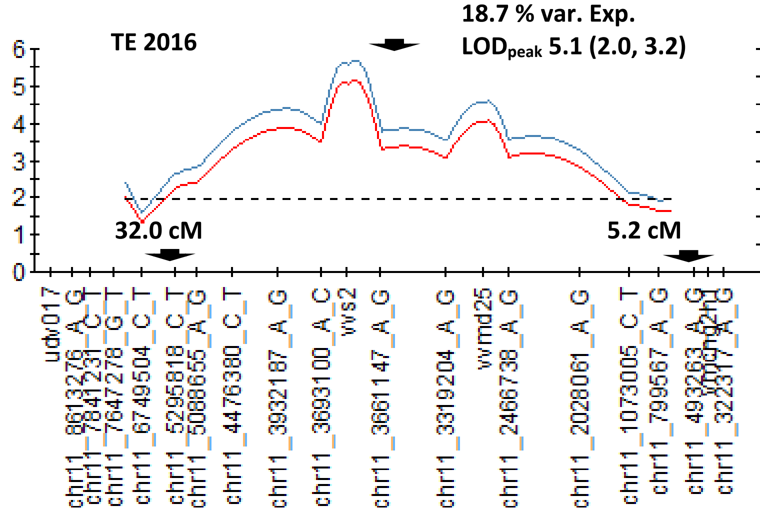
Considering the whole progeny, no common QTL were found between the two populations studied, and only putative QTL were detected in both progenies (Supplementary material 3.3.1 b, 3.3.3 b). Although QTL on LG2 was clearly linked to *Sex* locus, since VVMD34 was the closest marker associated to that QTL, neither large effects nor reproducibility of that QTL were observed in our experiment.

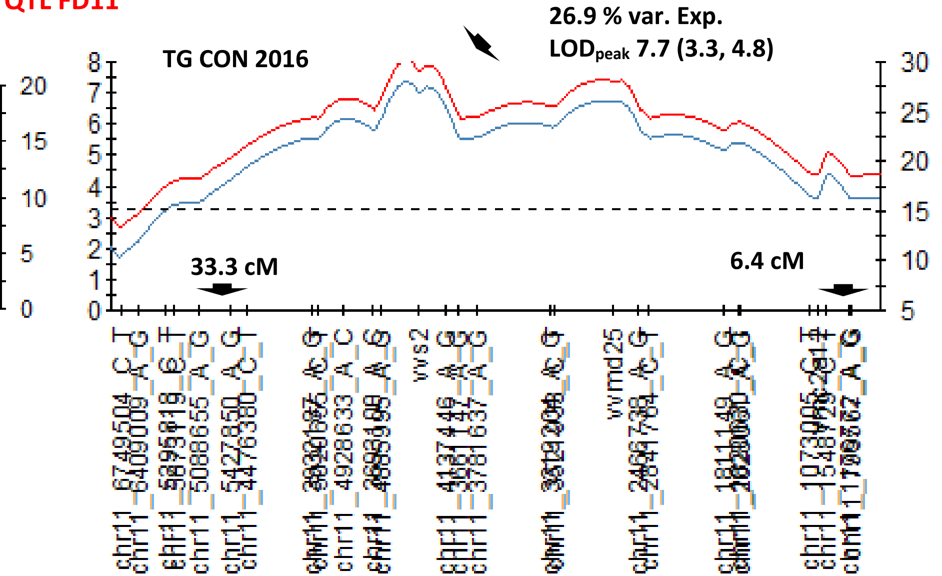
Figure 3.3.8. Summary of significant QTL for flower traits in T × G progeny. LOD (left axis) and % of explained variance (right axis). In parenthesis LOD (CW, GW). Horizontal dash line indicates CW.



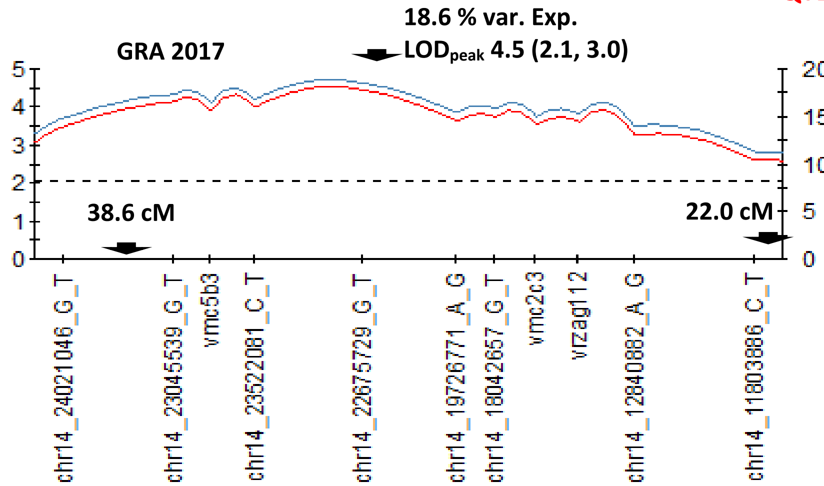
Flower diameter

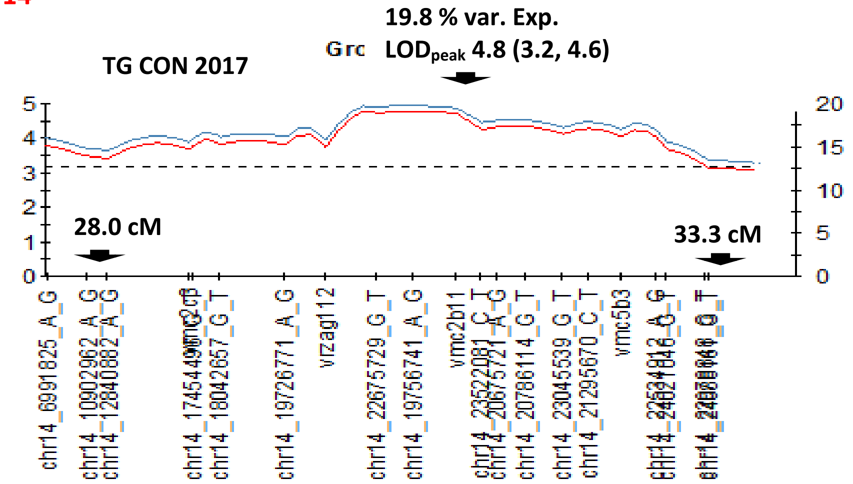
QTL FD11





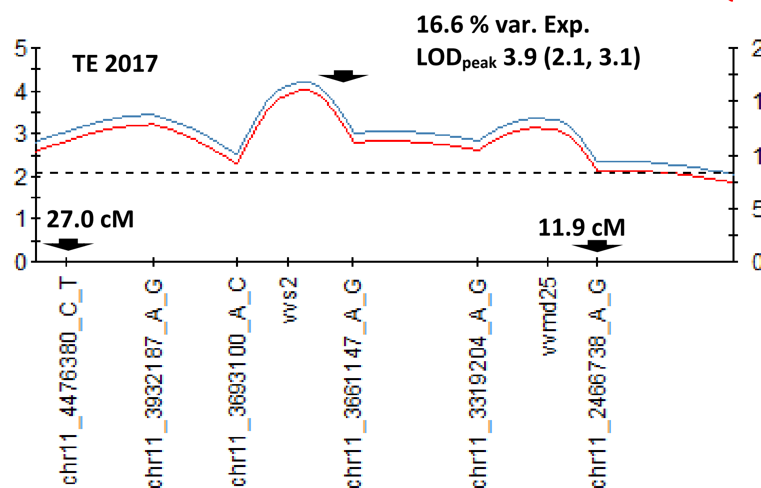
QTL FD14

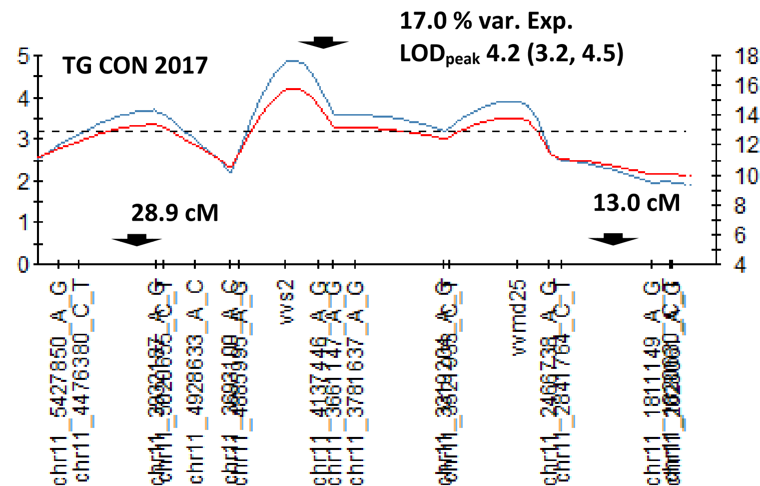




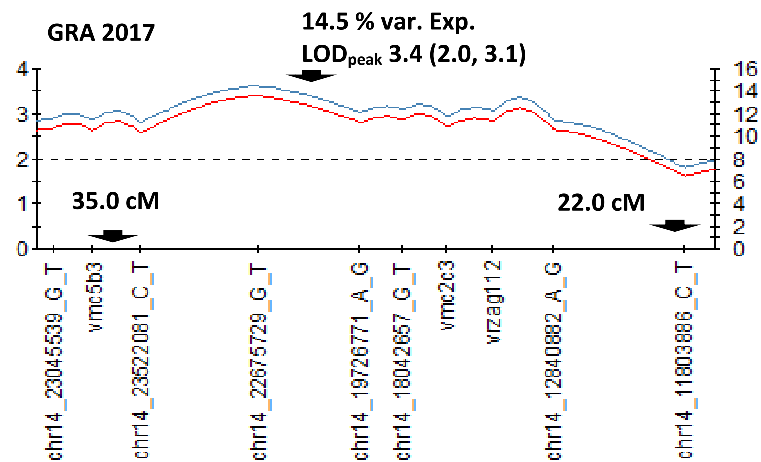
Ovary length

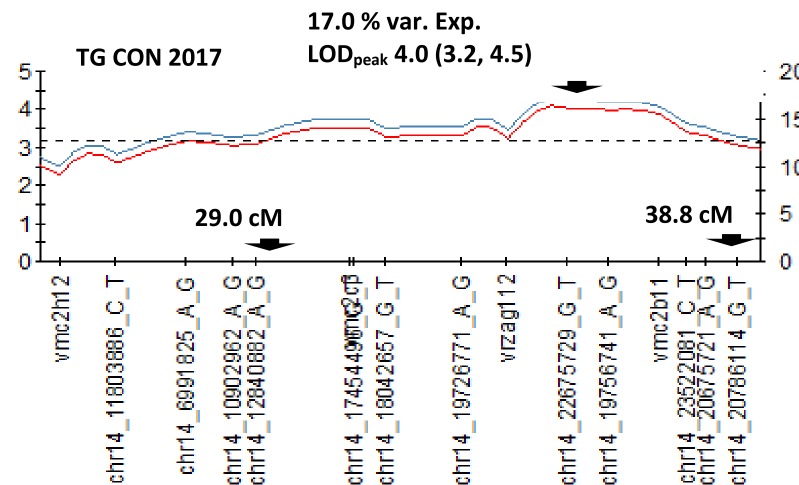
QTL OL11





QTL OL14





Pistil shape

QTL PS2

GRA 2016

25.5 % var. Exp.

LOD$_{peak}$ 7.2 (2.2, 3.1)

9.7 cM

38.3 cM

TE 2016

14.6 % var. Exp.

LOD$_{peak}$ 3.9 (1.9, 3.1)

45.0 cM

22.4 cM

TG CON 2016

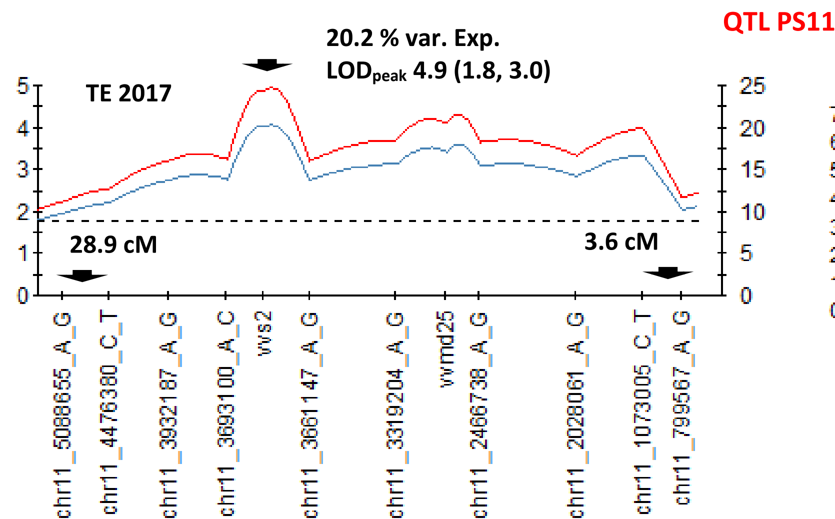
35.3 % var. Exp.

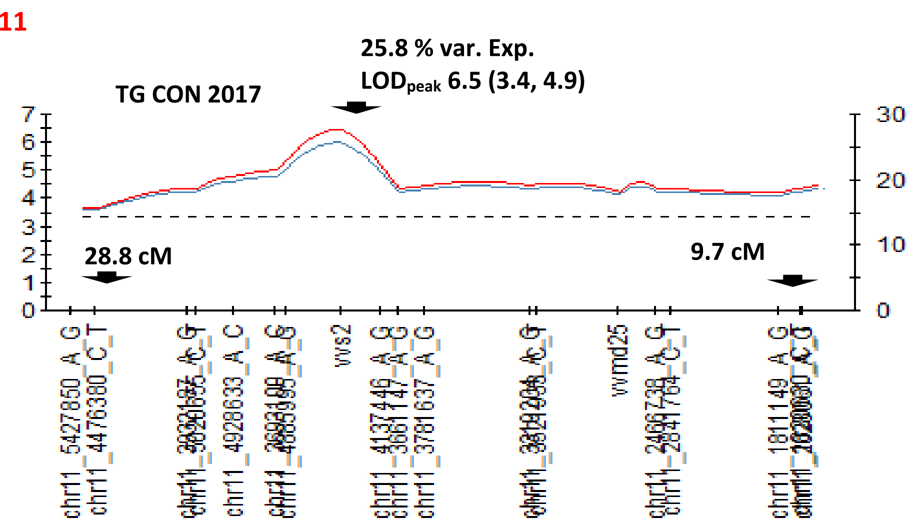
LOD$_{peak}$ 10.7 (3.3, 4.6)

1.7 cM

37.5 cM

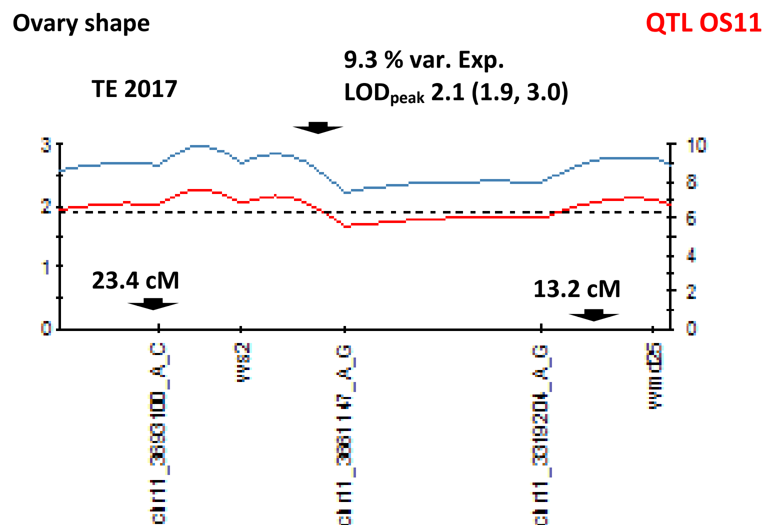
QTL PS11

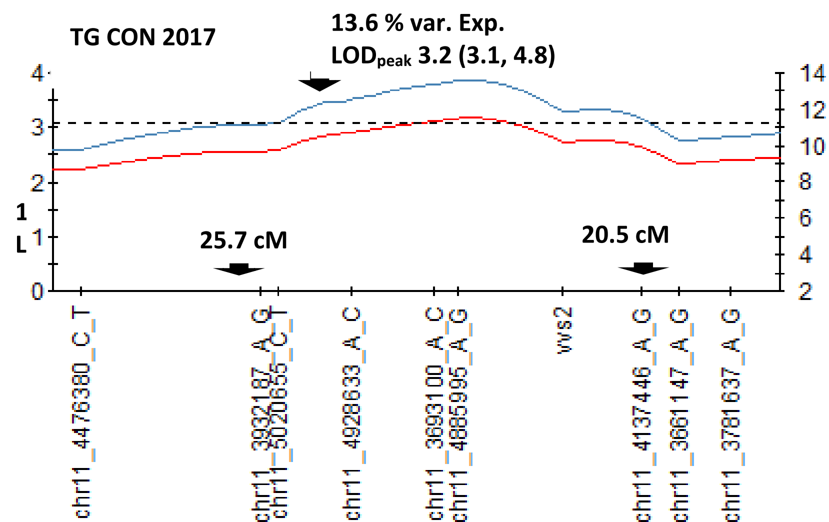


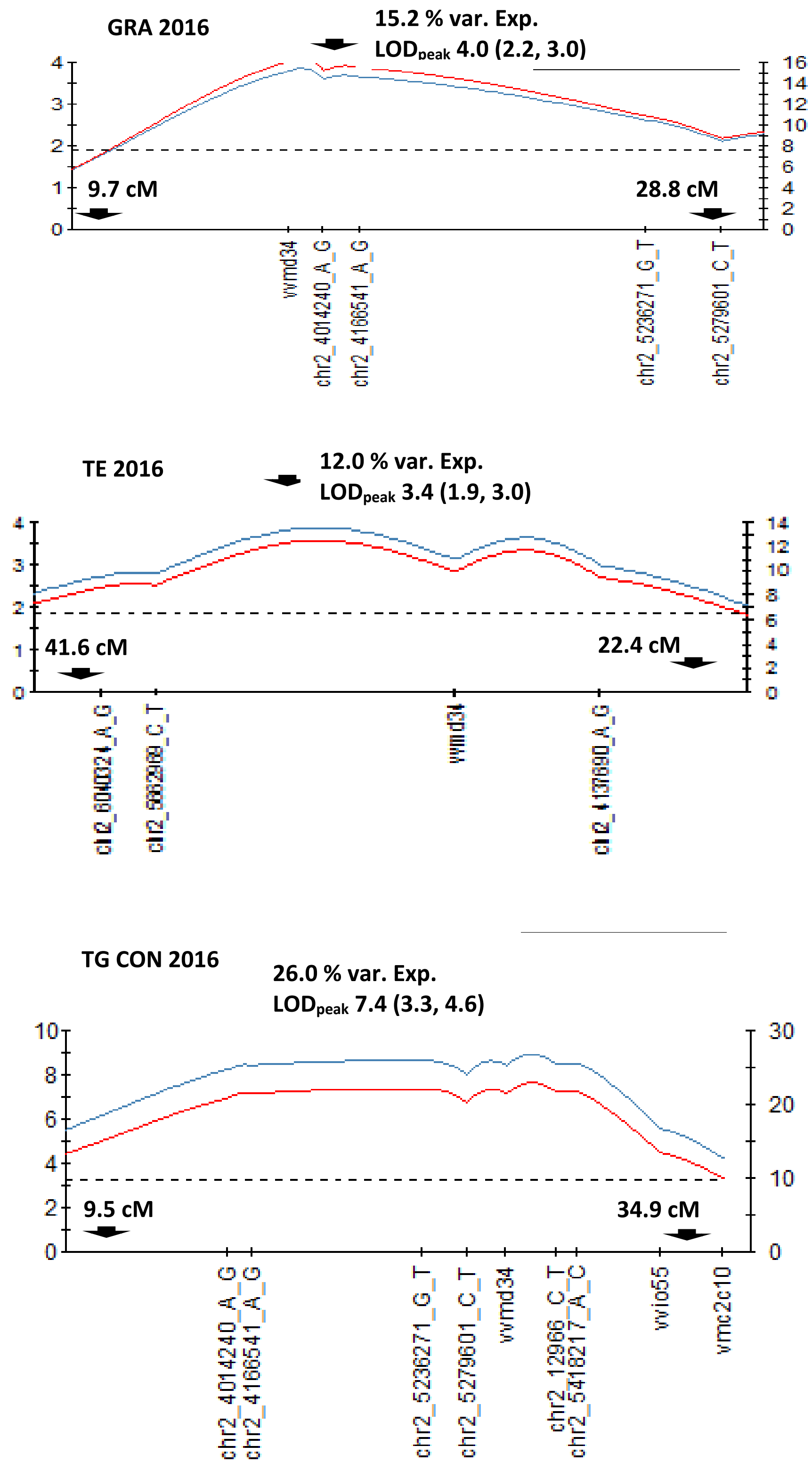


Ovary shape

QTL OS11






Ovary shape
QTL OS2
GRA 2016
15.2 % var. Exp.
LODpeak 4.0 (2.2, 3.0)
9.7 cM
28.8 cM
vvmd34
chr2_4014240_A_G
chr2_4166541_A_G
chr2_5236271_G_T
chr2_5279601_C_T
TE 2016
12.0 % var. Exp.
LODpeak 3.4 (1.9, 3.0)
41.6 cM
22.4 cM
vvmd34
TG CON 2016
26.0 % var. Exp.
LODpeak 7.4 (3.3, 4.6)
9.5 cM
34.9 cM
chr2_4014240_A_G
chr2_4166541_A_G
chr2_5236271_G_T
chr2_5279601_C_T
vvmd34
chr2_12966_C_T
chr2_5418217_A_C
vvio55
vmc2c10

When only hermaphrodite plants were considered, fewer QTL were detected as previously reported for flower and berry traits (Supplementary material 3.3.2 a and 3.3.4 a), indicating that they generate polymorphism to this trait. It must be highlighted that no QTL was found for flowering date in hermaphrodite T × G progeny, whilst in G × T progeny non-stable QTL on LG10 and in LG7 were detected (Supplementary material 3.3.2 b).

QTL associated to seed traits

Data for seed traits were available for only 1 year for T x G population, preventing a reliable QTL analysis comparison between populations. Thus, a combined analysis was established with previous T x G data by Song 2014 (unpublished). A QTL was identified in LG18 for seed traits in both progenies.

Female plants bore lower seed number compared with hermaphrodite genotypes in both populations, as reported in Chapter 3.1 (Table 3.1.2). Consequently, a stable and significant QTL was located in LG2 for seed number in both progenies and for seed weight (SW) in T × G population (Song 2014, unpublished) (Supplementary material 3.3.3 a, 3.3.2 b). Contrary to berry and flower QTL analysis, for seed traits the number of QTL detected in hermaphrodite subpopulations resulted higher than in the whole progeny.

In G × T progeny, the QTL detected in LG2 for seed number explained 18 % of the phenotypic variance (Supplementary material 3.3.1 a), whilst when the hermaphrodite population was analyzed, a QTL on LG6 was also found from Tempranillo, explaining up to 20 % of the phenotypic variance. In the hermaphrodite progeny, putative QTL were detected in LG 12, 13, 17 and 18 for seed weight and in LG1 for seed number (Supplementary material 3.3.2 b). QTL analysis for seed traits in this progeny was conducted with only two sets of data obtained in different plots, which may have affected the power of the analysis.

For T × G progeny, seed traits were analyzed only in 2016 and compared with results obtained by Song, (2014, unpublished) from three consecutive years (2008 - 2010). Based in 2016 analysis, only putative QTL were found for seed number and seed weight in LG18 and LG15, respectively (Supplementary material 3.3.3 a). Song (2014) reported stable and highly significant QTL on LG3 and LG5 in Graciano and Consensus maps reproducible over the three years, explaining up to 45 % of the phenotypic variance in seed number and seed weight traits (Supplementary material 3.3.3 a). Besides, Song (2014) also cited a stable QTL on LG2 suggesting the influence of *Sex* locus. As it is observed in Supplementary material 3.3.2 a. QTL on LG3 and LG5 co-locate with QTL for berry and flower-related traits in T × G progeny.

Remarkably, for seed number QTL on LG3 and LG5 the explained variance reached 70 %, whilst it was only 35 % in the analysis for the whole population, suggesting that the variance explained by QTL on the *Sex* locus, is attributed to these two QTL when female plants are removed. Putative QTL were also detected in LG18 for seed number and in LG1 and LG19 for seed weight traits. Interestingly, polymorphism in Graciano × Tempranillo is higher than between Tempranillo and Grenache for seed traits, which enhances the detection of a higher number and significant QTL, like with berry traits.

Comparison between the QTL detected in whole progeny and hermaphrodite plants

The working hypothesis was that *Sex* locus influences other traits besides flower determinism (Chapter 3.1). Seed number, flowering date and other traits such as flower diameter, pistil length, ovary shape and pistil shape proved to be influenced by flower sex at the phenotypic level.

Based on results of Chapter 3.1 differences in QTL identification were expected for all the traits mentioned above, however, only traits in Tables 3.3.5 a and 3.3.5 b presented differences at the QTL level. For G × T progeny, a QTL on LG2 was detected for ovary shape (OS) and seed number (SN) traits explaining up to 18 % of the variance for both traits. In this progeny, an additional QTL analysis considering SEX and VVIB23 marker as cofactors was performed. Although different results were obtained when each of the three approaches were performed, the significative effect in LG2 disappeared regardless of the analysis. In relation to ovary shape (OS), setting VVIB23 marker as cofactor reported the same result as removing female plants, identifying only one QTL on LG11 that accounted for 18 % of the phenotypic variance. The approach of setting SEX marker, reported a QTL on LG8, that explained only 12 % of the variance. Interestingly, QTL on LG8 and LG11 had been detected with the analysis performed in the whole population, thus no additional QTL were identified to account for the variance explained by LG2. For seed number (SN) though, the variance explained by QTL on LG2 is explained by QTL on LG1 and LG6 in the hermaphrodite subpopulation, and by QTL on LG1 and LG17 considering SEX as cofactor (Table 3.3.5 a). VVIB23 as cofactor did not detect more QTL than the one in LG2. The analysis without female plants represented more of the variance explained with around 20 % in LG1 and LG6 whilst in the analysis with cofactor only 12 % of the variance was explained by LG1 and LG17. Interestingly the QTL on LG17 co-localizes with QTL B17_2, that was found associated to berry length and berry weight in this study. Flowering date was shown to be influenced by flower sex in Chapter 3.1, but in this progeny no QTL was found in LG2. However, in the analysis removing female plants, a putative QTL on LG7 was found, whilst the analysis with cofactors gave the same previous results.

For T × G progeny, although HF plants presented longer pistils than females (Chapter 3.1), QTL analysis for pistil length delivered significant effects on LG5 and LG14 in the whole progeny, but significant effects were significant on LG11 and LG18 with the HF subpopulation; pointing at a flower sex influence, even though LG2 had no effect on PL. In ovary (OS) and pistil shape (PS) a significant effect was detected on LG2 when all the progeny was analyzed. The variance explained by the QTL on LG2 resulted in LG5, LG8 and LG13 for pistil shape and LG5, LG15 for ovary shape when SEX was set as cofactor of the analysis (Table 3.3.5 b). The QTL analysis for flowering date resulted drastically different considering the whole progeny and only hermaphrodite plants or SEX as cofactor. The last two approaches delivered only a putative QTL on LG7, whilst in the whole progeny different QTL were found in LG2, LG5, LG7 and LG14.

As a summary, different results were obtained with the analysis of QTL considering only hermaphrodite plants and cofactors, and sometimes the variance previously explained by LG2 in the whole progeny was not explained by other effects in other linkage groups when the effect of flower sex was removed, maybe due to a lower power of the analysis in a smaller population. That confirms the influence of *Sex* locus in pistil length, ovary shape, pistil shape, seed number, seed weight and flowering date and it depends on the progeny studied.

Table 3.3.5 a. Comparison between whole progeny and hermaphrodite plants in G × T population in the traits affected by *Sex* locus.

	LG	Map	Y	P	QTL	Marker	LOD peak	1-LOD interval	LOD CW	LOD GW	% Var.
OVARY SHAPE	2	**CON**	2015	21.5	OS2	2_4422552	5.16	18.5-28.3	3.2	4.7	17.1
		GAR	2015	16.4	OS2	2_2988540	3.42	14.3-25.6	1.9	3.2	11.7
		TE	2015	24.8	OS2	VVIB23	2.39	21-25.3	2.2	3.5	8.3
	8	**TE**	2016	22.8	OS8	8_5679508	2.45	21.5-24.3	2.1	3.5	9.7
		CON	2015	11.4	OS8	8_3071450	4.75	9.6-13.5	3.3	4.7	15.8
		GAR	2015	17.6	OS8	8_3071450	2.99	12.5-18.1	1.9	3.2	10.3
	11	**TE**	2016	20.1	OS11.1	11_1466955	4.05	10.2-32.1	2.2	3.5	15.5
		CON	2016	12.9	OS11.1	11_1466955	4.77	10.1-22.4	3.4	4.7	18.0
		TE	2016	41.8	OS11.2	11_4001363	3.06	35.1-48.7	2.2	3.5	11.9
SEED N°	2	**TE**	2016	13.8	SN2	2_3716600	2.98	4.1-19.3	2.0	3.5	12.9
		GAR	2015	26.7	SN2	-1_40570253	3.20	12-39.9	1.9	3.3	16.1
		CON	2015	30.7	SN2	VVIB23	3.23	25-45	3.2	4.7	16.2
		CON	2016	19.1	SN2	-1_31689073	4.23	14.5-32	3.4	4.8	17.9
FLOWERING	10	**CON**	2016	42.7	F10	10_2524063	4.54	30-51.8	3.1	4.9	16.6
		TE	2016	23.9	F10	10_1618757	2.45	20.4-29	1.7	3.5	9.3
		GAR	2016	44.7	F10	10_2524063	2.12	41.4-44.7	1.8	3.1	8.1
	15	**GAR**	2016	57.1	F15	15_11318578	3.50	48.6-64.8	1.9	3.1	13.1
		CON	2016	3.8	F15	-1_39397398	4.09	0-34.2	3.4	4.9	15.1

	C	LG	Map	Year	Pos	QTL	Marker	LOD peak	1-LOD interval	LOD CW	LOD GW	% Var.
OVARY SHAPE	SEX	8	**CON**	**2015**	11.4	OS_8	8_3071450	4.04	10.2-12.5	3.2	4.7	12.1
			GAR	**2015**	17.6	OS_8	8_3071450	2.64	13.5-18.6	1.9	3.1	8.1
	VVIB23	11	**CON**	**2016**	15.9	OS11	11_1694790	3.71	7.2-20.6	3.2	4.7	19.4
			TE	**2016**	16.9	OS11	11_1245158	2.71	11.9-29	2.2	3.6	13.7
	HF	11	**CON**	**2016**	15.8	OS11	11_1694790	3.71	7.2-20.6	3.2	4.7	19.4
			TE	**2016**	16.9	OS11	11_1245158	2.71	11.9-29	2.2	3.6	13.7
SEED N°	SEX	1	**CON**	**2016**	34.5	SN1	1_5972704	3.03	33.6-36.5	3.2	4.7	10.8
			GAR	**2016**	37.1		1_6074975	2.09	33.1-38.2	**1.9**	**3.3**	8.5
		17	**CON**	**2016**	26.4	NS17	17_3936107	3.52	25.1-28.2	3.1	4.7	12.6
			TE	**2016**	21.9	NS17	17_3676153	2.40	21.1-25.1	2.2	3.1	8.8
	HF	1	**GAR**	**2016**	52.7	SN1	1_9936972	2.51	48.3-56.1	1.9	3.3	13.9
			CON	**2016**	47.0	SN1	1_8440819	3.53	45.6-54.3	3.3	4.7	19.0
		6	**CON**	**2015**	40.3	SN6	6_6463639	3.33	38.5-42.4	3.2	4.7	21.0
			TE	**2015**	43.3	SN6	6_5519184	3.21	30.4-48.2	2.1	3.5	20.3
			TE	**2016**	30.4	SN6	6_4130249	2.28	28.8-40.3	2	3.5	12.7
		7	**CON**	2014	46.3	F7	-1_1108704	3.20	45.9-50.8	3.0	4.6	18.3
			TE	2014	48.5	F7	-1_19613096	2.22	43.9-48.4	2.1	3.4	14.4
FLOWERING	HF	10	**CON**	2016	42.7	F10	10_2524063	4.33	38.8-55.5	3.3	4.8	20.1
			GAR	2016	44.7	F10	10_2524063	2.23	43.8-45.2	1.9	3.2	10.9
			TE	2016	23.9	F10	10_1618757	2.13	22.5-27.7	2.0	3.3	10.0
	VVIB23/SEX	15	**CON**	2015	6.2	F15_1	15_10162460	6.33	5.8-16.5	3.2	4.8	20.0
			GAR	2016	13.5		15_11318578	3.48	12.6-32.3	1.8	3.2	12.9
			CON	2016	19.3	F15_2	15_13561872	4.08	17.3-22.4	3.2	4.8	14.5
			GAR	2016	37.8		15_13814474	3.20	36.2-43.1	1.8	3.2	11.9
		10	**CON**	2016	42.7	F10	10_2524063	3.67	41.1-54.3	3.1	4.8	13.2
			TE	2016	23.9		10_1618757	2.74	21.7-28.1	1.7	3.5	10.2

Left table contains the results of QTL analysis in the whole progeny in the traits affected by SEX locus. In the right table, C means analysis using markers VVIB23 and SEX as cofactors in the whole progeny. HF means that only hermaphrodite plants were considered.

Table 3.3.5 b. Comparison between whole progeny and hermaphrodite plants in T × G population in the traits affected by *Sex* locus.

	LG	Map	Year	Pos	QTL	Marker	LOD peak	1-LOD interval	LOD CW	LOD GW	% Var.
PISTIL LENGTH	5	TE	2016	46.9	PL5	chr5_19770665	1.92	42.2-47.8	1.8	3.0	7.5
		TE	2017	46.9	PL5	chr5_19770665	2.41	42.8-48.7	2.1	3.0	10.5
		CON	2017	47.7	PL5	chr5_7253933	3.33	41.5-57.3	3.3	5.5	13.5
	14	GRA	2016	26.3	PL14	vmc5b3	2.39	24.5-34.8	2.0	3.1	9.3
		GRA	2017	35.1	PL14	vrzag112	2.76	27.2-35	2.1	3.0	11.9
		CON	2017	31.4	PL14	vmc2c3	3.21	28-33.8	3.2	5.2	13.8
PISTIL SHAPE	2	CON	2016	23.5	PS2	chr2_5236271	10.7	15-25	3.3	4.6	35.3
		CON	2017	23.5	PS2	chr2_5236271	3.84	15-25	3.2	4.9	16.2
		TE	2016	25.9	PS2	chr2_4137690	3.86	10.2-28.5	1.9	3.1	14.6
		TE	2017	25.9	PS2	chr2_4137690	2.22	15-30	1.8	3.0	9.7
		GRA	2016	18.4	PS2	chr2_4166541	7.21	10.5-40	2.2	3.1	25.5
		GRA	2017	26.6	PS2	chr2_5236271	2.90	15-30.2	2.2	3.0	12.5
	11	CON	2016	42.0	PS11	vvs2	3.73	35-55	3.2	4.6	14.1
		CON	2017	42.0	PS11	vvs2	6.47	35-55	3.4	4.9	25.8
		TE	2016	50.0	PS11	chr11_2028061	1.85	35-52	1.8	3.1	7.3
		TE	2017	37.3	PS11	vvs2	4.90	35-55	1.8	3.0	20.2
		GRA	2016	58.1	PS11	chr11_1548729	1.95	50-60	1.9	3.1	7.6
OVARY SHAPE	2	CON	2016	23.5	OS2	chr2_5236271	7.39	10.2-30	3.3	4.6	26.0
		CON	2017	23.5	OS2	chr2_5236271	3.16	20-25	3.1	4.8	13.1
		TE	2016	21.8	OS2	vvmd34	2.86	10.2-30	1.9	3.0	12.0
		GRA	2016	16.3	OS2	vvmd34	4.03	10.2-30	2.2	3.0	15.2
		GRA	2017	26.6	OS2	chr2_5236271	2.24	22-26	1.9	3.0	9.8
	7	CON	2016	18.7	OS7	chr7_51263	3.43	18-20	3.2	4.6	13.0
		GRA	2016	0.1	OS7	chr7_51263	2.55	0-10	2.0	3.0	9.9
	8	CON	2016	50.0	OS8	chr8_16092315	3.44	50-59.6	3.2	4.6	13.1
		TE	2016	46.0	OS8	chr8_SNP865	1.86	44.6-59.7	1.8	3.0	7.3
		GRA	2017	72.1	OS8	chr8_21030434	2.16	59.0-73.9	2.0	3.0	9.5
	11	CON	2016	23.9	OS11	udv017	3.51	22-40	3.3	4.6	13.3
		CON	2017	40.6	OS11	chr11_4885995	3.19	38-42	3.1	4.8	13.6
		TE	2017	44.7	OS11	vvmd25	2.11	34-45	1.9	3.0	9.3
SEED NUMBER	2	TE	2009	13.4	SN2	chr2_5662969	2.5	13.4-18.4	1.6	2.9	8.9
		TE	2010	13.4	SN2	chr2_5662969	3.5	8.1-16.4	1.5	2.9	11.5
	3	CON	2008	54.9	SN3	chr3_10713706	10.6	48.7-60	3.2	4.7	35.5
		CON	2009	54.9	SN3	chr3_10713706	19.7	38.8-54.9	3.1	4.7	52.2
		CON	2010	54.9	SN3	chr3_10713706	19.4	50.9-54.9	3.1	4.6	49.9
		GRA	2008	54.2	SN3	chr3_10713706	10.5	47.3-54.2	1.7	2.9	35.2
		GRA	2009	54.2	SN3	chr3_10713706	19.3	50.4-54.2	1.7	2.9	51.4
		GRA	2010	54.2	SN3	chr3_10713706	19.2	48.8-54.4	1.6	2.9	48.8

	C	LG	Map	Year	Pos	QTL	Marker	LOD peak	1-LOD interval	LOD CW	LOD GW	% Var.
PISTIL LENGTH	HF	11	CON	2016	49.6	PL11	vvmd25	4.38	36.1-50.6	3.2	4.9	22.1
			TE	2016	33.4	PL11	chr11_3932187	2.03	32-38	1.8	3.0	9.5
			TE	2017	33.4	PL11	chr11_3932187	2.04	31-40	1.8	3.0	11.1
			GRA	2016	26.2	PL11	vmc5b3	2.43	23.6-34	1.8	3.1	11.7
			GRA	2017	26.2	PL11	vmc5b3	2.15	25.5-34.4	2.0	3.1	11.6
		18	CON	2016	54.8	PL18	chr18_9340550	4.09	48.9-61.1	3.2	4.7	15.3
			GRA	2016	53.4	PL18	chr18_13410273	1.95	48.3-60	1.8	3.1	7.6
PISTIL SHAPE	SEX	5	CON	2016	79.8	PS_5	chr5_SNP1071	2.99	79.4-82.4	3.2	4.7	7.8
			GRA	2016	83.1		chr5_SNP1071	2.07	77.6-86.2	2.2	3.5	5.5
		8	CON	2017	71.6	PS_8	chr8_20163834	3.47	65.0-73.2	3.2	4.7	12.8
			GRA	2017	2.5		chr8_21486439	2.36	1.0-5.5	2.2	3.5	9.0
		11	CON	2017	42.0	PS_11	vvs2	5.20	41.2-51.3	3.3	4.6	16.9
			TE	2017	37.3		vvs2	3.88	35.7-48	2.1	3.4	13.1
		13	CON	2017	55.1	PS_13	chr13_638880	4.01	54.5-63.4	3.2	4.7	14.6
			GRA	2017	40.6		chr13_638880	3.29	33.2-42.3	2.2	3.5	12.2
PISTIL SHAPE	HF	2	CON	2016	29.4	PS_2	chr2_5418217	3.69	22.2-30.6	3.2	4.8	17.2
			GRA	2016	16.3		vvmd34	1.98	16.1-21.9	1.9	3.2	9.6
		11	CON	2017	42.0	PS_11	vvs2	4.59	33.5-47.1	3.2	4.8	23.2
			TE	2017	37.3		vvs2	3.60	34.3-52.8	2.0	3.1	18.7
		13	CON	2017	51.4	PS_13	chr13_2265895	4.54	50.1-59.8	3.1	4.7	23.0
			GRA	2017	40.6		chr13_638880	2.53	34.4-42	2.0	3.1	13.6
OS	SEX	5	CON	2017	72.2	OS_5	chr5_5570145	3.21	58.3-73.4	3.2	4.8	11.7
			GRA	2017	65.9		chr5_7191560	2.25	57.2-66.2	2.2	3.4	8.3
		7	CON	2016	20.4	OS_7	chr7_3316137	3.16	13.1-21.4	3.2	4.7	9.5
			GRA	2016	75.7		chr7_1720231	2.34	69.1-75.3	2.2	3.5	7.1
		15	CON	2016	52.9	OS_15	vviv67	3.40	51.2-53.3	3.2	4.8	9.2
			GRA	2016	9.5		vviv67	1.93	8.2-12.1	2.2	3.5	5.4
OS	HF	5	CON	2017	72.2	OS_5	chr5_5570145	3.36	59.4-71.4	3.2	4.7	17.6
			GRA	2017	65.9	OS_5	chr5_7191560	2.34	58.6-70.4	2.1	3.2	12.6
SEED NUMBER	HF	3	CON	2008	54.8	SN3	chr3_10713706	13.18	30-60	3.1	4.7	46.5
			CON	2009	54.8	SN3	chr3_10713706	21.32	25-60	3.1	4.7	63.3
			CON	2010	51.8	SN3	chr3_11238850	27.78	30-60	3.1	4.7	70.1
			GRA	2008	52.9	SN3	chr3_11238850	26.80	38.1-56.4	2.1	3.2	68.8
			GRA	2009	52.9	SN3	chr3_11238850	20.64	38.1-56.4	2.0	3.1	62.1
			GRA	2010	52.9	SN3	chr3_11238850	26.80	38.1-56.4	2.2	3.3	68.8
		5	CON	2008	37.9	SN5	vmc3c7	13.01	20-52	3.0	4.7	46.1
			CON	2009	51.6	SN5	chr3_8560917	20.65	10.5-40	2.1	3.2	62.1

Trait	LG	Map	Year	Pos	QTL	Marker	LOD peak	1-LOD interval	LOD CW	LOD GW	% Var.
SEED NUMBER	5	CON	2008	60.8	SN5	vmc3c7	12.8	58.6-60.8	3.2	4.7	41.3
		CON	2009	60.8	SN5	vmc3c7	20.2	59.9-62	3.2	4.7	53
		CON	2010	60.8	SN5	vmc3c7	19.1	60.8-64.1	3.1	4.6	48.6
		GRA	2008	65	SN5	vmc3c7	9.4	51.4-70.1	1.9	2.9	32.3
		GRA	2009	65	SN5	vmc3c7	18.2	62.4-67.2	1.7	2.9	49.5
		GRA	2010	65	SN5	vmc3c7	16.2	49.5-65	1.8	2.9	43.1
	18	CON	2008	76.6	SN18	vmcng2f12	3.34	70.1-77	3.2	4.6	10.5
		GRA	2009	58.2	SN18	chr18_r_2677945	2.39	53.4-62.2	2	3.1	7.7
SEED WEIGHT	2	CON	2010	23.6	SW2	chr2_5236271	7.7	16.2-26.7	3.3	4.7	25.1
		CON	2008	23.6	SW2	chr2_5236271	10.7	18.1-24.6	3.2	4.6	31.2
		GRA	2009	26.6	SW2	chr2_5236271	4.6	14.7-36.1	1.6	2.9	15.8
		GRA	2010	26.6	SW2	chr2_5236271	5.2	26.6-28.6	1.5	2.9	16.5
	3	GRA	2016	53.0	SW3	chr3_11238850	2.1	14.8-53	1.6	2.9	7.7
		GRA	2016	53.0	SW3	chr3_11238850	2.3	40.6-53	1.7	2.9	7.8
	5	CON	2009	56.5	SW5	chr5_9640285	3.9	56.5-60.2	3.3	4.7	13.4
		CON	2010	56.5	SW5	chr5_9640285	4.3	45.3-64.2	3.2	4.6	14
		GRA	2009	58.5	SW5	chr5_9640285	3.4	58.5-63.3	1.7	2.9	11.9
		GRA	2010	58.5	SW5	chr5_9640285	3.3	49.3-58.5	1.9	2.9	9.7
	11	CON	2009	37.4	SW11	chr11_8616276	4.5	37.4-50.1	3.0	4.7	15.4
		CON	2010	37.4	SW11	chr11_8616276	4	37.4-41.4	3.1	4.6	12.9
		TE	2009	35.9	SW11	chr11_8616276	2.9	25.1-35.9	1.5	2.9	10.1
	15	CON	2010	8.6	SW15	chr15_1109421	3.78	1.3-9.6	3.3	4.6	11.9
		GRA	2009	7.4	SW15	chr15_9668745	1.99	4.2-7.5	1.8	3.1	6.1
FLOWERING DATE	2	CON	2010	26.7	F2	vvmd34	3.2	20.2-27.3	3.1	4.7	9.4
		GRA	2009	16.3	F2	vvmd34	2.4	13.3-21.4	2.1	3.1	8.8
	5	CON	2010	37.4	F5	vchr5a	3.2	35-38	3.1	4.7	13.1
		GRA	2009	37.7	F5	vchr5a	2.2	34-38	2.1	3.1	9.2
	7	CON	2016	38.3	F7	chr7_8211796	3.01	52-55	3	4.8	10.2
		TE	2016	50.9	F7	chr7_8211796	2.59	46-55	2	3	9.5
	14	CON	2016	34.7	F14	vrzag112	3.86	34-50	3.3	4.7	16.4
		GRA	2016	35.1	F14	vrzag112	1.95	34-38	1.8	3.1	7.8

Trait	C	LG	Map	Year	Pos	QTL	Marker	LOD peak	1-LOD interval	LOD CW	LOD GW	% Var.
SEED NUMBER	HF	5	CON	2010	35.7	SN5	chr5_14699639	25.41	20-52	3.2	4.7	66.8
			GRA	2008	37.1	SN5	vmc3c7	12.88	19-55	2.0	3.2	45.7
			GRA	2009	37.1	SN5	vmc3c7	21.23	10.5-55	2.0	3.1	63.1
			GRA	2010	36.2	SN5	chr5_14699639	25.25	19-55	2.2	3.2	66.6
		18	CON	2016	76.6	SN18	vmcng2f12	3.79	72.5-83.6	3.3	4.7	14.8
			GRA	2016	58.2	SN18	chr18_r_2677945	1.91	55.1-59.6	2.0	3.2	7.8
SEED WEIGHT	SEX	15	CON	2016	51.5	SW_15	chr15_1109421	3.97	51.1-59.7	3.2	4.7	14.1
			GRA	2016	7.4		chr15_9668745	1.60	3.5-8.4	1.9	3.2	5.9
	HF	1	CON	2009	41.9	SW1	chr1_11027925	3.35	34.5-42.5	3.2	4.8	14.6
			TE	2009	28.6	SW1	chr1_11027925	2.58	27.8-34.2	1.8	3.2	11.4
		3	CON	2009	46.4	SW3	chr3_7157449	4.86	30-60	3.2	4.8	20.4
			CON	2010	51.8	SW3	chr3_11238850	5.26	30-60	3.2	4.7	20.4
			GRA	2009	16.9	SW3	chr3_11238850	3.92	10.5-45	2.1	3.1	16.8
			GRA	2010	16.9	SW3	chr3_11238850	4.95	10.5-45	2.1	3.2	19.4
		5	CON	2009	35.7	SW5	chr5_14699639	4.24	25-55	3.1	4.8	18.0
			CON	2010	35.7	SW5	chr5_14699639	5.77	25-55	3.4	4.7	22.2
			GRA	2009	36.2	SW5	chr5_14699639	3.98	19-55	2.2	3.1	17.0
			GRA	2010	36.2	SW5	chr5_14699639	5.37	19-55	2.2	3.2	20.8
		10	CON	2009	56.7	SW10	chr10_918773	3.90	52.4-59	3.2	4.8	16.8
			CON	2010	56.7	SW10	chr10_918773	3.29	52.4-59	3.1	4.7	13.3
			GRA	2009	57.9	SW10	chr10_918773	3.41	54.1-63.4	2.1	3.1	14.8
			GRA	2010	57.9	SW10	chr10_918773	2.88	54.1-64	2.1	3.2	11.8
		19	CON	2008	39.6	SW19	vmc6c7	3.41	38.8-40.8	3.3	4.8	14.9
			CON	2009	26.5	SW19	chr19_5200598	3.3	23.3-26	3.1	4.8	12.9
			GRA	2008	26.9	SW19	vmc6c7	3.27	25.9-47.9	2.0	3.1	14.4
F	SEX	7	CON	2016	38.3	F_7	chr7_8211796	2.68	36.5-44.2	3.2	4.7	10.3
			TE	2016	52.1		vmc1a2	2.89	47.1-51.9	2.2	3.5	11.1

Left table contains the results of QTL analysis in the whole progeny in the traits affected by *Sex* locus. In the right table, C means analysis using markers VVIB23 and SEX as cofactors in the whole progeny. HF means that only hermaphrodite plants were considered. OS ovary shape and F flowering date. Data in grey colour means Song 2014. Highly significant values are highlighted in red.

QTL association analysis

Main associations between QTL identified for berry, flower and seed traits in both genetic backgrounds are summarized in Table 3.3.6 a and Table 3.3.6 b. Associations between berry traits: berry length, diameter and weight were found in G × T progeny in LG17 and LG18 and in T × G population in LG3 and LG5. Genomic regions associated to flower morphology traits were found in LG8 and LG11 in G × T progeny and in LG11 and LG14 in T × G , being LG14 also associated to flowering date. QTL regions for berry length and shape traits appeared in LG10 in G × T and LG1 in T × G progeny. Besides, in T × G progeny QTL regions in LG3 and LG5 were detected for berry and seed traits, being the QTL on LG5 also associated to pistil length. QTL on LG2 associated to *Sex* locus were detected for flower shape and seed traits in both progenies and for berry traits in G × T progeny, confirming the influence of *Sex* locus.

Table 3.3.6 a. Matrix with the main associations between QTL regions in G × T progeny

	LG1	LG2	LG3	LG8		LG9	LG10	LG11		LG13	LG17	LG18
	50-75	15-30	47-63	5-20	35-52	43-60	40-60	10-25	35-42	45-60	14-40	30-50
BL												2
BD		2	2									2
BS	2						2					
BW			3									2
PL					2			2				
FD												
OL					2			2				
PS				2								
OS												
SN		2										
SW												
F												

Table 3.3.6 b. Matrix with the main associations between QTL regions in T × G progeny

	LG1	LG2	LG3		LG5	LG11	LG14	LG18	
	30-45	**15-25**	**25-50**	**55-65**	**30-60**	**5-30**	**28-45**	**50-57**	**75-85**
BL									
BD			2		2				
BS	2								
BW			3	2	3				S 2
PL					2		2		
FD						2	2		
OL						2	2		
PS		2				2			
OS		2				2			
SN		S2	3	3	3				
SW		S2	2						
F									

Colour legend: LOD 3.0-3.5 (green), LOD 3.5-4 (turquoise), LOD 4-4.5 (grey), LOD 4.5-6 (light blue), LOD 6-8(dark blue), LOD > 8 (purple), LOD > 10 (black).BL Berry length, BD berry diameter, BS berry shape, BW berry weight, PL pistil length, FD flower diameter, OL ovary length, PS pistil shape, OS ovary shape, SN seed number, SW seed weight, F flowering date.. Numbers 2 and 3 inside cells indicate the number of years in which the QTL was found. S means reported by Song 2014.

Discussion

Mapping of *Sex* locus

Both populations in this study segregate for flower sex because parents Graciano, Grenache and Tempranillo present the *Hf* heterozygous genotype (hermaphrodite-female). Results from our study confirmed that sex determinism is under the control of a single major region of grapevine genome located on LG2, being VVIB23 and VVMD34 the closest SSR markers. The region around the flower *Sex* locus covers a similar physical distance than that proposed by Fechter et al. (2011), herein located between 4422552 and 4579843 Mbp on the physical map of PN40024. In this work, VVIB23 marker located closely to *Sex* locus in Grenache, Tempranillo and Consensus maps at 0.3 cM, 1.4 cM and 0.4 cM respectively, being the number of recombinants similar to those reported by Battilana et al. (2013) in their intra-specific hybrid progeny of 91 plants. However, those values are lower than the distance between VVIB23 marker and *Sex* locus reported by Riaz et al. (2006) and Margueritt et al. (2009) with inter-specific progenies with 181 and 138 plants (being the distances 1.5 and 4.5 cM, respectively).

Genetic analysis of berry morphology

Berry and seed traits are key selection criteria in grape breeding. This study reports QTL for berry morphology and seed traits in two wine-grape segregation populations. Although berry weight and berry size (diameter) are reported to be highly correlated, allowing the analysis of only one trait (Mejia et al. 2007), berry length, diameter, shape and weight were considered in this study, in order to gather as much information as possible for berry morphology.

Berry weight is affected by numerous factors such as grape variety, clone, berry position in a bunch, number of berries per bunch, cell wall mechanisms, growth regulators etc, that conduct to a great variability (0.5 to 11g) on berry fresh weight at maturity (Houel et al. 2013). The quantitative nature of the trait and its complexity, with different causal polymorphisms segregating in different populations as reported by Doligez et al. (2013), explains the wide range of results reported. Moreover, these authors observed substantially different patterns between Consensus and parental maps relative to reproducibility of QTL likewise in the present study. The high correlations ($r = 0.8 - 0.9$, $p < 0.01$) (Table 3.3.3) found between berry length, diameter and berry weight in both populations, were confirmed by the detection of common QTL in this analysis. However, a very low correlation was observed between berry weight and berry shape ($r = 0.1 - 0.2$, $p < 0.05$) in both progenies.

In the present work, two QTL were identified for berry weight in LG3 and LG18 in common for both populations. QTL on LG18, was significant in G × T population (18 % of the variance LOD = 5.1), and in T x G hermaphrodite subpopulation (13 % variance explained; LOD = 3.3) was located near the position cited by Doligez et al. (2013) linked to locus *SDI* (Seed Development Inhibitor). QTL associated with berry weight on LG18 have been widely cited by other authors as Fanizza et al. (2005), Cabezas et al. (2006), Constantini et al. (2008), Guo et al. (2019). Besides, locus *SDI* has been previously identified as having a major effect in the determination of berry size. It explained up to 70 % of the phenotypic variance in seed related traits being this gene also the responsible of the inheritance of seedlessness in grapes. Seed abortion, caused by an amino acid substitution in seed morphogenesis regulator gene AGAMOUS-LIKE11 (VviAGL11) has been recently reported as the major cause of seedlessness

in cultivated grapevine (Royo et al. 2018), being related with stenospermocarpy and berry weight. However, *SDI* gene is not a candidate gene for QTL BW18 significant for berry traits in G × T progeny.

A QTL on LG3 (B3) resulted highly significant and reproducible in T × G progeny associated with berry traits explaining up to 28 % variance for berry length, and up to 29 % for berry diameter and weight. It resulted only reproducible and significative related to berry diameter (25 %) and berry weight (14.4 %) in G × T hermaphrodite subpopulation, being significative for both traits in the whole progeny. Moreover, for Graciano x Tempranillo population, (T × G); a significant and reproducible QTL for berry traits, BL, BD and BW; was also detected in LG5 (B5), close to VMC3C7 explaining up to 21 % of the variance for berry weight. Both QTL have significant effects on seed number and seed weight in this population.

Remarkably, the stable QTL on LG3 and LG5 for berry traits in this work had been previously detected for the T × G population by Song (2014) in a different experiment in two years. QTL associated to berry weight on LG5 were previously reported by Fischer et al. (2004), Fanizza et al. (2005) and have been related to the *Arabidopsis thaliana FERONIA (FER)* locus. FERONIA/FER-like transcription factors encode for receptor kinases and have been implicated in multiple signaling pathways in fruit development and ripening (Li et al. 2017), plant stress responses (Guo et al. 2018), plant growth and development (Haruta et al. 2014), and traits like seed weight or fresh weight (Azevedo et al. 2018) in several climacteric and non-climacteric fruits.

Stable QTL found in LG17 associated to berry weight in G × T population, had been also detected by Doligez et al. (2013) and Guo et al. (2019) and the putative QTL on LG11 by Viana et al. (2013), and Ban et al. (2016). A search for candidate genes for B17_2 (explaining up to 20 % of the variance for berry length, diameter and weight) identified VIT_17s0000g04670 gene, an ortholog of AT5G08160 gene of *Arabidopsis thaliana,* annotated as a putative serine/threonine-protein kinase.

Few studies have addressed berry shape genetics, even though a wide range of variability had been previously reported for this trait (Houel et al. 2013). Guo et al. (2019) did not identify any stable QTL for berry shape. In this work, QTL for berry shape were detected on LG1 and LG9 in G × T and T × G hermaphrodite progeny. As shown in the correlation matrix (Table 3.3.3), berry length was the variable that contributed most to berry shape ($r = 0.4$, $p < 0.01$) in both populations, as previously reported by Pereira (2014). Although QTL on LG1 and LG9 are not shared between berry length and shape, a putative QTL found in LG10 in G x T hermaphrodite progeny for berry length co-localizes with berry shape, supporting the hypothesis that the longitudinal dimension is what determines berry shape. In T × G progeny, the putative QTL found in LG1 for berry length, did not co-localize with a QTL for berry shape. In other crops such as tomato, berry shape has been deeply studied, detecting QTL linked to a family of genes such as LOCULE NUMBER (WOX), OVATE (Ovate, OFP) (Rodriguez et al. 2011). The mutation OVATE has been associated to elongated fruits in tomato (Monforte 2017) and pepper (Tsaballa et al. 2011).

QTL analysis of seed traits

In the present work, berry weight was correlated with seed weight ($r = 0.5$, $p < 0.01$, 0.3, $p < 0.01$) and seed number (0.2, $p < 0.01$, 0.3, $p < 0.01$) in G × T and T x G progeny respectively.

Song (2014) found a lower correlation between berry weight and seed traits (0.2, $p < 0.01$) in T x G progeny.

QTL for seed traits were identified in LG2 for both populations and on LG3, LG5 and LG18 (T × G) and on LG17 (G × T) with different LOD and reproducibility. The *Sex* locus region in LG2 harbours a QTL for seed number in T × G (LOD = 3.5, 11.0 %) and G x T populations (LOD = 4.2, 17.8 %) and for seed weight only in T × G population (LOD = 7.7, 35 %), in agreement with Constantini et al. (2008), Doligez et al. (2013), and Royo et al. (2018). Moreover, many studies (Cabezas et al. 2006, Constantini et al. 2008, and Doligez et al. 2002, 2013) have also identified a QTL on LG18, related to the previously cited *SDI* locus. Interestingly, a putative QTL (LOD = 3.2, 10.5 %) in LG18 for seed number in T × G population (2016) co-localizes with the one found by Song (2014,) associated to berry weight (LOD = 3.3, 10.8 %) and co-localizes with *SDI* gene reported by Royo et al. (2018), presumably a candidate for that QTL.

The putative QTL found in LG17 for seed weight SW_17 in G × T population co-localizes with the stable B17_2 QTL in two years for BL, BD and BW. This association was expected as berry diameter is correlated with berry weight (0.9, $p < 0.01$), seed number (0.3, $p < 0.01$), and seed weight (0.5, $p < 0.01$) in the correlation analysis (Table 3.3.3). Although variance explained for SW is 17.4 %, this finding supports the hypothesis of a QTL influencing at the same time seed and berry cell numbers during early development, or maybe that seed growth regulators affect cell expansion as reported by Houel et al. (2015) who found co-localization on LG7 of QTL for seed number and berry weight.

Stable QTL for seed number and weight were detected in LG3 and LG5 only in T x G population. These QTL co-localize with QTL B3 and B5 for berry length (one year), berry diameter (2 years) and berry weight (3 years), suggesting that the genetic control of seed and berry traits is shared. Interestingly, in the hermaphrodite progeny, these QTL explained up to 70 % of seed number, twice the value reported in the whole progeny, confirming the effect of flower sex in this trait. A QTL on LG5 was also found by Doligez et al. (2002) for seed dry matter percentage, and appeared to be linked to *FER* locus region mentioned above. FERONIA (AT3G51550) is a receptor-like protein kinase mapped in LG3 in *Arabidopsis*, with an orthologue, VIT_05s0020g00200 and one paralogue VIT_05s0020g00080 in *Vitis vinifera* LG5. Both genes are within the region of B5 and SN5 QTL, significant for berry traits and seed number in T x G progeny. Both VIT_05s0020g0080 and VIT_05s0020g00360 genes, also placed in the confidence interval of the QTL, present Bucentaurus (BCNT) domain. This domain is characteristic of the BCNT gene family, which in eukaryotes take part in the multiproteic SWR1 complex involved in chromatin organization and epigenetic control of gene expression (Messina et al. 2017, Sun & Luk 2017). Besides, for QTL B3 for berry and SN3 for seed number both in LG3, two candidate genes were identified VIT_03s0091g00280 with a protein-kinase activity and VIT_03s0097g00530 one orthologue of *Arabidopsis thaliana* AT5G09590 gene associated to mitochondrial protein cellular response to heat.

QTL peak in LG5 correspond to VMC3C7 marker, and in the QTL interval apart from *FER* locus, other candidate genes were identified: VIT_05s0029g00680 one ortholog of Beta-galactosidase *Arabidopsis thaliana* AT2G32810 gene with a function related to cell wall, integral component of the membrane, and VIT_05s0029g01310 an actin-related protein, putatively involved in actin filament polymerization, and thus in actin cytoskeleton that is responsible for mediating various important cellular processes such as cell structural support.

The fact that in T x G progeny, QTL on LG3 and LG5 were associated to both berry and seed traits agrees with the widely accepted positive correlation between berry size and seed content, due to a positive association between these traits within seeded cultivars (May 2000, Walker et al. 2005, Friend et al. 2009).

QTL analysis of flower morphology

A relationship between flower morphology and final berry size has been reported by Houel et al. (2013), Fernández et al. (2013) and Nicolas et al. (2013) based on the strong correlation observed between berry weight at veraison, berry size at ripeness and ovary size. Table 3.3.3 lists moderate significant associations between berry weight (BW) and flower diameter (FD), ovary length (OL) and pistil length (PL) ($r = 0.3 - 0.6$, $p < 0.01$) in both populations, being higher in T x G progenie. Besides, pistil shape (PS) resulted associated with berry length (BL), pistil length (PL) and berry shape (BS) (0.2 - 0.4, $p < 0.01$) (Table 3.3.3).

The main hypothesis for these relationships is that processes of cell division around flowering time and cell expansion after anthesis are important components of the variation in the final berry size observed among grape cultivars (Coombe 1992, Gray & Coombe 2009, Houel et al. 2013, Fernandez et al. 2013, Nicolas et al. 2013) as it happens in watermelon, where final fruit shape is similar to pistil shape (Périn et al. 2002). In tomato some genes associated to berry size have a role in the modification of pistil shape before anthesis (OVATE, SLELF1) (Liu et al. 2002, Chusreeaeom et al. 2014) and its development post-anthesis (SUN) (Wu et al. 2011). A similar result has been reported in pepper in other works (Chaim et al. 2003, Borovsky & Paran 2011, Tsaballa et al. 2011). A study by Mejía et al. (2011), proposed VviAGL11 gene, an ortholog of a MADS-box gene involved in ovule differentiation in Arabidopsis and petunia, as candidate for berry weight, suggesting again a relationship between ovary and berry size and shape. In a recent work, Chialva et al. (2016) also identified VviANT1 as a putative transcription factor associated with the determination of the final berry size through its relationship with cell division during the flower development. In their study they discovered that VviANT1 mRNAs accumulate predominantly in the inflorescences, while its expression patterns strongly correlate with number of cells/ovary, the ovary size and the final berry size. In this way, flowers with larger ovaries conducted to larger berries, although pistil perimeter did not change. All these considerations justify the interest of studying flower morphology in order to assess its impact in final berry morphology. Chialva et al. (2016), found in their work that berry size was more determined by ovary size than by seed number per berry, due to the presence of greater number of cells per ovary in larger berry plants. This relationship between berry size and ovary size is more stable than with seed number in both genetic backgrounds studied here.

Significant QTL for flower traits were identified in LG2, LG8, LG11 and LG14 in both populations, and on LG5 and LG18 only for T × G and LG 13 only on G × T. The major QTL on LG11 was significant for all the flower morphology traits studied, explaining up to 30 % and 27 % of the phenotypic variance in G × T and T × G, respectively, consistently in two years and in different plots. This result agrees with Chialva et al. (2016), that located one candidate gene VviAIL5 regulating ovary cell division associated to a QTL on LG11. In their study that QTL co-localizes with a stable region for mean berry weight (Doligez et al. 2013). In the present work, a significant QTL on LG11 identified in G × T progeny, co-localizes with QTL for berry shape and berry weight in one year. However, VviAIL5 proposed by Chialva et al. (2016), is not a candidate gene for the QTL on LG11 for flower morphology for both progenies. QTL FD11 associated to

flower traits in both progenies and explaining up to 30 % of the variance of flower morphology-related traits as pistil length, flower diameter, ovary length and pistil shape resulted located close to VVS2 marker in T x G progeny. A candidate gene VIT_11s0016g04100.t01 gene, ARF guanine-nucleotide exchange factor with a function linked to pollen tube morphology and pollen germination, was identified. Also in the same region, VIT_11s0016g03650 gene was located, it is an ortholog of AT4G28980 protein gene described in *Arabidopsis thaliana* with a putative protein kinase activity. AT5G39000, a gene paralogue of FERONIA has one orthologue in *Vitis vinifera* VIT_08s0040g00010 situated in the region of the QTL PS8_2 detected in G x T progeny for pistil shape trait.

In the same population, B17_2 a significant region in LG17 for berry traits co-localize with a putative QTL found for ovary length (OL). Another QTL on LG14 was also detected in both populations associated mainly to flower diameter (19 % of variance explained for T × G). Margueritt et al. (2009) also reported two QTL on LG14 and LG17 associated to inflorescence morphology. In the searching of candidate genes for these QTL regions in LG14 and LG17, two genes were found VIT_14s0083g01030, and VIT_17s0000g04990 of the Agamous-like MADS-box (AT5G60910) gene in *Arabidopsis thaliana*, associated to VviAGL11 gene with a function related to ovule differentiation and berry size (Mejia et al. 2011). Besides, AT5G38990 gene present one orthologue in LG14 in *Vitis vinifera* VIT_14s0068g00010 that is situated in the region of the QTL obtained in T x G progeny for flower morphology traits as pistil length, flower diameter or ovary length.

Remarkably, QTL regions found in LG18 and LG5 for berry diameter and berry weight traits co-localize with pistil length (PL) and flower diameter (FD), being QTL on LG5 more robust. QTL on LG5 related with *FER* locus, co-localizes with regions for pistil length (PL), flower diameter (FD), berry length (BL), berry diameter (BD), berry weight (BW), seed number (SN) and seed weight (SW), in T × G population suggesting that the genetic codification of these traits is somehow shared. Putative QTL on LG18 have been also reported by Margueritt et al. (2009) related to wing morphology in the inflorescence. Moreover, Chialva et al. (2016) reported that gene VviANT1, also associated to ovary cell division, co-localizes in LG18 with a stable QTL detected for berry weight in both table grape (Cabezas et al. 2006) and wine grape segregating progenies (Doligez et al. 2013).

Regarding flower shape, a QTL on LG2 in the *Sex* locus region was detected in both populations. This QTL on LG2 was also reported in the work of Margueritt et al. (2009), associated to other flower morphology traits such as filament length, number of inflorescences per shoot and flowering date. In that work, the analysis with flower SEX as cofactor triggered the detection of more QTL. In this region of LG2 VIT_02s0241g00040 gene with deoxyribodipyrimidine photo-lyase activity related to AT1G12370 gene in Arabidopsis thaliana was found, or VIT_02s0025g05110 gene inside MATE efflux family protein DTX1-like, with membrane functions. Here, when female plants were removed, new QTL were detected in LG15 and LG10 related to pistil length and shape respectively. Margueritt et al. (2009), also cited a QTL on LG10 associated to flower morphology. However, the main influence of *Sex* locus was found associated with ovary shape. In both populations four QTL were detected on LG2, LG7, LG8, and LG11 when all the progeny was considered, and only one QTL on LG5 for T × G population and one in LG11 for G × T when female plants were removed from the analysis.

QTL analysis of flowering date

Phenology traits are particularly difficult to analize because they are controlled by many loci such as regulation of flowering time (Costantini et al. 2008). A recent study (Kamal et al. 2019) has confirmed the role of VvFT/TFL1 gene family in the timing of flowering. The fact that the expression of VvFT (ortholog of *A. thaliana* FLOWERING LOCUS T) or VvFL (ortholog LEAFY) has been associated to flowering date, the development of inflorescences, flowers, and fruits (Joly et al. 2004, Sreekantan et al. 2006), suggests a relationship between flower date, flower and berry traits.

However, according to Table 3.3.3 no consistent relationships were found between flowering date and berry and/or flower traits in the populations studied. Flowering (F) appeared positively correlated with flower related traits such as pistil length (PL) ($r = 0.5$, $p < 0.01$) in G × T progeny but negative correlated in T × G population (- 0.5 $p < 0.01$). No consistent QTL were identified in this analysis for both populations. QTL on LG2, LG5, LG7 and LG14 for T × G and LG10 and LG15 for G ×T represented small effects, the highest explaining only 16 % of the variance (LG14). The fact that Graciano is a late variety and Tempranillo an early one explains the higher number of QTL detected compared with G × T progeny.

The QTL on LG2, detected for Flowering (F) in T × G (when all the progeny was considered in the analysis), was located at marker VMD34, likewise Costantini et al. (2008), suggesting an association between SEX marker, flowering date and seed traits. The putative QTL on LG7 for flowering time, was also identified by Song (2014), Duchene et al. (2012) and Grzeskowiak et al. (2013). A locus region in LG7 contains genes related to flower morphology as the before mentioned VvFT (FLOWERING LOCUS T) (Carmona et al. 2007, Díaz-Riquelme et al. 2009, Kamal et al. 2019). In T × G progeny, the putative QTL on LG7, co-localizes with the putative QTL region found for ovary shape and pistil shape, what supports the hypothesis that genes of VvFT family are involved in flowering time and also in flower development (Kamal et al. 2019). This is also supported by the correlations between flower diameter, pistil length, pistil shape and flowering date (0.4, $p < 0.01$) in G × T progeny. However, the region of this putative QTL on LG7 not correspond with the ortholog gene VIT_07s0129g00650 of VvFT previously mentioned to be related to FLOWERING LOCUS T in *Arabidopsis thaliana.*

Two more putative QTL were also found in LG10 as reported by Fechter et al. (2014) and LG15 (Carreño-Ruiz 2012). QTL regions associated to these traits have been also identified in chromosomes 1, 6, 14 or 18, supporting the hypothesis of a complex trait with numerous genes involved in flower development and flowering date (Fechter et al. 2014). In this study the QTL found in LG10 for Flowering (F) date co-localizes with the region found for berry length (BL) and berry shape (BS). This result is supported by the correlations between berry shape (BS), BL (0.4, $p < 0.01$) and F (0.4, $p < 0.01$).

The detection of a great number of QTL with small effect and not consistent across years, could be related to large environmental variation. Weather conditions were extremely different, being 2014 a low productivity year due to the impact of powdery-mildew, 2016 a very productive one, and 2017 a really warm year. Alternate bearing has not been reported in grapevine, but in the light of the results of these years, it could be a plausible hypothesis, that affected the detection of more stable QTL.

Conclusions

This is the first study in the detection of QTL for berry and flower morphology traits and seed parameters in two wine-grape segregant populations, and to assess the influence of *Sex* locus in these parameters. QTL with different level of significance were detected for berry diameter and berry weight in LG3 and LG18, and for berry shape in LG1 and LG9 in both genetic backgrounds. QTL on LG17 resulted highly significant related to berry traits in G × T progeny, whilst a significant effect was detected in LG3 and LG5 in T × G progeny related to berry and seed traits. Concerning flower morphology, QTL on LG11 and LG14 were detected for flower diameter (FD), in LG8 and LG11 for ovary length (OL), in LG11 for pistil shape (PS) and in LG18 related to seed traits in both populations. The influence of *Sex* locus was confirmed in traits like pistil shape, ovary shape and seed number. From the study of candidate genes, QTL on LG18 for T x G progeny associated to seed traits resulted in the same region of locus *SDI*, and QTL on LG5 for berry, seed and flower traits in T x G progeny covered the region of *FERONIA* locus, presenting orthologues in LG8 and LG14 also associated to the significant region found for flower morphology traits in this work. A candidate gene VIT_11s0016g03650 with a function associated to pollen morphology is proposed associated to the highly significant QTL detected in LG11 for flower traits in both progenies. These results contribute to the elucidation of the genetic control of berry, seed and flower morphology- traits and may support decision making in grapevine breeding program.

Supplementary material

Supplementary material 3.3.1. a. Summary of significant (LOD > GW) and stable (reproducible in at least two years/environments) QTL detected in G × T progeny (130 genotypes).

	LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD	1-LOD	CW	GW	% Var	KW
BL	**8**	TE	2015	UR	27.9	BL8	8_13549535	1.9	20.4-28.2	1.7	3.3	7.6	3
		TE	2016	V	27.9	BL8	8_13549535	1.9	20.4-28.2	1.7	3.3	7.6	2
		CON	2015	UR	40.1	BL8	8_16288664	3.5	37.6-43.1	3.0	4.9	14.2	1
	17	GAR	2015	UR	46.2	BL17_1	17_9096321	2.1	17.2-25.1	1.8	3.2	9.0	4
		GAR	2016	UR	46.2	BL17_1	17_9096321	2.6	18.9-22.7	1.8	3.2	18.0	6
		CON	2016	UR	51.5	BL17_1	17_8987545	3.2	45.1-49.9	3.2	4.8	21.6	4
		CON	2015	UR	5.9	BL17_2	17_3259402	3.2	5.4-30.7	3.1	4.7	16.0	3
		CON	2016	V	26.4	BL17_2	17_3936107	5.5	9.4-30.8	3.1	4.7	22.5	4
		GAR	2016	V	11.6	BL17_2	17_2887367	4.2	4.9-20.9	1.8	3.2	17.7	7
	18	GAR	2015	UR	35.9	BL18	18_13651383	2.2	27.7-45.8	2.1	3.2	9.1	4
		GAR	2016	UR	30.9	BL18	18_9890724	1.9	29.2-31.4	1.9	3.2	13.4	2
		CON	2015	UR	40.5	BL18	18_8778958	3.2	38.9-51.7	3.2	4.7	12.1	2
BD	**15**	TE	2014	UR	23.7	BD15	15_13788169	2.1	19.9-42.7	2.0	3.4	7.4	3
		TE	2016	UR	59.1	BD15	15_19195249	2.3	36.4-61.6	2.0	3.5	15.8	3
		CON	2016	UR	24.1	BD15	15_14596752	3.5	19.3-28.4	3.2	4.8	23.0	2
		CON	2014	UR	24.1	BD15	15_14596752	4.1	19.3-28.4	3.2	4.8	15.1	3
	17	CON	2016	V	26.4	BD17_2	17_3936107	5.5	9.9-30.4	3.1	4.7	22.2	4
		GAR	2016	V	15.4	BD17_2	17_4156201	3.9	4.9-20.9	1.8	3.2	16.6	7
BS	**1**	CON	2015	UR	47.7	BS1	1_8668231	3.2	47.5-49.6	3.1	5.1	13.2	2
		CON	2016	V	47.7	BS1	1_8668231	3.3	47.5-49.6	3.2	4.9	14.0	3
		TE	2015	UR	63.1	BS1	1_11602411	2.2	55.4-67.6	2.0	3.5	8.3	2
		GAR	2016	V	31.3	BS1	1_9035171	2.2	42.7-51.6	2.0	3.3	9.7	4
	6	CON	2015	UR	52.1	BS6	6_12192504	3.1	49.7-54	3.0	5.1	12.4	3
		CON	2016	V	53.5	BS6	6_9366264	4.8	49.7-53	3.3	4.8	19.9	4
		TE	2016	V	58.9	BS6	6_10945251	2.5	55.2-58.9	2.1	3.6	12.8	3
		GAR	2016	UR	82.3	BS6	6_18797523	1.8	77.4-83.7	1.7	3.1	12.8	3
	9	CON	2015	UR	34.8	BS9	9_5017151	4.1	25.9-55.8	3.1	4.8	14.9	2
		GAR	2015	UR	37.4	BS9	9_9402637	2.5	31.7-42.5	1.8	3.1	10.3	4
		GAR	2016	UR	29.4	BS9	9_7542886	1.9	30.9-40.4	1.7	3.1	13.1	4

Continue

	LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD	1-LOD	CW	GW	% Var	KW
BS	**10**	CON	2015	UR	61.6	BS10_1	10_8296023	3.7	49.9-62.6	3.6	5.1	15.1	7
		GAR	2015	UR	19.7	BS10_1	10_8296023	3.4	15.3-21.3	1.8	3.1	14.1	7
		CON	2015	UR	50.4	BS10_2	10_5307494	3.3	48.8-52.1	3.6	5.1	13.7	7
		CON	2016	UR	50.4	BS10_2	10_5307494	3.5	48.8-52.1	3.3	4.8	22.9	6
		GAR	2015	UR	10.6	BS10_2	10_5307494	3.2	6.4-13.7	1.8	3.1	13.3	7
		GAR	2016	UR	10.6	BS10_2	10_5307494	2.5	6.4-13.7	1.8	3.1	17.1	7
		GAR	2016	V	10.6	BS10_2	10_5307494	2.9	1.9-13.7	1.9	3.1	10.1	4
	15	CON	2014	UR	25.2	BS15	15_13788169	3.5	24.1-29	3.3	4.8	12.9	2
		GAR	2014	UR	37.8	BS15	15_13814474	2.8	32.6-38.3	1.8	3.1	10.7	7
		GAR	2015	UR	38.3	BS15	15_14341194	2.4	33.3-389	1.9	3.1	10.0	4
BW	**17**	GAR	2015	UR	32.3	BW17_1	17_9096321	3.2	38.3-49.5	1.9	3.2	10.7	7
		GAR	2016	UR	46.2	BW17_1	17_9096321	2.1	43.2-49.0	1.9	3.2	13.2	4
		GAR	2016	V	30.3	BW17_1	17_6994088	2.2	30-39.0	1.8	3.1	9.6	4
		CON	2016	V	47.6	BW17_1	17_9346152	3.6	51.5-56.1	3.1	4.8	15.3	2
		GAR	2016	V	15.4	BW17_2	17_4156201	3.7	13.3-19.9	1.8	3.1	15.7	7
		CON	2016	V	26.5	BW17_2	17_3304673	4.8	21.9-29.1	3.1	4.7	19.8	7
	18	GAR	2015	UR	30.9	BW18	18_13651383	3.5	29.9-49.3	2.0	3.2	13.3	4
		GAR	2017	UR	31.7	BW18	18_17293665	2.5	27-36.9	1.9	3.2	10.0	1
		CON	2015	UR	48.5	BW18	18_10207857	5.1	36.2-53.9	3.2	4.8	16.7	2

Significant QTL were highlighted in red. LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome-wide (CW) and genome-wide (GW) LOD threshold ($p < 0.05$), % Var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal-Wallis significance level, given by the p-value ("1": 0.1, "2": 0.05, "3": 0.01, "4": 0.005, "5": 0.001, "6": 0.0005, "7": 0.00001; "-": no significance). BL berry length, BD berry diameter, BS berry shape, BW berry weight.

Supplementary material 3.3.1 b. Summary of putative (LOD > CW) QTL in G × T progeny (130 genotypes).

Traits	LG	Map	Year	Plot	Position (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD threshold 0.95		% Var. expl	KW
										CW	GW		
BL	15	CON	2016	UR	56.800	BL15	15_19677720	3.5	55.2-58.7	3.5	4.8	23.1	3
		TE	2016	UR	61.301	BL15	15_19573227	2.2	56.9-62.7	1.9	3.5	15.4	3
BD	8	TE	2016	UR	44.901	BD8	8_16587973	2.0	33.7-48.4	1.9	3.2	8.8	2
		CON	2015	UR	40.113	BD8	8_16288664	3.1	39.4-44.8	3.0	4.8	12.7	1
	17	CON	2016	UR	51.451	BD17_1	17_8987545	3.2	45.1-49.9	3.1	4.7	21.6	4
		GAR	2016	UR	34.447	BD17_1	17_7211570	2.7	31.4-35.5	1.8	3.2	18.1	7
	18	CON	2015	UR	40.458	BD18	18_8778958	3.2	38.9-51.7	3.1	4.7	12.7	2
		GAR	2015	UR	35.919	BD18	18_13651383	2.4	27.8-45.9	1.9	3.2	10.1	2
BS	8	CON	2016	V	30.215	BS8	8_12530373	3.8	25.4-31.4	3.2	4.8	15.8	7
		GAR	2016	V	34.936	BS8	8_12280700	3.0	31.7-37.4	1.9	3.2	12.8	7
BW	11	TE	2016	UR	62.418	BW11	11_8368537	3.1	59.2-67.2	2.3	3.7	19.2	7
		CON	2016	UR	62.444	BW11	11_10541565	3.9	57.5-66.1	3.3	4.7	23.5	4
	16	GAR	2016	V	56.900	BW16	16_19309935	4.1	48.3-61.8	1.8	3.1	17.2	7
		CON	2016	V	43.233	BW16	16_18020389	4.9	39.6-55.5	3.2	4.8	20.3	7
PL	10	**CON**	2015	UR	11.845	PL10	-1_15201277	4.6	6.1-15.1	3.7	7.1	15.3	5
		TE	2016	V	23.872	PL10	10_1618757	3.0	20.9-27.4	1.7	3.3	11.8	7
FD	14	**GAR**	2015	UR	81.046	FD14	14_27351673	2.7	70.5-84.3	1.9	3.2	9.1	6
		TE	2016	V	58.174	FD14	14_24821273	2.7	54.4-63.6	2.3	3.6	10.7	6
		CON	2016	V	71.208	FD14	14_26684001	3.4	62.7-73.9	3.5	4.7	13.2	6
OL	13	**GAR**	2016	V	53.445	OL13	13_19664441	2.0	49.2-55.1	1.8	3.2	7.9	3
		CON	2016	V	68.693	OL13	13_19848842	4.0	65.5-69.7	3.2	4.8	15.2	4
	17	**GAR**	2016	V	21.7	OL17	17_5296971	3.2	17.5-22.7	1.8	3.3	12.3	6
		CON	2016	V	31.245	OL17	17_5296971	3.3	26.5-31.6	3.1	4.8	13.9	6
PS	7	**CON**	2016	V	62.672	PS7	-1_26957175	4.2	52.2-71.4	3.2	4.6	16.0	4
		GAR	2016	V	75.662	PS7	-1_11278013	2.4	71.7-83.7	1.9	3.3	9.5	4
OS	7	**CON**	2016	V	63.845	OS7	-1_29865398	3.8	47.8-74.2	3.3	4.7	14.6	2
		GAR	2016	V	39.609	OS7	7_6270357	2.6	31.8-54.7	2	3.2	10.2	5
F Date	10	**CON**	2016	V	42.684	F10	10_2524063	4.5	30-51.8	3.1	4.9	16.6	6
		TE	2016	V	23.872	F10	10_1618757	2.5	20.4-29	1.7	3.5	9.3	7
		GAR	2016	V	44.739	F10	10_2524063	2.1	41.4-44.7	1.8	3.1	8.1	6
	15	**GAR**	2016	V	57.151	F15	15_11318578	3.5	48.6-64.8	1.9	3.1	13.1	7
		CON	2016	V	3.809	F15	-1_39397398	4.1	0-34.2	3.4	4.9	15.1	3
SW	12	**GAR**	2015	UR	0.000	SW12	12_3448072	1.9	0.0-13.4	1.8	3.1	7.9	3
		CON	2015	UR	2.000	SW12	12_238801	3.8	0.0-8.7	3.2	4.7	15.6	5
	17	**TE**	2016	UR	25.771	SW17	17_4051401	2.3	21.4-28.1	2.1	3.4	10.3	4
		GAR	2016	UR	10.800	SW17	17_3161035	1.7	4.9-11.7	1.6	3.1	7.6	2
		CON	2016	UR	26.417	SW17	17_3936107	4.1	15.3-27.1	3.1	4.9	17.4	4

LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome-wide (CW) and genome-wide (GW) LOD threshold ($p < 0.05$), % Var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal-Wallis significance level, given by the p-value ("1": 0.1, "2": 0.05, "3": 0.01, "4": 0.005, "5": 0.001, "6": 0.0005, "7": 0.00001; "-": no significance).

Supplementary material 3.3.2.a. Summary of significant (LOD > GW) and stable (reproducible in at least two years/environments) QTL detected in G × T hermaphrodite population (102 genotypes).

Traits	LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD threshold 0.95		% Var. expl	KW
										CW	GW		
BL	17	GAR	2015	UR	22.262	B17_1	17_8779899	2.3	20.8-22.6	1.8	3.2	12.2	4
		GAR	2016	UR	36.453	B17_1	17_6994088	2.5	20.3-37.7	1.8	3.2	21.5	3
		CON	2016	UR	51.500	B17_1	17_8987545	3.3	45.3-49.8	3.2	4.8	26.0	2
		GAR	2016	V	4.900	B17_2	17_2141301	3.2	0.0-15.4	1.8	3.2	17.6	7
		CON	2015	UR	5.900	B17_2	17_3259402	3.2	5.4-20.7	3.1	4.7	16.0	3
		CON	2016	V	18.668	B17_2	17_2012240	3.7	12.8-26.5	3.2	4.8	19.9	7
	18	GAR	2015	UR	35.919	B18	18_13651383	2.3	34.2-40.7	1.9	3.2	12.4	4
		GAR	2016	UR	48.264	B18	18_10432197	2.8	37.1-56.1	1.8	3.1	23.7	5
		CON	2016	UR	40.458	B18	18_8778958	2.6	38.9-51.7	3.2	4.8	12.1	2
BD	2	CON	2015	UR	15.700	BD2	2_4579843	2.4	15.5-17.7	3.0	4.7	16.2	-
		CON	2016	UR	18.340	BD2	-1_40569945	4.2	15.4-18.6	3.3	4.8	23.9	-
		TE	2016	UR	2.900	BD2	2_726776	1.8	0.0-3.3	2.0	3.3	11.7	-
	3	TE	2015	UR	51.404	B3	3_17524653	2.9	42.5-59.9	2.1	3.4	15.5	4
		TE	2016	UR	51.404	B3	3_17524653	2.4	50.7-52.1	2.1	3.4	20.8	4
		CON	2016	UR	57.805	B3	3_9523848	3.0	51.8-63.0	3.0	4.7	25.3	2
	18	GAR	2015	UR	35.919	B18	18_13651383	2.0	34.3-39.5	1.8	3.2	10.9	3
		GAR	2016	UR	48.264	B18	18_10432197	2.3	47.4-56.3	1.8	3.2	19.9	4
		CON	2016	UR	40.458	B18	18_8778958	2.9	38.9-51.7	3.1	4.7	9.2	2
BS	1	CON	2015	UR	52.565	BS1	1_10724157	3.9	53.4-74	3.5	5.1	20.1	2
		TE	2015	UR	53.919	BS1	1_9936875	3.4	53.3-71.5	2.1	3.2	17.9	4
		TE	2016	V	53.919	BS1	1_9936875	1.8	52.5-66.5	1.8	3.1	10.3	2
	10	CON	2015	UR	61.636	B10	10_8296023	4.8	48.5-65.5	3.6	5.1	23.9	7
		CON	2016	UR	50.432	B10	10_5307494	5.9	50.4-63.1	3.3	4.8	44.0	7
		CON	2016	V	50.432	B10	10_5307494	3.2	50.4-63.1	3.1	4.6	16.0	7
		GAR	2015	UR	7.900	B10	10_5699252	4.6	5.4-18.5	2.1	3.5	23.2	6
		GAR	2016	UR	10.600	B10	10_5307494	3.9	6.4-18.5	2.1	3.4	31.7	6
BW	3	TE	2015	UR	51.404	B3	3_17524653	3.5	47.8-59.3	2.2	3.4	14.4	7
		TE	2016	UR	51.404	B3	3_17524653	2.2	50.7-52.4	2.0	3.3	18.0	4
		TE	2017	UR	48.487	B3	3_8186352	2.2	47.1-58.9	2.0	3.2	11.3	3
		CON	2017	UR	53.966	B3	3_8413122	3.1	51.9-56.1	3.0	4.6	15.6	3
	18	CON	2015	UR	43.200	B18	18_9245197	3.2	38-5-51.7	3.1	4.7	12.5	2
		CON	2016	V	43.200	B18	18_9245197	3.2	38-5-49	3.1	4.7	13.1	2
		GAR	2015	UR	29.900	B18	18_8633419	3.3	28.3-52.1	2.1	3.3	13.9	2
PL	8	CON	2015	UR	48.044	PL8	8_17165461	4.2	45.5-57.8	3.2	6.1	17.4	3
		CON	2016	V	40.113	PL8	8_16288664	6.4	32.8-56.9	3.2	4.7	29.1	6
		TE	2015	UR	56.225	PL8	8_17664677	3.1	53.4-58.6	2.2	3.6	13.1	6
		TE	2016	V	44.901	PL8	8_16587973	4.3	25.8-53.9	2.2	3.3	20.9	7
		GAR	2015	UR	40.234	PL8	8_15058381	2.0	34.9-41.5	1.8	3	10.7	2
	11	CON	2015	UR	12.781	PL11	11_1569201	6.1	7.7-24.8	3.1	6.1	24.2	7
		CON	2016	V	15.854	PL11	11_1694790	4.3	15.2-33.3	3.2	4.7	20.6	4
		TE	2015	UR	14.821	PL11	11_1120935	4.1	7.9-40.5	2.2	3.6	17.2	7
		TE	2016	V	7.881	PL11	11_599295	2.7	7.1-24.8	1.9	3.3	13.5	4

Continue

Traits	LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD threshold 0.95		% Var. expl	KW
FD	11	CON	2015	UR	43.045	FD11_1	11_5758657	4.1	32-59.8	3.2	4.7	17.1	4
		CON	2016	V	50.109	FD11_1	11_6736262	3.9	35.5-59.8	3.2	4.7	18.9	6
		TE	2015	UR	50.130	FD11_1	11_6333949	3.7	36-58.9	2	3.4	15.4	7
		TE	2016	V	51.660	FD11_1	11_5911892	2.9	33-58.9	2.1	3.6	14.7	6
		CON	2015	UR	12.898	FD11_2	11_1466955	6.6	7.8-31.9	3.2	4.7	25.8	7
		CON	2016	V	15.854	FD11_2	11_1694790	8.1	7-35.3	3.2	4.7	36.0	6
		TE	2015	UR	14.821	FD11_2	11_1120935	5.0	11.6-32.5	2	3.4	20.2	6
		TE	2016	V	16.957	FD11_2	11_1245158	4.8	9.9-37.4	2.1	3.6	26.0	7
	13	CON	2016	V	67.562	FD13	-1_2647261	4.5	61.1-68.5	3.2	4.8	21.8	2
		GAR	2016	V	53.445	FD13	13_19664441	2.0	48.3-55	1.8	3.2	10.4	4
		GAR	2016	V	35.608	FD13	13_12748868	3.0	35.1-39.5	1.8	3.2	14.8	3
OL	8	CON	2015	UR	54.809	OL8	8_18403932	4.0	48-57	3.2	4.8	16.8	2
		TE	2015	UR	56.225	OL8	8_17664677	3.3	50.9-58	2.2	3.4	14.0	7
		TE	2016	UR	44.901	OL8	8_16587973	2.5	41.3-58.8	2.1	3.4	12.5	4
	11	CON	2015	UR	46.681	OL11_1	11_5210688	3.6	36-58.9	3.2	4.8	15.3	6
		TE	2015	UR	51.660	OL11_1	11_5911892	3.2	35.5-59.8	2.2	3.3	13.4	6
		CON	2015	UR	12.781	OL11_2	11_1569201	5.4	8.1-31.9	3.2	4.8	21.9	6
		CON	2016	V	15.854	OL11_2	11_1694790	4.0	14-22	3.2	4.7	19.4	5
		TE	2015	UR	14.821	OL11_2	11_1120935	4.0	7.2-32.5	2.2	3.3	16.7	6
		TE	2016	V	12.268	OL11_2	11_814957	2.1	5.7-12.6	2	3.3	10.9	4
PS	11	CON	2015	UR	15.854	PS11	11_1694790	3.7	14.9-17.7	3.2	4.7	18.2	3
		CON	2016	V	12.898	PS11	11_1466955	3.8	8.7-22.8	3.2	4.7	18.4	4
		TE	2016	V	20.053	PS11	11_1466955	2.1	17.2-22.4	2	3.3	10.8	4

Significant QTL were highlighted in red. LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome-wide (CW) and genome-wide (GW) LOD threshold ($p < 0.05$), % Var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal-Wallis significance level, given by the p-value ("1": 0.1, "2": 0.05, "3": 0.01, "4": 0.005, "5": 0.001, "6": 0.0005, "7": 0.00001; "-": no significance). BL berry length, BD berry diameter, BS berry shape, BW berry weight, PL pistil length, FD flower diameter, OL ovary length, PS pistil shape.

Supplementary material 3.3.2.b. Summary of putative (LOD > CW) QTL in G × T Hf progeny (102 genotypes).

	LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD threshold 0.95		% Var. expl	KW
										CW	GW		
BL	10	CON	2016	UR	38.849	BL10	-1_36434224	3.4	38.8-53.2	3.2	4.7	8.0	-
		GAR	2016	UR	1.900	BL10	10_2707460	2.5	0.0-15.3	1.8	3.2	22.0	4
BD	9	CON	2014	UR	48.733	BD9	9_3064943	3.7	43.7-59.9	3.1	4.8	16.5	4
		GAR	2014	UR	52.797	BD9	9_2958692	3.3	48.3-59.6	1.8	3.2	14.8	7
	17	CON	2016	UR	51.500	BD17_1	17_8987545	3.4	43.1-50.3	3.1	4.8	28.0	2
		GAR	2016	UR	36.453	BD17_1	17_6994088	3.2	31.7-38.3	2.0	3.2	26.9	4
		CON	2016	V	26.471	BD17_2	17_3304673	3.6	15.6-26.7	3.1	4.7	19.4	7
		GAR	2016	V	61.798	BD17_2	17_2141301	3.0	38.4-62.9	2.0	3.2	16.5	7
BS	3	TE	2015	UR	20.157	BS3	3_3253543	3.2	18.9-25.7	2.2	3.4	16.8	5
		CON	2015	UR	23.122	BS3	3_5097640	3.6	20.1-26.3	3.1	4.8	18.1	7
		GAR	2016	UR	4.000	BS3	3_2132387	2.2	3.6-16.7	2.0	3.2	19.2	4
	9	CON	2014	UR	50.298	BS9	9_2721760	4.2	33.8-50.7	3.1	4.8	18.5	2
		GAR	2014	UR	57.187	BS9	9_1879718	3.3	43.7-61.1	1.8	3.2	14.8	6
	11	CON	2016	UR	15.854	BS11	11_1694790	4.2	10.2-20.4	3.3	4.7	33.5	7
		TE	2016	UR	20.053	BS11	11_1466955	3.4	14.8-24.9	2.3	3.7	28.4	4
	16	CON	2016	UR	76.760	BS16	16_21734101	3.6	75.1-76.8	3.2	4.8	29.5	5
		TE	2016	UR	72.326	BS16	16_21734101	3.1	67.2-72.5	2.1	3.4	26.0	3
BW	2	CON	2016	V	18.200	BW2	2_8125744	2.1	16.4-20.4	3.2	4.8	8.0	-
		TE	2016	V	23.473	BW2	-1_12236882	2.9	21.3-23.4	2.1	3.4	15.5	2
	11	TE	2016	UR	62.418	BW11_1	11_8368537	2.9	53.1-62.7	2.1	3.4	22.8	7
		CON	2016	UR	62.444	BW11_1	11_10541565	4.2	50.1-66.1	3.2	4.7	30.9	3
		CON	2017	UR	38.218	BW11_2	11_4929080	3.1	35.7-41.5	3.1	4.6	15.6	1
		GAR	2017	UR	4.900	BW11_2	11_4447089	2.4	0.0-9.9	1.9	3.1	11.9	5
	12	CON	2014	UR	73.300	BW12	12_20150176	2.5	69.9-76.8	3.2	4.7	15.1	2
		GAR	2014	UR	58.100	BW12	12_20527469	1.8	56.4-59.2	1.8	3.1	7.9	2
	17	CON	2016	V	26.471	BW17	17_3304673	3.5	25.2-26.5	3.3	4.8	18.7	4
		GAR	2016	V	16.200	BW17	17_4575721	2.5	10.8-19.9	2.0	3.2	13.8	7
PL	10	CON	2015	UR	18.997	PL10	-1_10633306	4.2	12.4-19.6	3.6	6.1	17.5	4
		TE	2016	V	23.872	PL10	10_1618757	2.2	20.7-25.1	1.8	3.3	11.3	5
	15	CON	2015	UR	24.6	PL15	15_15060974	3.9	19.3-28.4	3.3	4.8	16.2	2
		TE	2015	UR	14.256	PL15	15_10162460	2.3	13.5-16.4	2.1	3.3	9.8	4
OL	13	CON	2016	V	68.693	OL13	13_19848842	4.1	66-69.2	3.2	4.8	19.9	4
		GAR	2016	V	53.445	OL13	13_19664441	2.2	48.3-55	2	3.3	11.1	4
	17	CON	2016	V	17.817	OL17	17_2254157	4.0	15.3-18.7	3.2	4.7	19.5	4
		GAR	2016	V	4.900	OL17	17_2141301	2.7	2.9-13.3	1.9	3.3	14.0	4
PS	7	CON	2016	V	62.672	PS7	-1_26957175	4.0	61.6-63.7	3.2	4.7	19.3	4
		GAR	2016	V	75.662	PS7	-1_11278013	2.0	75.4-83.7	1.8	3.1	10.2	4
	11	CON	2016	V	15.854	OS11	11_1694790	3.7	7.2-20.6	3.2	4.7	19.4	6
		TE	2016	V	16.957	OS11	11_1245158	2.7	11.9-29	2.2	3.6	13.7	5

Continue

	LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD threshold 0.95		% Var. expl	KW
F	7	CON	2014	UR	46.273	F7	-1_1108704	3.2	45.9-50.8	3.0	4.6	18.3	2
		TE	2014	UR	48.503	F7	-1_19613096	2.2	43.9-48.4	2.1	3.4	14.4	2
	10	CON	2016	V	42.684	F10	10_2524063	4.3	38.8-55.5	3.3	4.8	20.1	4
		GAR	2016	V	44.739	F10	10_2524063	2.2	43.8-45.2	1.9	3.2	10.9	4
		TE	2016	V	23.872	F10	10_1618757	2.1	22.5-27.7	2.0	3.3	10.0	4
SN	**1**	GAR	2016	V	52.758	SN1	1_9936972	2.5	48.3-56.1	1.9	3.3	13.9	4
		CON	2016	V	47.036	SN1	1_8440819	3.5	45.6-54.3	3.3	4.7	19.0	4
	6	CON	2015	UR	40.281	SN6	6_6463639	3.3	38.5-42.4	3.2	4.7	21.0	4
		TE	2015	UR	43.300	SN6	6_5519184	3.2	30.4-48.2	2.1	3.5	20.3	6
		TE	2016	V	30.400	SN6	6_4130249	2.3	28.8-40.3	2	3.5	12.7	4
SW	**12**	GAR	2015	UR	8.400	SW12	12_3909227	2.2	0-10.5	1.9	3.1	11.7	4
		CON	2015	UR	2.000	SW12	12_238801	3.6	0-15.1	3.2	4.7	18.3	7
	13	TE	2015	UR	5.466	SW13	13_3017582	2.1	3.6-8.2	2	3.3	11.0	4
		CON	2015	UR	15.011	SW13	13_3017582	3.6	2.9-16	3.3	4.8	18.5	4
	17	CON	2016	V	26.417	SW17	17_3936107	3.3	24.3-26.7	3.1	4.9	17.4	2
		TE	2016	V	25.771	SW17	17_4051401	2.3	21.9-28.1	2.1	3.4	13.5	3
	18	TE	2016	V	0.000	SW18	18_9650943	2.1	0.0-4.1	1.9	3.2	9.0	2
		CON	2016	V	43.200	SW18	18_9245197	3.8	40.5-44.1	3.3	4.7	20.0	2

Significant QTL were highlighted in red. LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome-wide (CW) and genome-wide (GW) LOD threshold ($p < 0.05$), %var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal-Wallis significance level, given by the p-value ("1": 0.1, "2": 0.05, "3": 0.01, "4": 0.005, "5": 0.001, "6": 0.0005, "7": 0.00001; "-": no significance). BL berry length, BD berry diameter, BS berry shape, BW berry weight, PL pistil length, FD flower diameter, OL ovary length, PS pistil shape, F flowering date, SN seed number, SW seed weight.

Supplementary material 3.3.3.a. Summary of significant (LOD > GW) and stable (reproducible in at least two years/environments) QTL detected in T × G progeny (151 genotypes).

	LG	Map	Year	Pos(cM)	QTL	Marker	LOD	1-LOD	LOD		% Var.	KW
									CW	GW		
BL	1	CON	2016	38.853	BL1	chr1_9996086	5.0	30.7-43.2	3.1	4.8	18.6	2
		GRA	2016	22.673	BL1	vvin61	2.2	20.7-26	1.9	3.0	8.8	4
	3	GRA	2012	39.660	BL3	chr3_5968917	3.4	38.2-69.9	1.9	3.2	14.2	6
		CON	2012	55.467	BL3	chr3_r_294211	5.9	41.1-64.9	3.1	4.8	23.4	7
	5	GRA	2012	65.000	BL5	vmc3c7	4.2	56.2-80.8	2.0	3.2	17.1	6
		CON	2012	64.000	BL5	chr5_19770665	4.6	59.9-77.3	3.1	4.7	18.8	7
BD	3	CON	2012	55.642	BD3	chr3_10844728	6.1	37.0-69.9	3.2	4.8	24.1	7
		GRA	2012	41.500	BD3	chr3_6512337	5.2	22.2-69.9	1.9	3.1	20.9	6
		GRA	2016	46.400	BD3	chr3_7157449	2.6	38.2-64.9	1.9	3.1	10.3	5
	5	CON	2012	63.000	BD5	chr5_14699639	5.6	56.2-67.3	3.3	4.6	22.5	6
		CON	2016	60.800	BD5	vmc3c7	4.3	59.9-65.1	3.2	4.8	16.2	6
		GRA	2012	65.000	BD5	vmc3c7	4.6	56.2-80.8	2.0	3.1	17.5	4
		GRA	2016	65.800	BD5	chr5_14699639	2.2	56.2-70.6	1.9	3.1	8.8	6
BW	3	CON	2012	60.195	BW3	chr3_16593567	6.9	42.1-62.0	3.3	4.8	26.6	7
		GRA	2012	60.200	BW3	chr3_16593567	5.8	41.5-69.9	2.1	3.2	16.5	7
		GRA	2016	46.400	BW3	chr3_7157449	2.7	41.5-60.2	2.0	3.1	10.4	4
	5	CON	2012	60.800	BW5	vmc3c7	5.3	59.9-67.3	3.2	4.8	21.3	6
		CON	2016	60.800	BW5	vmc3c7	4.9	59.9-70.4	3.2	4.7	18.2	6
		GRA	2012	65.000	BW5	vmc3c7	4.9	56.6-80.8	2.1	3.2	19.7	7
		GRA	2016	69.200	BW5	chr5_18607493	2.4	56.2-80.8	2.0	3.1	9.5	4
PL	5	TE	2016	46.941	PL5	chr5_19770665	1.9	42.2-47.8	1.8	3.0	7.5	4
		TE	2017	46.941	PL5	chr5_19770665	2.4	42.3-51.2	2.1	3.0	10.5	4
		CON	2017	47.740	PL5	chr5_19770665	3.3	56.2-65.1	3.3	5.5	13.5	3
	14	GRA	2016	26.250	PL14	vmc5b3	2.4	27.2-35.9	2.0	3.1	9.3	4
		GRA	2017	35.087	PL14	vrzag112	2.8	24.2-35.9	2.1	3.0	11.9	4
		CON	2017	31.456	PL14	vmc2c3	3.2	28-33.8	3.2	5.2	13.8	4
FD	5	CON	2016	60.776	FD5	chr5_7367897	3.2	44-61.4	3.1	4.8	12.2	5
		CON	2017	47.740	FD5	chr5_7253933	3.3	41.5-57.3	3.1	4.6	14.2	2
		TE	2016	29.021	FD5	chr5_6343083	2.1	28.2-35.1	2.0	3.2	8.3	4
		TE	2017	29.021	FD5	chr5_6343083	1.9	28.2-33.4	1.9	3.0	8.5	4
		GRA	2017	57.675	FD5	chr5_7992188	2.2	53.4-64.4	2.2	3.0	9.4	4
	11	CON	2016	21.600	FD11	vvs2	7.7	6.4-33.3	3.3	4.8	26.9	7
		CON	2017	21.600	FD11	vvs2	6.8	6.4-33.3	3.2	4.6	26.9	7
		TE	2016	20.700	FD11	vvs2	5.1	5.2-32.0	2.0	3.2	18.7	7
		TE	2017	20.700	FD11	vvs2	6.3	5.2-32.0	1.9	3.0	25.1	7
		GRA	2016	58.132	FD11	chr11_1548729	3.5	5.1-25.8	2.1	3.1	13.4	6
	14	GRA	2016	29.908	FD14	chr14_22675729	3.0	28-40	2.0	3.1	11.6	7
		GRA	2017	29.908	FD14	chr14_22675729	4.5	22.0-38.6	2.1	3.0	18.6	7
		CON	2017	36.824	FD14	chr14_19756741	4.8	28.0-33.3	3.2	4.6	19.8	6

	LG	Map	Year	Pos(cM)	QTL	Marker	LOD	1-LOD	LOD		% Var.	KW
OL	11	TE	2016	20.700	OL11	vvs2	2.4	5.2-32.0	2.0	3.1	9.2	5
		TE	2017	20.700	OL11	vvs2	3.9	11.9-27.0	2.1	3.1	16.6	7
		CON	2017	21.600	OL11	vvs2	4.2	13.9-28.9	3.2	4.5	17.6	4
	14	GRA	2016	26.250	OL14	vmc5b3	2.4	25.3-40	2.1	3.1	9.3	4
		GRA	2017	29.908	OL14	chr14_22675729	3.4	22.0-35.0	2.0	3.1	14.5	7
		CON	2017	35.975	OL14	chr14_22675729	4.0	29.0-38.8	3.2	4.5	17.0	7
PS	2	CON	2016	23.552	PS2	chr2_5236271	10.7	1.7-37.5	3.3	4.6	35.3	5
		CON	2017	23.552	PS2	chr2_5236271	3.8	15-25	3.2	4.9	16.2	5
		TE	2016	25.868	PS2	chr2_4137690	3.9	22.2-45.0	1.9	3.1	14.6	6
		TE	2017	25.868	PS2	chr2_4137690	2.2	15.5-30.5	1.8	3.0	9.7	5
		GRA	2016	18.356	PS2	chr2_4166541	7.2	9.7-38.3	2.2	3.1	25.5	6
		GRA	2017	26.576	PS2	chr2_5236271	2.9	15-30.2	2.2	3.0	12.5	5
	11	CON	2016	21.600	PS11	vvs2	3.7	12.5-30.2	3.2	4.6	14.1	7
		CON	2017	21.600	PS11	vvs2	6.5	9.7-28.8	3.4	4.9	25.8	7
		TE	2016	20.700	PS11	chr11_2028061	1.9	5.6-23.5	1.8	3.1	7.3	4
		TE	2017	20.700	PS11	vvs2	4.9	3.6-28.9	1.8	3.0	20.2	7
		GRA	2016	58.132	PS11	chr11_1548729	2.0	5.0-12.5	1.9	3.1	7.6	4
OS	2	CON	2016	23.552	OS2	chr2_5236271	7.4	10.2-30	3.3	4.6	26.0	6
		CON	2017	23.552	OS2	chr2_5236271	3.2	20-25	3.1	4.8	13.1	4
		TE	2016	21.822	OS2	VVMD34	2.9	22.4-41.6	1.9	3.0	12.0	4
		GRA	2016	16.262	OS2	VVMD34	4.0	9.5-35.9	2.2	3.0	15.2	7
		GRA	2017	26.576	OS2	chr2_5236271	2.2	22-26	1.9	3.0	9.8	4
	11	CON	2016	42.500	OS11	udv017	3.5	22.0-43.5	3.3	4.6	13.3	4
		CON	2017	23.000	OS11	chr11_4885995	3.2	20.5-25.7	3.1	4.8	13.6	2
		TE	2017	44.713	OS11	vvmd25	2.1	13.2-23.4	1.9	3.0	9.3	4
F	2	CON	2016	26.705	F2	VVMD34	3.2	20.2-27.3	3.1	4.7	9.4	2
		GRA	2016	16.262	F2	VVMD34	2.4	13.3-21.4	2.1	3.1	8.8	5
	5	CON	2017	37.369	F5	vchr5a	3.2	35-38	3.1	4.7	13.1	3
		GRA	2017	37.718	F5	vchr5a	2.2	34-38	2.1	3.1	9.2	4
	7	CON	2016	38.338	F7	chr7_8211796	3.0	52-55	3	4.8	10.2	2-
		TE	2016	50.907	F7	chr7_8211796	2.6	46-55	2	3	9.5	4
	14	CON	2017	34.731	F14	vrzag112	3.9	34-50	3.3	4.7	16.4	5
		GRA	2017	35.087	F14	vrzag112	2.0	34-38	1.8	3.1	7.8	5

Continue

	LG	Map	Year	Pos(cM)	QTL	Marker	LOD	1-LOD	LOD		% Var.	KW
SN	2	**TE**	2009	13.400	SN2	chr2_5662969	2.5	13.4-18.4	1.6	2.9	8.9	4
		TE	2010	13.400	**SN2**	chr2_5662969	3.5	8.1-16.4	1.5	2.9	11.5	6
	3	**CON**	2008	54.900	SN3	chr3_10713706	10.6	31.7-60	3.2	4.7	35.5	7
		CON	2009	54.900	SN3	chr3_10713706	19.7	38.8-60.9	3.1	4.7	52.2	7
		CON	2010	54.900	SN3	chr3_10713706	19.4	30.9-60.9	3.1	4.6	49.9	7
		GRA	2008	54.200	SN3	chr3_10713706	10.5	37.3-65.2	1.7	2.9	35.2	7
		GRA	2009	54.200	**SN3**	chr3_10713706	19.3	30.4-65.2	1.7	2.9	51.4	7
		GRA	2010	54.200	SN3	chr3_10713706	19.2	38.8-65.4	1.6	2.9	48.8	7
	5	**CON**	2008	60.800	SN5	vmc3c7	12.8	48.6-70.8	3.2	4.7	41.3	7
		CON	2009	60.800	SN5	vmc3c7	20.2	59.9-62	3.2	4.7	53	7
		CON	2010	60.800	SN5	vmc3c7	19.1	50.8-64.1	3.1	4.6	48.6	7
		GRA	2008	65.000	**SN5**	vmc3c7	9.4	51.4-70.1	1.9	2.9	32.3	7
		GRA	2009	65.000	SN5	vmc3c7	18.2	52.4-67.2	1.7	2.9	49.5	7
		GRA	2010	65.000	SN5	vmc3c7	16.2	49.5-65	1.8	2.9	43.1	7
SW	2	**CON**	2009	23.600	SW2	chr2_5236271	7.7	16.2-26.7	3.3	4.7	25.1	7
		CON	2010	23.600	SW2	chr2_5236271	10.7	18.1-24.6	3.2	4.6	31.2	7
		GRA	2009	26.600	**SW2**	chr2_5236271	4.6	14.7-36.1	1.6	2.9	15.8	7
		GRA	2010	26.600	SW2	chr2_5236271	5.2	26.6-28.6	1.5	2.9	16.5	7
	5	**CON**	2009	56.500	SW5	chr5_9640285	3.9	56.5-67.2	3.3	4.7	13.4	7
		CON	2010	56.500	SW5	chr5_9640285	4.3	45.3-64.2	3.2	4.6	14	7
		GRA	2009	58.500	SW5	chr5_9640285	3.4	48.5-63.3	1.7	2.9	11.9	7
		GRA	2010	58.500	**SW5**	chr5_9640285	3.3	49.3-58.5	1.9	2.9	9.7	7

Significant QTL were highlighted in red. Song 2014 results are coloured in grey. LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome-wide (CW) and genome-wide (GW) LOD threshold ($p < 0.05$), % Var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal-Wallis significance level, given by the p-value ("1": 0.1, "2": 0.05, "3": 0.01, "4": 0.005, "5": 0.001, "6": 0.0005, "7": 0.00001; "-": no significance). BL berry length, BD berry diameter, BW berry weight, PL pistil length, FD flower diameter, OL ovary length, PS pistil shape, OS ovary shape, F flowering date, SN seed number, SW seed weight.

Supplementary material 3.3.3.b. Summary of putative (LOD > CW) QTL in T × G progeny (151 genotypes).

	LG	Map	Year	Pos (cM)	QTL	Marker	LOD	1-LOD	LOD		% Var	KW
BS	**1**	**CON**	2012	63.736	BS1_1	vvio61	3.8	60.2-65	3.1	4.6	15.7	4
		TE	2016	8.538	BS1_1	vvio61	1.8	8.2-16.5	1.8	3.1	7.3	3
		CON	2016	15.982	BS1_2	chr1_1372222	3.8	10.1-17	3.2	4.7	14.5	3
		GRA	2016	11.173	BS1_2	chr1_6218393	3.0	8.8-14	2.1	3.2	11.7	6
BW	**16**	**CON**	2012	51.608	BW16	chr16_19543957_A_C	3.5	50.5-57	3.2	4.8	14.5	4
		TE	2012	41.772	BW16	chr16_19543957_A_C	2.0	37.8-43.2	1.9	3	8.7	4
PL	**18**	**CON**	2016	54.795	PL18	chr18_9340550	4.1	48.9-61.1	3.2	4.7	15.3	3
		GRA	2016	53.424	PL18	chr18_13410273	2.0	48.3-60	1.8	3.1	7.6	4
FD	**18**	**CON**	2016	51.717	FD18	chr18_7446110_A_C	4.2	48.9-61.1	3.2	4.8	15.8	3
		GRA	2016	49.583	FD18	chr18_12077018	2.0	43.9-51	1.9	3.1	7.8	4
OL	**5**	**CON**	2017	39.218	OL5	chr5_10288195	3.8	36.2-55.4	3.1	4.5	15.9	2
		TE	2017	46.941	OL5	chr5_19770665	2.8	44.3-53.5	2.0	3.1	12.0	4
	8	**CON**	2016	68.764	OL8	chr8_19730855	3.1	65.3-68.4	3.1	4.6	11.6	6
		TE	2016	67.70	OL8	chr8_19730855	2.9	62.7-70.6	1.9	3.1	11.1	6
	18	**GRA**	2016	49.583	OL18	chr18_12077018	2.2	43.9-51	2.0	3.1	8.4	4
		CON	2016	61.065	OL18	chr18_12012282	3.9	49.6-63.8	3.2	4.6	14.7	3
OS	**7**	**CON**	2016	18.699	OS7	chr7_51263	3.4	18-20	3.2	4.6	13.0	5
		GRA	2016	0.000	OS7	chr7_51263	2.6	0-10	2.0	3.0	9.9	5
	8	**CON**	2016	50.042	OS8	chr8_16092315	3.4	50-59.6	3.2	4.6	13.1	2
		TE	2016	46.00	OS8	chr8_SNP865_80	1.9	44.6-59.7	1.8	3.0	7.3	2
		GRA	2017	72.100	OS8	chr8_21030434	2.2	59.0-73.9	2.0	3.0	9.5	4
SN	**18**	**CON**	2016	76.574	SN18	vmcng2f12	3.3	70.1-77	3.2	4.6	10.5	4
		GRA	2016	58163	SN18	chr18_r_2677945	2.4	53.4-62.2	2	3.1	7.7	3
SW	**3**	**GRA**	2009	53	SW3	chr3_11238850	2.1	14.8-53	1.6	2.9	7.7	4
		GRA	2010	53	SW3	chr3_11238850	2.3	40.6-53	1.7	2.9	7.8	6
	11	**CON**	2009	37.4	SW11	chr11_8616276	4.5	37.4-50.1	3.0	4.7	15.4	4
		CON	2010	37.4	SW11	chr11_8616276	4.0	37.4-41.4	3.1	4.6	12.9	2
		TE	2009	35.9	SW11	chr11_8616276	2.9	25.1-35.9	1.5	2.9	10.1	4
	15	**CON**	2016	8.600	SW15	chr15_1109421	3.8	1.3-9.6	3.3	4.6	11.9	1
		GRA	2016	7417	SW15	chr15_9668745	2.0	4.2-7.5	1.8	3.1	6.1	2

LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome-wide (CW) and genome-wide (GW) LOD threshold ($p < 0.05$), % Var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal-Wallis significance level, given by the p-value ("1": 0.1, "2": 0.05, "3": 0.01, "4": 0.005, "5": 0.001, "6": 0.0005, "7": 0.00001; "-": no significance). BS berry shape, BW berry weight, PL pistil length, FD flower diameter, OL ovary length, OS ovary shape, SN seed number, SW seed weight.

Supplementary material 3.3.4.a. Summary of significant (LOD > GW) and stable (reproducible in at least two years/environments) QTL detected in T × G Hf progeny (120 genotypes).

	LG	Map	Year	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var. expl	KW
									CW	GW		
BL	3	CON	2012	60.8	BL3_1	chr3_17217402	5.9	50-65	3.1	4.8	28.4	7
		GRA	2012	9.7	BL3_1	chr3_16593567	5.3	5.5-29.5	1.9	3.2	26.0	7
		CON	2012	42.0	BL3_2	chr3_6812820	4.9	35-50	3.1	4.8	23.9	7
		GRA	2012	39.7	BL3_2	chr3_5968917	3.5	30-45	1.9	3.2	17.8	6
	5	CON	2012	37.9	BL5	vmc3c7	4.9	30-45	3.1	4.7	23.9	6
		GRA	2012	37.1	BL5	vmc3c7	4.3	30-50	2.0	3.2	21.7	7
BD	3	CON	2012	60.8	BD3_1	chr3_17217402	6.2	50-65	3.1	4.8	29.6	7
		GRA	2012	8.4	BD3_1	chr3_16721456	5.5	5.2-29.8	1.9	3.2	26.7	6
		CON	2012	37.9	BD3_2	vmc3c7	5.7	30-45	3.2	4.8	27.6	7
		CON	2012	42.0	BD3_2	chr3_6812820	5.4	35-50	3.1	4.8	26.4	6
		GRA	2012	30.7	BD3_2	chr3_5968917	4.2	30-45	3.1	4.8	21.2	7
		GRA	2016	23.5	BD3_2	chr3_7157449	2.5	10.2-30.5	2.0	3.2	12.1	4
	5	CON	2016	35.7	BD5	chr5_14699639	3.7	30-50	2.0	3.2	17.4	4
		GRA	2012	36.2	BD5	chr5_14699639	4.5	30-50	2.0	3.2	22.4	7
		GRA	2016	37.7	BD5	vchr5a	2.2	30.2-40.4	2.0	3.2	10.7	4
BS	1	CON	2012	25.0	BS1	chr1_6673914	3.5	17.7-28.8	3.2	4.8	18.0	4
		GRA	2012	12.8	BS1	chr1_6673914	2.1	12.3-14	2.0	3.1	11.4	4
		GRA	2016	11.2	BS1	chr1_6218393	2.0	10.1-12.6	1.9	3.1	9.9	3
	9	TE	2012	38.6	BS9	chr9_3157020	2.6	35.8-44.4	1.9	3.2	13.7	5
		TE	2016	38.6	BS9	chr9_3157020	2.3	37.9-49.4	1.9	3.0	11.0	4
		CON	2016	45.9	BS9	chr9_2448248	3.0	44.6-49.3	3.1	4.7	14.3	2
BW	3	CON	2012	60.2	BW3_1	chr3_16593567	6.1	55-65	3.1	4.8	28.9	7
		GRA	2012	9.7	BW3_1	chr3_16593567	5.8	5.6-29.6	2.2	3.3	27.6	7
		CON	2012	41.1	BW3_2	chr3_6512337	4.0	35-50	3.1	4.8	20.0	7
		GRA	2012	30.7	BW3_2	chr3_5968917	3.0	30.1-45.3	2.2	3.3	15.5	6
		GRA	2016	23.5	BW3_2	chr3_7157449	2.8	7.8-29.5	2.1	3.1	13.1	4
	5	CON	2012	37.9	BW5	vmc3c7	5.0	25-45	3.3	4.9	24.5	7
		CON	2016	37.9	BW5	vmc3c7	4.7	32.5-39.2	3.1	4.8	21.1	7
		GRA	2012	37.1	BW5	vmc3c7	3.9	28.5-47	2.1	3.1	19.8	4
		GRA	2016	37.7	BW5	vchr5a	2.8	30.5-40	2.2	3.3	13.1	5
PL	11	CON	2016	49.6	PL11	vvmd25	4.4	36.1-50.6	3.2	4.9	22.1	2
		TE	**2016**	**33.4**	**PL11**	chr11_3932187	2.0	32-38	1.8	3.0	9.5	4
		TE	**2017**	**33.4**	**PL11**	chr11_3932187	2.0	31-40	1.8	3.0	11.1	4
		GRA	**2016**	**26.3**	**PL11**	vmc5b3	2.4	23.6-34	1.8	3.1	11.7	3
		GRA	**2017**	**26.3**	**PL11**	vmc5b3	2.2	25.5-34.4	2.0	3.1	11.6	3

Continue

	LG	Map	Year	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var. expl	KW
									CW	GW		
FD	**11**	**CON**	**2016**	**49.6**	**FD11**	vvmd25	5.4	36.1-50.6	3.3	4.9	24.1	7
		CON	**2017**	**42.0**	**FD11**	vvs2	4.8	36.1-50.6	3.3	4.8	24.3	6
		TE	**2016**	**37.3**	**FD11**	vvs2	4.0	33.5-40.3	1.9	3.2	18.7	7
	14	**GRA**	**2016**	**26.3**	**FD14**	vmc5b3	2.7	24.2-34.7	2.1	3.4	12.9	7
		GRA	**2017**	**25.4**	**FD14**	chr14_23045539	3.3	24.9-39.6	2.1	3.0	17.4	7
		CON	2017	38.5	FD14	chr14_23522081	3.7	35-42	3.3	4.8	19.3	6
OL	**11**	**TE**	**2016**	**37.3**	**OL11**	vvs2	2.1	32.5-39.5	1.9	3.2	10.2	4
		TE	**2017**	**37.3**	**OL11**	vvs2	3.4	36.4-49.4	1.9	3.2	17.5	6
		CON	2017	42.0	OL11	vvs2	3.8	37.4-49.8	3.2	4.8	19.7	4
	14	**GRA**	**2016**	**26.3**	**OL14**	vmc5b3	2.3	25.1-35.6	2.0	3.1	10.9	4
		GRA	**2017**	**29.9**	**OL14**	chr14_22675729	2.6	25.1-35.6	2.0	3.1	13.9	5
		CON	2017	37.9	OL14	vmc2b11	3.8	32.8-38.9	3.1	4.7	19.6	4
PS	**11**	CON	2017	42.0	PS11	vvs2	4.6	33.5-47.1	3.2	4.6	23.2	6
		TE	2017	37.3	PS11	vvs2	3.6	34.3-52.8	2.0	3.1	18.7	6
	13	CON	2017	51.4	PS13	chr13_2265895	4.5	50.1-59.8	3.1	4.6	23.0	3
		GRA	2017	40.6	PS13	chr13_638880	2.5	34.4-42	2.0	3.1	13.6	2
SN	**3**	CON	2008	54.9	SN3	chr3_10713706	13.2	30-60	3.1	4.7	46.5	7
		CON	2009	54.9	SN3	chr3_10713706	21.3	25-60	3.1	4.7	63.3	7
		CON	2010	51.8	SN3	chr3_11238850	27.8	30-60	3.1	4.7	70.1	7
		GRA	2008	52.9	SN3	chr3_11238850	26.8	38.1-56.4	2.1	3.2	68.8	7
		GRA	2009	52.9	SN3	chr3_11238850	20.6	38.1-56.4	2.0	3.1	62.1	7
		GRA	2010	52.9	SN3	chr3_11238850	26.8	38.1-56.4	2.2	3.3	68.8	7
	5	CON	2008	37.9	SN5	vmc3c7	13.0	20-52	3.0	4.7	46.1	7
		CON	2009	51.6	SN5	chr3_8560917	20.7	10.5-40	2.1	3.2	62.1	7
		CON	2010	35.7	SN5	chr5_14699639	25.4	20-52	3.2	4.7	66.8	7
		GRA	2008	37.1	SN5	vmc3c7	12.9	19-55	2.0	3.2	45.7	7
		GRA	2009	37.1	SN5	vmc3c7	21.2	10.5-55	2.0	3.1	63.1	7
		GRA	2010	36.2	SN5	chr5_14699639	25.3	19-55	2.2	3.2	66.6	7
SW	**3**	CON	2009	46.4	SW3	chr3_7157449	4.9	30-60	3.2	4.8	20.4	7
		CON	2010	51.8	SW3	chr3_11238850	5.3	30-60	3.2	4.7	20.4	7
		GRA	2009	16.9	SW3	chr3_11238850	3.9	10.5-45	2.1	3.1	16.8	7
		GRA	2010	16.9	SW3	chr3_11238850	5.0	10.5-45	2.1	3.2	19.4	7
	5	CON	2009	35.7	SW5	chr5_14699639	4.2	25-55	3.1	4.8	18.0	7
		CON	2010	35.7	SW5	chr5_14699639	5.8	25-55	3.4	4.7	22.2	7
		GRA	2009	36.2	SW5	chr5_14699639	4.0	19-55	2.2	3.1	17.0	7
		GRA	2010	36.2	SW5	chr5_14699639	5.4	19-55	2.2	3.2	20.8	7

Significant QTL were highlighted in red. LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome-wide (CW) and genome-wide (GW) LOD threshold ($p < 0.05$), % Var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal-Wallis significance level, given by the p-value ("1": 0.1, "2": 0.05, "3": 0.01, "4": 0.005, "5": 0.001, "6": 0.0005, "7": 0.00001; "-": no significance). BL berry length, BD berry diameter, BS berry shape, BW berry weight, PL pistil length, FD flower diameter, OL ovary length, PS pistil shape, F flowering date, SN seed number, SW seed weight.

Supplementary material 3.3.4.b. Summary of putative (LOD > CW) QTL in T × G Hf population (120 genotypes).

	LG	Map	Year	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var.	KW
									CW	GW		
BD	**18**	GRA	2016	56.1	BD18	chr18_18040316	1.9	56.1-62.2	1.8	3.1	8.3	3
		CON	2016	59.1	BD18	chr18_18040316	3.1	67.4-78	3.1	4.7	14.7	3
BW	**8**	TE	2012	36.4	BW8	chr8_14172672	2.2	45.5-48.2	1.9	3.0	11.8	4
		CON	2016	14.4	BW8	chr8_4740668	3.2	10.8-15.4	3.1	4.7	14.8	2
	18	GRA	2016	43.9	BW18	chr18_10156555	1.8	43.1-55.4	1.8	3.1	8.1	2
		CON	2016	51.7	BW18	chr18_7446110	3.5	51.5-56.8	3.3	4.9	16.3	2
FD	**5**	CON	2017	40.1	FD5	chr5_r_361363	3.5	38.8-55.3	3.2	4.8	18.1	4
		GRA	2017	48.7	FD5	chr5_8835832	2.6	48-57	2.2	3.0	14.0	7
OL	**15**	CON	2016	55.7	OL15	chr15_8655099	3.5	51.1-56.4	3.2	4.8	16.5	3
		GRA	2016	7.4	OL15	chr15_9668745	2.3	7.4-12.6	2.0	3.1	11.0	4
PS	**2**	CON	2016	29.4	PS2	chr2_5418217	3.7	22.2-30.6	3.2	4.8	17.2	2
		GRA	2016	16.2	PS2	VVMD34	2.0	16.1-21.9	1.9	3.2	9.6	4
OS	**5**	CON	2017	72.2	OS5	chr5_5570145	3.4	59.4-71.4	3.2	4.7	17.6	3
		GRA	2017	65.9	OS5	chr5_7191560	2.3	58.6-70.4	2.1	3.2	12.6	4
SN	**18**	CON	2016	76.6	SN18	vmcng2f12	3.8	72.5-83.6	3.3	4.7	14.8	5
		GRA	2016	58.2	SN18	chr18_r_2677945	1.9	55.1-59.6	2.0	3.2	7.8	3
SW	**1**	CON	2009	41.9	SW1	chr1_11027925	3.4	34.5-42.5	3.2	4.8	14.6	4
		TE	2009	28.6	SW1	chr1_11027925	2.6	27.8-34.2	1.8	3.2	11.4	4
	10	CON	2009	56.7	SW10	chr10_918773	3.9	52.4-59	3.2	4.8	16.8	7
		CON	2010	56.7	SW10	chr10_918773	3.3	52.4-59	3.1	4.7	13.3	6
		GRA	2009	57.9	SW10	chr10_918773	3.4	54.1-63.4	2.1	3.1	14.8	6
		GRA	2010	57.9	SW10	chr10_918773	2.9	54.1-64	2.1	3.2	11.8	7
	19	CON	2008	39.6	SW19	vmc6c7	3.4	38.8-40.8	3.3	4.8	14.9	4
		CON	2009	26.5	SW19	chr19_5200598	3.3	23.3-26	3.1	4.8	12.9	-
		GRA	2008	26.9	SW19	vmc6c7	3.3	25.9-47.9	2.0	3.1	14.4	6

LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome-wide (CW) and genome-wide (GW) LOD threshold ($p < 0.05$), % Var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal-Wallis significance level, given by the p-value ("1": 0.1, "2": 0.05, "3": 0.01, "4": 0.005, "5": 0.001, "6": 0.0005, "7": 0.00001; "-": no significance). BD berry diameter, BW berry weight, FD flower diameter, OL ovary length, PS pistil shape, SN seed number, SW seed weight.

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3.4. QTL analysis for productivity, phenology and must composition traits in a Grenache × Tempranillo population

Abstract

Selecting grapevine (*Vitis vinifera* L.) cultivars adapted to the visible changes produced by global warming in must composition and phenological stages is a great challenge in the present and forthcoming years. Deciphering the genetic control of productivity traits like yield, must composition parameters such as acidity, and phenological stages, especially ripening date, is crucial for optimizing variety performance in this new scenario. In the present work we evaluated the phenological, fruitfulness and must composition variability present in a wine-grape segregating population derived from a cross between Grenache and Tempranillo in four consecutive years at two different locations. The progeny was genotyped with genotyping-by-sequencing (GBS) using a genome-wide association approach based on SNP markers; and QTL mapping was performed to identify regions significantly associated with the fourteen traits evaluated.

For productivity traits; yield, fertility index, cluster number, and cluster weight main effect QTL were detected in LG17 and LG18. A QTL on LG1 was detected for sugar content, and for total acidity five stable QTL were identified on LG4, LG12, LG13, LG14 and LG17. Concerning phenological traits, QTL were detected on LG10 and LG14 for sprouting, and on LG7 and LG10 for flowering. Veraison showed significant associations with genomic regions in LG11 and LG17, being ripening dates significantly associated to LG8, LG11 and LG13. A region in LG2 linked to *Sex* locus was found correlated with productivity traits (yield, fertility index, cluster number and cluster weight), and phenological stages (start and end veraison), evidencing the influence of sex in the genetic determinism of these characters. A QTL on LG2 located in the region where berry colour was mapped, resulted also associated to total acidity and ripening date. A QTL region in LG17 was found significantly associated to berry size, productivity traits, and phenology stages, suggesting close linkage or pleiotropic effects. These results give more insight into the processes that determine grapevine productivity and phenology in order to improve the performance of current varieties for the future climatic conditions.

Introduction

Grapevine is the fruit crop with major relevance worldwide. Most of the grape production is based on the cultivation of *Vitis vinifera* L. varieties, mostly grown for wine elaboration (OIV 2019). Nowadays, there is a growing demand for new wine grape varieties adapted to sustainable wine production in a changing climate. Climate change would result in severe water stress and would need to make the greatest adaptation efforts to face global warming, with higher costs to maintain quality and productivity (Fraga et al. 2013, 2016, Resco et al. 2016). Besides, different studies reported advancement in harvest dates up to two weeks (Koufos et al. 2018) or a decrease in quality due to less acidity and higher pH (Webb et al. 2007, Sweetman et al. 2014).

In the last decade, the study of the genetic control via simple sequence repeat (SSR) markers in biparental populations has been performed for productivity traits (Constantini et al. 2008, Grzeskowiak et al. 2013), phenology stages (Duchene et al. 2012, Fechter et al. 2014, Kamal et al.,2019) and must composition parameters (Ban et al. 2016, Bayo-Canha et al. 2019).

However, the irruption of next-generation sequencing (NGS) has allowed the obtention of genome-wide coverage markers in many species including grapevine, being genotyping-by sequencing (GBS) an excellent tool used in current genome wide association studies (GWASs) (Elshire et al. 2011). As a result, QTL detection of traits using GBS genotyping in grapevine has been recently reported for must composition parameters (Chen et al. 2015, Guo et al. 2019).

Understanding the genetic basis of productivity and phenology-related traits in segregating progenies is crucial for their potential adaptative benefits in the current climate change context, but deciphering the genetic architecture of traits and their interactions is complex due to polygenic control (Costantini et al. 2008, Fechter et al. 2014). Thus, QTL related to fertility, growth and phenology have been mapped in F_1 segregating progenies in other experiments (Costantini et al. 2008, Doligez et al. 2010, Duchene et al. 2012, Grzeskowiak et al. 2013, Fechter et al. 2014, Kamal et al. 2019). Earlier bud burst could trigger yield loss due to spring frost, and berry ripening under higher temperatures will increase sugar content and decrease acidity in grapes (Fraga et al. 2013, Kamal et al. 2019). Many studies have focused on the detection of QTL regions for phenology developmental stages with different results, explained by the varying degree of phenotypic plasticity in grapevine phenology, being budburst, flowering and ripening dates greatly influenced by variety, climate and environment factors (Sadras et al. 2009; Grzeskowiak et al. 2013).

Phenology traits could have an influence in must composition properties, since genes related to abscisic acid response and sugar metabolism were detected in the same QTL regions as veraison time (Duchene et al. 2012). The optimal balance between sugar and acidity is capital in wine stability, acidity dramatically influencing colour stability and sensory properties of red wines (Sáenz -Navajas et al. 2013, Nimii et al. 2015). Organic acid composition depends on the cultivar, cultural practices, environment, and interaction between genotype and environment (Bayo-Canha et al. 2019) making difficult the study of their inheritance. Despite being complex quantitative traits, several works have focused in the inheritance patterns of total soluble solids, pH and acidity (Liu et al. 2007, Viana et al. 2013, 2016, Chen et al. 2015, Zhao et al. 2015, 2016, Ban et al. 2016, Yang et al. 2016, Bayo-Canha et al. 2019). However, the current understanding of their genetic bases remains very limited with all the studies except Houel et al. (2015) and Bayo-Canha et al. (2019) being conducted in interspecific crosses. In the present study we performed QTL mapping in a biparental population derived from Grenache and Tempranillo genotyped with GBS methodology with the aim of getting an deeper insight on the genetic control of traits relevant for adaptation to climate change.

Materials and Methods

Plant material

A F_1 population of 130 plants (one plant per genotype) obtained from controlled crosses between the wine grape cultivars Grenache (female parent) and Tempranillo (male parent) (G × T) was used for our investigation. Between three and five plants of each parent were evaluated each vintage. Population has been described in Chapter 3.1.

Phenotypic evaluation

Fourteen traits including productivity, must composition and phenology characteres were evaluated in the G × T population in four seasons (2014 - 2017) in two different plots. The number

of genotypes that bore fruit varied each year due to bird attack during veraison - ripening stages and powdery mildew especially in 2017. Thus, 127, 111, 117 and 120 genotypes were harvested in 2014, 2015, 2016 and 2017, respectively. The experimental field consisted of two plots at different locations: one at Viveros Provedo (Varea, La Rioja, Spain), and a second one at University La Rioja experimental field (UR), both belonging to Rioja Alta. Climatic data and soil characteristics are reported in Chapter 3.1.

At harvest date, 200 whole berries from each genotype were sampled at random from different positions within the cluster to avoid sun exposition effects. Grapes were squeezed and parameters of resulting musts were evaluated by triplicate. Sugar content (total soluble solids (TSS) expressed as ° Brix) was measured with an Atago Master-Brix refractometer (Atago, Tokio, Japan); pH and total acidity (expressed as g / L tartaric acid) were measured with a TitroMatic 1S - 1B (Crison, Barcelona, Spain). Phenology and fertility related traits were recorded between 2014 - 2017 as reported in chapter 3.1. Veraison length (VL, time between the veraison of first berry and that of all the berries), interval from flowering to start veraison (F-SV), and interval from end veraison to ripening (EV - R) were calculated as described by Costantini et al. (2008), Duchene et al. (2010). For each genotype, yield per vine and number of clusters per vine (CN), were measured at harvest, and the average weight of clusters (CW) was calculated. The fertility index (FI) was scored as the number of inflorescences per young shoot. Mean berry weight (BW) was estimated from 2 replicates of 100 berries randomly taken from three regions of the cluster in each genotype.

Figure 3.4.1 illustrates the vintages and environments for which must composition, productivity and phenological traits were studied.

Statistical analysis

A t-test was carried out to detect differences between parents. Analysis of variance with LSD test was used to evaluate mean value differences across years. The normality of each trait distribution was checked by the Kolmogorov-Smirnov test. Data that significantly deviated from normality were analyzed by non-parametrical tests. Phenotypic correlations among traits were determined in each year with the Spearman rank-correlation coefficient ($p < 0.05$). Correlation analysis between years was used to evaluate the genotype stability across years for each trait. Year effect was tested with analysis of variance and non-parametric Kruskal-Wallis test. A principal component analysis was conducted on the population to identify variables accounting for the variability present.

Figure 3.4.1. Summary of the vintages and environments considered for G x T progeny

	Trait	Abr.	Vintage/ Plot
Productivity	Yield Cluster number Cluster weight Fertility index	Y CN CN FI	4 Vintages, 2 Plots Vintages 2014 - 2017 (UR) Vintages 2014 - 2017 (Varea)
Must composition	Brixº pH Total Acidity	ºB/TSS pH TA	4 Vintages, 2 Plots Vintages 2014 - 2017 (UR) Vintages 2016 & 2017 (Varea)
Phenological stages	Start Sprouting End Sprouting Sprouting Length	SS ES SL	1 Vintage, 2 Plots Vintage 2016 UR & Varea
	Flowering date	F	3 Vintages, 2 Plots Vintages 2014 - 2016 (UR) Vintage 2016 (Varea)
	Start Veraison End Veraison Veraison Length	SV EV VL	4 Vintages, 2 Plots Vintages 2014 - 2017 (UR) Vintages 2014 - 2017 (Varea)
	Ripening date	RD	4 Vintages, 2 Plots Vintages 2014 - 2017 (UR) Vintages 2016 - 2017 (Varea)
	Flowering - Veraison	F - SV	3 Vintages, 2 Plots Vintages 2014 - 2016 (UR) Vintage 2016 (Varea)
	Veraison - Ripening	EV - R	3 Vintages, 2 Plots Vintages 2014 - 2017 (UR) Vintages 2016 & 2017(Varea

QTL analysis

DNA extraction, GBS methodology and genetic linkage map construction was fully explained in Chapter 3.2. QTL analysis was carried out on the parental and Consensus maps with MapQTL 6.0 software (Van Ooijen 2009) using interval mapping and Kruskal-Wallis analysis as previously described (Chapter 3.3). Phenotypic data from each year were separately analyzed. Logarithm of odds (LOD) thresholds corresponding to $\alpha = 0.05$ genome-wide (GW) or chromosome wide (CW) were determined using 1000 permutations (Churchill & Doerge 1994) of the phenotypic data. As detailed in Chapter 3.3, QTL analysis was carried out on the parental and Consensus maps separately using Map QTL 6.0 software and the phenotypic data from each year (Van Ooijen 2009).

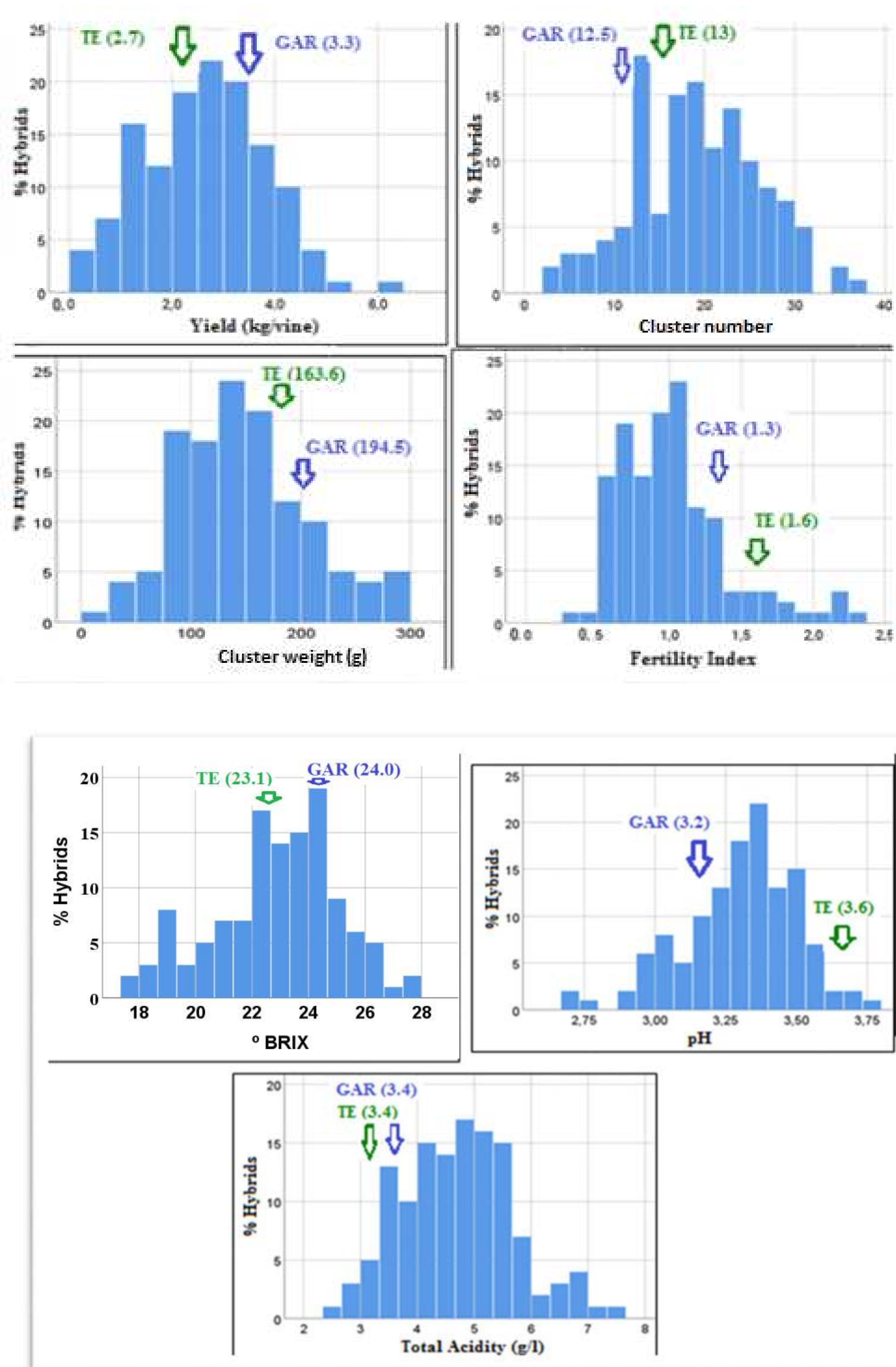
Non-parametric Kruskal-Wallis (KW) rank sum test was applied to the data using a stringency significant level of $p = 0.005$ (****). Interval mapping was then conducted to detect significant QTL regions. Maximum LOD values were used to estimate QTL peak position. Confidence intervals (1 - LOD) were estimated in cM and corresponded to a LOD score drop of one on either side of the likelihood peak. QTL analysis were performed first considering the whole population and then only with the hermaphrodite plants in both genetic backgrounds in order to assess sex influence. A QTL was considered significant when the maximum LOD exceeded the genome - wide (GW) and putative when it exceeded the chromosome - wide (CW) threshold. A QTL was considered stable when detected in at least two seasons or two environments. Putative QTL were also retained since several relevant agronomical traits are controlled by multiple genes each making a small contribution to the genetic determinism of the character.

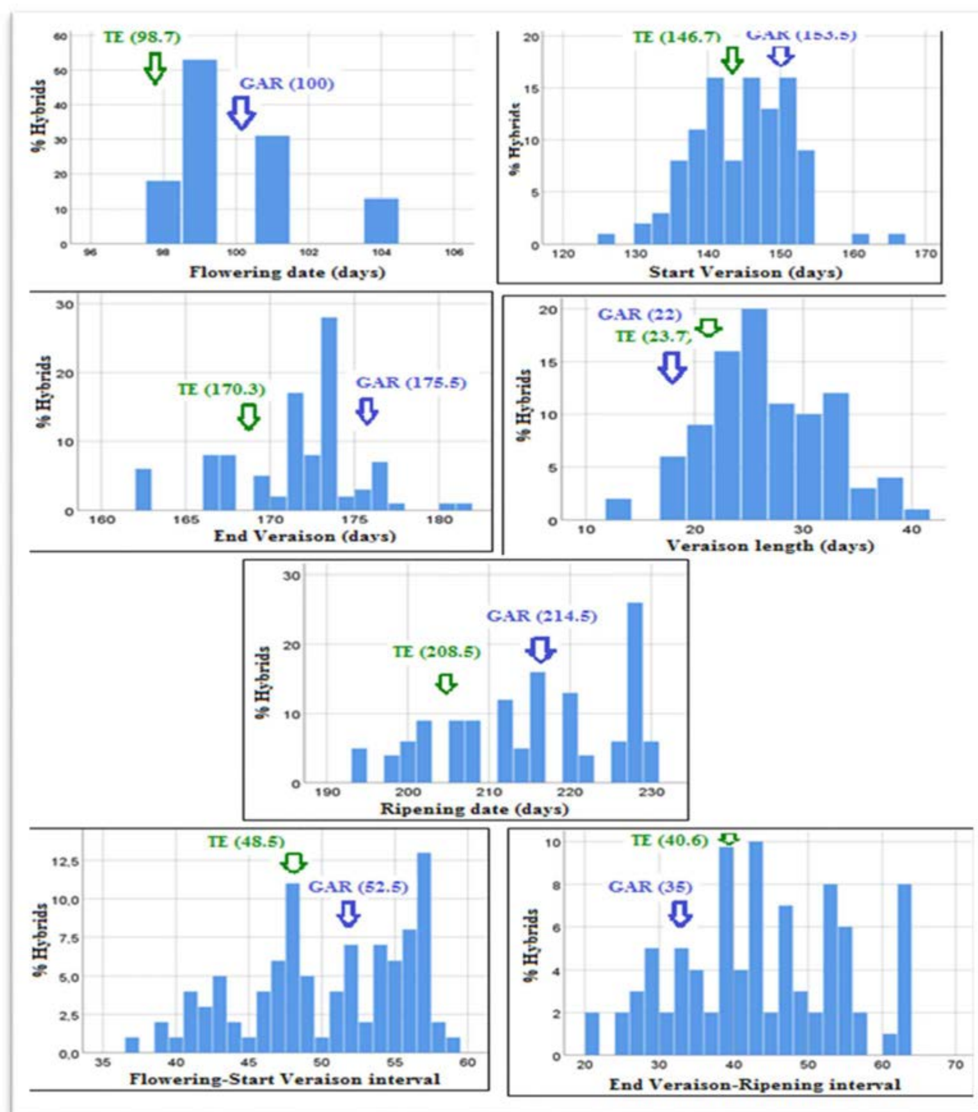
Results and discussion

The evaluation of the phenotypic variability present in a progeny obtained by the crossing of two of the most Spanish relevant wine varieties, Grenache and Tempranillo, was performed in some key traits with the aim of selecting improved hybrids to cope with climate change scenario. Statistical differences were found between both parents in fertility index, pH, acidity and phenology-related traits. All the traits studied presented transgressive segregation and continuous variation confirming the quantitative nature of the traits and the wide range of variability presented in the progeny. Phenotypic data distributions were similar for the four years analyzed, therefore, only data for 2015 are shown (Fig.3.4.2). Continuous variation and transgressive segregation were observed, confirming the quantitative nature of the traits studied and indicating genetic variability for the traits in the progeny.

A t-test (Table 3.4.1) detected significant differences between Grenache and Tempranillo in fertility index, pH, total acidity, end veraison date and end veraison-ripening interval ($p < 0.05$) The Kolmogorov–Smirnov test indicated that yield per vine, cluster number and all the phenological traits significantly deviated from normality. Year and plot effect ($p < 0.05$) were found for all the traits studied by Kruskal–Wallis test (Supplementary material 3.4.1 and 3.4.2). In must composition, Grenache had lower pH and higher total acidity than Tempranillo, and the distribution of values in the progeny was intermediate compared to the parents for pH (ranging between 2.5 and 3.8), although presenting in average higher acidity values, ranging between 2.8 and 8 g / L. In phenology, Tempranillo presented earlier start and end veraison dates (SV, EV) and shorter veraison length (VL).

Figure 3.4.2. Phenotypic distribution of productivity, must and phenological traits in G × T population in 2015. Parental data are indicated: Grenache (GAR) and Tempranillo (TE).





The number of clusters in the progeny showed intermediate values compared to both parents, whereas for yield and fertility index, the population presented in average, lower values compared with their progenitors. Besides, Tempranillo had a higher fertility index compared with Grenache (Table 3.4.1) and mean progeny value. Productivity traits showed significant differences according to year with higher values in 2015 and 2016 vintages compared to 2014 and 2017 (Table 3.4.1), presumably due to differences in weather conditions in each vintage (Chapter 3.1). Distributions of productivity traits showed (Figure 3.4.2) that 38% of the progeny had low production (below 2.0 kg), 53% presented low cluster weight (< 150 g) and 44% exhibited low fertility index (< 1.0), being these the standard values set by DOCa Rioja Regulatory Council for vines in commercial fields. Yield registered a significant reduction in 2014 and 2017, due to higher pluviometry during flowering (45 L / m^2 compared to 25 L / m^2 in 2015 - 2016), that caused flower drop.

Average value for total acidity resulted higher in the progeny compared to the parents, which is desirable in a climate change context, where grapes tend to present lower acidity values due to higher temperatures. Must sugar content (expressed as Brix degree) in the population and

parents presented an average value close to 23.4 ºBrix, the value set for grape harvest. Mean pH value for G × T progeny was intermediate compared with the progenitors, with Grenache showing a lower pH than Tempranillo (Table 3.4.1).

Table 3.4.1. Mean values of traits studied in Grenache × Tempranillo (G × T) and parents.

	Year	G × T population				Grenache		Tempranillo	
		N	Mean ± SD	Min	Max	N	Mean ± SD	N	Mean ± SD
Yield (kg/vine)	**2014**	128	1.7 ± 1.0a	0.2	5.2				
	2015	130	2.6 ± 1.2b	0.1	6.4	2	3.3 ± 0.0	5	2.7 ± 0.3
	2016	112	2.8 ± 2.1b	0.1	6.1	3	4.5 ± 1.0	5	4.9 ± 1.2
	2017	110	1.8 ± 1.0a	0.2	5.3	2	3.4 ± 0.9	4	3.4 ± 1.2
	Mean	110	2.2 ± 0.5	0.1	6.4	3	3.5 ± 1.0	5	3.7 ± 1.3
Cluster number	**2014**	128	9.3 ± 4.4a	1	23				
	2015	130	18.8 ± 7.0c	3	25	2	14.0 ± 5.7	5	13.4 ± 1.8
	2016	112	14.8 ± 6.7b	3	26	3	12.5 ± 6.4	5	13 ± 1.4
	2017	110	16.1 ± 6.7b	4	32	2	11.5 ± 0.7	4	11.3 ± 3.9
	Mean	110	12.8 ± 4.0	1	32	3	12.4 ± 3.7	5	12.6 ± 2.4
Cluster weight (g)	**2014**	128	185 ± 79bc	45	384				
	2015	130	151 ± 66b	22	380	2	142 ± 35	5	116 ± 12
	2016	112	234 ± 65c	31	367	3	**195 ± 18***	**5**	**164 ± 16**
	2017	110	118 ± 55a	27	284	3	110 ± 19	3	134 ± 38
	Mean	110	197 ± 95	38	384	3	155 ± 42	5	138 ± 30
Fertility index	**2014**	128	1.2 ± 0.6a	0.3	1.8				
	2015	130	1.0 ± 0.4b	0.4	1.9	2	1.3 ± 0.4	3	1.6 ± 0.1
	2016	130	0.9 ± 0.3b	0.2	2.1	3	1.3 ± 0.2	3	1.4 ± 0.1
	2017	130	1.0 ± 0.4b	0.3	2.3				
	Mean	128	1.01 ± 0.1	0.2	2.3	3	**1.3 ± 0.2***	**3**	**1.5 ± 0.2**
ºBrix	**2014**	128	13.2 ± 0.9a	11	15				
	2015	123	12.5 ± 1.2b	9	15	2	13.1 ± 0.9	5	12.3 ± 0.6
	2016	112	12.0 ± 1.0c	10	14	3	12.9 ± 0.2	5	12.6 ± 0.9
	2017	121	12.7 ± 0.8b	11	15	3	14.2 ± 0.6	3	14.3 ± 0.9
	Mean	112	12.6 ± 0.9	9.2	15.1	3	13.3 ± 0.7	5	12.8 ± 1.1
pH	**2014**	127	3.52 ± 0.19a	3.1	4.7				
	2015	127	3.32 ± 0.23b	2.72	3.70	2	**3.18 ± 0.21 ****	**5**	**3.60 ± 0.10**
	2016	112	3.41 ± 0.27b	2.51	3.88	3	3.32 ± 0.08	5	3.62 ± 0.31
	2017	120	3.45 ± 0.15a	2.88	3.67	3	3.19 ± 0.11	3	3.44 ± 0.10
	Mean	112	3.37 ± 0.31	2.51	3.88	3	**3.23 ± 0.14 ***	**5**	**3.55 ± 0.20**
Total acidity (g/L)	**2014**	128	5.1 ± 1.1ab	3.1	6.8				
	2015	127	4.7 ± 1.0a	2.5	7.4	2	3.4 ± 0.0	5	3.4 ± 0.2
	2016	112	4.9 ± 1.2a	2.2	6.9	3	**6.7 ± 1.0 ****	**5**	**4.5 ± 0.7**
	2017	116	5.3 ± 1.1b	2.8	7.0	3	5.4 ± 0.5	3	5.4 ± 0.6
	Mean	112	5.1 ± 1.1	2.2	8.0	3	**5.5 ± 1.5 ***	**5**	**4.3 ± 0.9**
Flowering date	**2014**	97	103 ± 3a	97	108				
	2015	127	94 ± 2b	92	462	2	93 ± 0	5	93 ± 1
	2016	115	100 ± 2b	98	104	4	100 ± 1	3	99 ± 1
	Mean	97	102 ± 2	92	108	4	97 ± 1	5	96.± 1
Start Veraison	**2014**	97	154 ± 5a	126	176				
	2015	97	145 ± 9b	133	166	2	**154 ± 1***	**3**	**147 ± 2**
	2016	97	158 ± 10a	151	182	4	161 ± 6	3	154 ± 2
	2017	95	144 ± 11b	138	154				
	Mean	95	150 ± 7	126	182	4	157 ± 3*a	3	152 ± 4

Continue

	Year	GxT population				Grenache		Tempranillo	
		N	Mean ± SD	Min	Max	N	Mean ± SD	N	Mean ± SD
End Veraison	**2014**	97	177 ± 6c	166	186				
	2015	97	171 ± 4b	152	181	2	**176 ± 1***	3	**170 ± 2**
	2016	97	175 ± 4c	164	165	4	**182 ± 2****	3	**173 ± 2**
	2017	95	159 ± 7a	131	86				
	Mean	95	171 ± 8	131	181	4	**177 ± 2***	3	**172 ± 3**
Veraison length	**2014**	97	23 ± 6b	7	25				
	2015	95	27 ± 6c	6	22	2	22 ± 0	3	24 ± 2
	2016	97	17 ± 5a	10	29	4	20 ± 3	3	18 ± 4
	2017	98	16 ± 5a	7	24				
	Mean	95	21 ± 5	6	25	4	**21 ± 3***	3	**21 ± 4**
Ripening date	**2014**	128	228 ± 9c	212	240				
	2015	130	215 ± 11b	194	229	2	215 ± 9	5	208 ± 9
	2016	117	216 ± 10b	191	230	4	227 ± 1	3	223 ± 2
	2017	123	197 ± 11a	182	222	4	196 ± 4	4	194 ± 7
	Mean	117	214 ±13	182	240	4	215 ± 17	5	210 ± 12
F - SV interval	**2014**	64	50 ± 7	42	58				
	2015	96	50 ± 6	44	61	2	**523 ± 7***	2	**49 ± 6**
	2016	81	57 ± 3	50	63	4	**55 ± 7***	4	**50 ± 6**
	Mean	81	54 ± 8	42	63	4	54 ± 7	4	53 ± 5
EV - R interval	**2014**	97	51 ± 9b	41	60				
	2015	88	43 ± 11ab	30	55	2	35 ± 8*a	3	41 ± 9b
	2016	86	42 ± 10ab	31	53	4	45 ± 5*a	3	50 ± 4b
	2017	89	36 ± 11a	26	49				
	Mean	86	44 ± 11	26	60	4	**37 ± 8***	3	**45 ± 7**

Values with different letters show differences between years in the progeny and between parents in the same year. * $p < 0.05$, ** $p < 0.01$, according to Tukey test. Mean (Mean), minimum (Min) and maximum (Max) values and standard deviation (SD) were represented for the 2014 - 2017 years.

Significant statistical differences between years were observed, being pH higher in 2017, an extremely warm season, and lower in years 2015 and 2016. The fact that in 2014 and 2017 radiation accumulated in August and September and the temperature maximum was higher (around 700 MJ / m^2, 35ºC), than in 2015 and 2016 (around 600 MJ / m^2, 32 ºC), likely explains the higher values of Brix degree and pH values registered in those years.

Differences in veraison and ripening dates were observed among years, especially with 2017 vintage (with the highest temperature between May and August, 36.2 ºC) that presumably had an effect in the advancement of veraison end and ripening dates and the shortening of veraison period. As a result, in 2017 end veraison and ripening dates came about up to two weeks earlier in comparison to other years. Apart from vintage, plot also had an influence in phenological stages, and ripening date was influenced by plot and by plot*vintage interaction (Supplementary material 3.4.2). Grenache showed a longer flowering-veraison interval (F-V), in comparison with Tempranillo, due to a delay in the beginning and end of veraison dates. However, no differences were found in ripening date, with a shorter EV-R interval in Grenache relative to Tempranillo (Table 3.4.1). G × T population showed on average intermediate values between parents, being particularly interesting the fact that around 30% of the population presented a delay in the ripening date compared with their parents, mainly due to a longer veraison length, and flowering-start veraison and end veraison - ripening intervals (Figure 3.4.2). These genotypes could be especially suitable in the context of climate change.

The distributions of total acidity, veraison length, ripening date and veraison-ripening period showed an additive model of inheritance, agreeing with Liang et al. (2009) and Song (2014). That is a remarkable result since the main effects of climate change are the reduction of growth-cycle leading to an incomplete phenolic maturity (Resco et al. 2016) and a decrease in grape quality, due to less acidity, excess sugar content (Webb et al. 2008, Fraga et al. 2013, Sweetman et al. 2014).

Phenotypic correlations

Correlation coefficient between years (Table 3.4.2) and between traits in the same year (Table 3.4.3) were calculated in order to assess the effect of genetic and environmental factors on the parameters studied.

Table 3.4.2. Phenotypic correlations (Spearman) between years in G × T progeny

Traits	2014-2015	2014-2016	2014-2017	2015-2016	2015-2017	2016-2017
Yield	0.3 **	0.5 **	0.2 *	0.7 **	0.3 **	0.4 **
Cluster number	ns	0.3 **	ns	0.4 **	0.2 *	0.7 **
Cluster weight	0.5 **	0.5 **	0.3 **	0.8 **	0.4 **	0.4 **
Fertility Index UR	ns	0.2 *	ns	0.2 *	0.2 *	0.3 **
Fertility Index V	ns	ns	0.3 **	0.4 **	0.4 **	0.4 **
Total soluble solids (Brix º)	ns	ns	ns	ns	0.2 *	ns
pH	0.3 **	0.3 **	0.3 **	0.4 **	0.4 **	0.4 **
Total Acidity	0.4 **	0.3 **	0.5 **	0.7 **	0.7 **	0.7 **
Flowering date	ns	ns	-	ns	-	-
Start Veraison UR	ns	ns	0.3 **	0.3 **	0.4 **	0.5 **
Start Veraison V	0.4 **	0.5 **	ns	0.5 **	0.4 **	0.4 **
End Veraison UR	ns	0.3 **	ns	0.3 *	ns	0.4 **
End Veraison V	0.4 **	0.4 **	0.5 **	0.5 **	0.5 **	0.6 **
Veraison length UR	ns	0.2 **	ns	ns	ns	ns
Veraison length V	ns	ns	0.3 *	ns	ns	ns
Ripening date	ns	ns	ns	0.3 **	0.4 **	ns
F - SV interval	0.3 *	0.4 **	-	ns	-	-
EV - R interval	ns	ns	ns	0.5 **	0.4 **	0.4 **

Correlations significant at $p < 0.01$ (**); and not significant (ns). Missing data (-). V refers to Varea plot.

Correlations between years were highly significant ($p < 0.01$) for all the parameters studied except for veraison length and F - SV interval ($p < 0.05$). Production traits (yield, cluster number, cluster weight and fertility index) showed high variability between years, with coefficients ranging between very low ($r = 0.2$ $p < 0.05$) and high ($r = 0.8$ $p < 0.01$). Among must parameters, total soluble solids (TSS) presented the lowest reproducibility, whereas pH with values between $r = 0.3 - 0.4$ ($p < 0.01$) and total acidity $r = 0.3 - 0.7$, ($p < 0.01$) resulted moderately correlated. Lastly, among phenology - related traits, start and end veraison dates, and veraison - ripening interval seem to be the most reproducible across years, with coefficients between 0.3 to 0.6 ($p < 0.01$). However, ripening and specially flowering dates showed a strong influence by other factors.

Plot effect was also evaluated for those traits studied in the same year in both plots (Supplementary material 3.4.1). Fertility index was higher in UR plot compared to Varea in all years whereas berry weight resulted higher in Varea, maybe due to a less productivity in consequence of soil characteristics. Soil characteristics are expected to have an impact in pH and total acidity, being that influence modulated by weather conditions. In this work, plot influenced acidity and pH composition in musts, pH resulted higher in Varea plot, maybe also due to different soil composition.

Analysis of variance and Kruskal-Wallis test showed a significant year effect ($p < 0.01$) for all traits (Supplementary material 3.4.2). Year had an effect in must composition affecting TSS, acidity and pH. Regarding phenology, UR plot showed a delay in veraison and ripening dates compared to Varea. In general, phenology-related traits show a low consistency between years, having vintage effect as found by Constantini et al. (2008) or Song (2014), being explained mainly by temperature differences among years (Duchene et al. 2012, Fraga et al. 2013).

The consistency of the traits that characterized these genotypes over years is a capital issue, so phenotypic correlations between years were calculated. They resulted moderate for acidity-related traits (0.4 - 0.7), being productivity and phenology stages highly affected by genotype x year interaction. End veraison date had the highest correlation between years (0.4 - 0.6), and any correlation for flowering time (as reported by Song 2014). These moderate-weak correlations between years will affect to the detection of stable QTL for the traits considered.

Table 3.4.3. Correlation matrix for productivity, must composition and phenology traits

	BW	Y	CN	CW	FI	ºB	pH	TA	F	SV	EV	VL	R	FSV	ER
BW	**1**	0.5**	0.4**	0.7**	-0.2**										
Y		**1**													
CN		0.8**	**1**												
CW		0.8**	0.7**	**1**											
FI		0.8**	0.9**	0.3**	**1**										
ºB		-0.3**	-0.3**			**1**									
pH	0.2**	-0.4**	-0.3**			0.4**	**1**								
TA		0.2*		0.3**	0.2**		-0.3**	**1**							
F		-0.3**	-0.3 **			0.2*	0.2*	0.2*	**1**						
SV	0.3**								0.5**	**1**					
EV	-0.3 **	-0.3**	-0.3**	0.3**					0.4*	0.7**	**1**				
VL									-0.3**	-0.4**	0.4**	**1**			
RD		0.4**	0.2**	0.2**		-0.6**	-0.3**			0.4*	0.4*	0.4*	**1**		
FSV									-0.3**	0.9**	0.4**			**1**	
EVR						-0.6**	-0.6**	0.3**	-0.4**	-0.3**	-0.3**		0.9**		**1**

BW berry weight, Y yield, CN cluster number, CW cluster weight, FI fertility index, ºB Brix degree, TA Total acidity, F flowering date, SV start veraison date, EV end veraison date, RD ripening date, FSV flowering - start veraison interval, EVR end veraison ripening interval. Correlations significant at $p < 0.01$ (**); and $p < 0.05$(*).

Within each year, Spearman rank correlation coefficient (Table 3.4.3) revealed several associations between traits studied in the present study. Coefficients observed across four years varied in some cases, so the values reported in the table correspond to the highest value found in at least two years. High significant ($p < 0.01$) correlations were found between productivity traits such as yield, cluster number and cluster weight (0.8) as previously found by Fanizza et al. (2005) and Song et al. (2014) and moderately correlated with berry weight ($r = 0.3 - 0.6$, $p < 0.01$), being all key traits for breeding programs and viticulturists. Cluster weight correlated with yield and cluster number (0.7 - 0.9, $p < 0.01$) but a low correlation was found with fertility index (0.3, $p < 0.01$). Besides, pH and total acidity showed a quite high correlation ($r = 0.3 - 0.7$, $p < 0.01$) between the years of study (Table 3.4.3). Berry weight and pH showed a positive correlation (0.4, $p < 0.01$whereas a low and negative correlation was found between TSS and berry weight (- 0.2, $p < 0.01$). Few relationships are reported between berry and must composition; Gil et al. (2015) found that musts from smaller berry cultivars presented lower pH, in agreement with our results.

In phenology, veraison -ripening period resulted highly correlated with ripening date (0.9) and flowering - veraison period with start veraison (0.9) as reported by Costantini et al. (2008). Veraison length resulted positively correlated with start veraison date (0.7, $p < 0.01$). Negative correlations were found between flowering, end veraison dates and productivity traits such as yield and cluster number (- 0.3, $p < 0.01$), and a positive correlation between yield and ripening date (0.4, $p < 0.01$), that may suggest later vines would be more productive, although correlations were low. TSS expressed as º Brix and pH were negatively correlated with ripening date and veraison - ripening period (0.3 - 0.6, $p < 0.01$, 0.6, $p < 0.01$, respectively). Genotypes unable to reach maturity accumulate less sugars, while pH is still low. This negative relationship between ripening date and acidity was previously reported by Bayo-Canha et al. (2012), and Song et al. (2014). A delay in ripening date is probably due to the longest period between veraison and ripening (0.9, $p < 0.01$) and start veraison date presented a high correlation with flowering-start veraison interval (0.9, $p < 0.01$).

A MANOVA was conducted with parent data for the different years and plots (Supplementary material 3.4.3). Parent resulted significant different for pH, total acidity, and all the phenology traits except veraison length. as expected, since Grenache presented lower pH, higher acidity and a delay in developmental stages compared with Tempranillo. Vintage presented an effect in TSS (º Brix), total acidity, seed weight and all phenology related traits with the exception of end veraison date. Climatic conditions were very distinct depending on the year, so it is expected that parameters as TSS and acidity be affected. Even though seed traits are likely to have high heritability, in this study, vintage influenced their values. The interaction parental - vintage resulted significant only for productivity traits such as cluster weight.

QTL detection

All QTL were detected at p = 0.05 Genome Wide (GW) and Chromosome Wide (CW) applying interval mapping method. Since Grenache and Tempranillo resulted statistically different in acidity and phenology dates a higher variability is expected in the progeny.

Female plants presented lower yield, higher cluster number and hence higher fertility index compared to hermaphrodite genotypes. Moreover, a delay in flowering and veraison dates, but a shorter veraison period and a longer Flowering - Start Veraison (F - SV) interval were observed in female genotypes (Table 3.1.3, Chapter 3.1). For that reason, parallel to Chapter 3.3, QTL analysis were performed both with all and only with the hermaphrodite subpopulation in order to assess the influence of *Sex* locus in the parameters studied.

QTL detection for productivity traits

QTL for productivity traits were analyzed in (G × T) population with data of four consecutive years 2014 - 2017 in two different environments (Table 3.4.4). As reported for flower traits removing female genotypes decreased variability and reduced the number of stable QTL, since no significant QTL were found in the hermaphrodite progeny analysis, being all putative. (Supplementary material 3.4.5). The influence of flower sex on productivity traits was confirmed by the presence of a QTL on LG2 close to *Sex* locus for yield, cluster number and weight, and fertility index, that was not detected when female genotypes were removed, with the exception of fertility index, that co-localized with the *colour* locus. (Table 3.4.4).

One stable QTL (Y17_1) and one significant QTL (Y17_2) were detected for **yield (Y, kg / vine)** in LG17. First one was found in Tempranillo and Consensus maps, being one stable during two years for both maps, explaining 14 % and 15 % of the total variance respectively

(Table 3.4.4). Putative QTL were also detected in LG2, LG7 and LG18 (Supplementary material 3.4.4). QTL located in LG2 was found in one year in Grenache and Consensus maps explaining only 6 % and 11 % of the phenotypic variance.

Three QTL for **cluster number (CN)** were found on LG2, LG17 and LG18. QTL on LG2 (CN2) resulted reproducible in two years in Grenache (14 % explained variance, LOD = 2.2) and Consensus maps (22 % of the variance explained, LOD = 3.3). Two putative QTL were found on LG17, CN17_1 in Tempranillo and CN17_2 in Grenache, only in one year of study, with 10 % and 7 % variance explained in Consensus, Grenache and Tempranillo maps, respectively (Supplementary material 3.4.4). QTL CN18 found as putative for yield on LG18 (Y18) was detected for cluster number in Consensus (20 %) and Grenache maps (9 %) in one year (Table 3.4.5).

Four reproducible QTL were detected for **cluster weight (CW)** on LG2, LG10 and two on LG17. QTL on LG2 resulted stable during two years, in Grenache and Consensus maps, explaining up to 14 % and 18 % of the variance. This QTL (CW2) was significative one year in Tempranillo map, being the closest marker VVIB23 and explaining 9 % of the variance. QTL on LG10 (CW10) was stable during two years in Consensus and Grenache maps, explaining 12.5 % and 10.8 % of the variance. QTL were detected on LG17 over two years in Tempranillo and Consensus maps, (10 % and 16 % of the variance explained, respectively).

Five QTL were found for **fertility index (FI)**, on LG2, LG9 (Supplementary material 3.4.4) and on LG3 and LG17. The QTL on LG3 (FI3) was found in Grenache, Tempranillo and Consensus maps over two different years, explaining up to 9 %, 12.3 % and 12.6 % of the phenotypic variance respectively. QTL on LG17 (FI17) was stable on Tempranillo map over two years and in Consensus in only one, explaining in both cases up to 10 % of the variance.

In summary, for productivity traits, one QTL on LG2 and two QTL on LG17 were found in common. QTL on LG2 suggest the influence of *Sex* locus on these traits as reported in Chapter 3.3 and both QTL on LG17 are in the same region as the QTL detected for berry traits (BL17_2, BD17_2 or BW17_2). In the analysis conducted in hermaphrodite plants, a putative QTL was detected for yield, cluster number and cluster weight in LG17 and one QTL on LG9 for cluster weight and fertility index. Only a putative QTL on LG2 was found for fertility index trait but located in the colour region in this new analysis, evidencing the influence of *Sex* locus in this trait.

Table 3.4.4. Summary of significant (LOD > GW) and stable (reproducible in at least two years/environments) QTL detected for productivity traits in G × T progeny (130 genotypes).

		LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1 - LOD interval	LOD 0.95 CW	LOD 0.95 GW	% Var	KW
PRODUCTIVITY TRAITS	Y	17	TE	2015	UR	12.4	Y17_1	17_2012240	4.0	0.0 - 16.7	2.0	3.4	13.3	6
			TE	2017	UR	6.8	Y17_1	17_1364051	3.7	0.0 - 13.3	2.2	3.5	14.4	4
			CON	2015	UR	15.3	Y17_1	17_2141301	4.7	6.1 - 21.9	3.4	4.7	15.3	6
			CON	2017	UR	1.5	Y17_1	17_213104	4.3	0.0 - 17.8	3.1	4.8	16.3	4
			TE	2015	UR	19.8	Y17_2	17_3449228	4.2	21.9 - 43.2	2.0	3.4	13.9	7
			CON	2015	UR	27.7	Y17_2	17_4790506	4.6	22.6 - 39.5	3.4	4.7	15.1	3
	CN	2	GAR	2015	UR	2.9	CN2	2_662505	2.2	0.9 - 3.7	1.9	3.1	7.4	4
			GAR	2016	UR	3.7	CN2	2_905604	2.2	0.9 - 9.3	1.8	3.2	14.2	4
			CON	2016	UR	10.4	CN2	2_3159930	3.3	9.9 - 14.5	3.3	4.8	20.5	4
			CON	2017	UR	13.5	CN2	2_4297673	3.7	11.1 - 14.4	3.4	4.7	14.2	2
			TE	2017	UR	15.7	CN2	2_4075810	2.6	9.2 - 19.6	2.1	3.5	10.4	4
		18	CON	2017	UR	87.7	CN18	18_27984510	5.5	80.8 - 91.2	3.3	4.9	20.7	4
			GAR	2017	UR	72.3	CN18	18_24968109	2.3	68.2 - 78.4	1.9	3.2	9.2	3
	CW	2	GAR	2014	UR	16.4	CW2	2_2988540	3.0	14.6 - 24.9	1.8	3.1	10.2	4
			GAR	2016	UR	14.6	CW2	2_2286699	2.2	12 - 19.9	1.8	3.2	13.9	3
			CON	2014	UR	26.9	CW2	2_4863957	3.1	25.2 - 32.6	3.2	5.0	10.2	3
			CON	2016	UR	29.5	CW2	2_5386803	3.1	24.9 - 32	3.2	5.0	18.0	2
			TE	2016	UR	24.8	CW2	VVIB23	1.5	24.8 - 25	2.0	3.2	9.3	2
		10	GAR	2014	UR	15.3	CW10	10_8053703	1.7	10.6 - 19.7	1.6	3.2	6.0	2
			GAR	2015	UR	15.3	CW10	10_8053703	3.2	10.6 - 19.7	1.9	3.4	10.8	5
			CON	2015	UR	64.5	CW10	10_9919995	3.8	58.5 - 65.5	3.4	5.0	12.5	3
			CON	2017	UR	64.3	CW10	10_9405617	3.1	61.6 - 65.5	3.1	4.8	12.3	2
		17	TE	2015	UR	6.8	CW17_1	17_1364051	2.7	2.1 - 13.3	2.1	3.4	9.0	4
			TE	2017	UR	6.8	CW17_1	17_1364051	2.6	2.2 - 13.3	2.1	3.5	10.2	5
			CON	2015	UR	14.2	CW17_1	17_1626071	4.9	12.4 - 17.1	3.2	5.0	16.0	4
			CON	2017	UR	17.8	CW17_1	17_2254157	3.1	12.4 - 17.1	3.0	4.8	12.1	4
			TE	2015	UR	19.8	CW17_2	17_3449228	2.3	17 - 20.2	2.1	3.4	7.8	4
			TE	2017	UR	19.8	CW17_2	17_3449228	2.2	17 - 20.2	2.1	3.5	8.9	5
			CON	2015	UR	26.4	CW17_2	17_3936107	3.5	25.2 - 30.8	3.2	5.0	11.8	3
			CON	2017	UR	27.7	CW17_2	17_4790506	3.2	25.2 - 30.8	3.0	4.8	12.4	4
	FI	3	GAR	2014	V	34.9	FI3	3_5207753	1.9	34.5 - 40.1	1.7	3.3	9.1	2
			GAR	2015	V	32.8	FI3	3_6912508	1.7	32.0 - 39.2	1.6	3.2	7.2	4
			TE	2015	V	58.1	FI3	3_9986740	2.9	53.4 - 58.9	2.4	3.6	12.3	4
			TE	2016	V	58.1	FI3	3_9986740	2.2	53.7 - 58.9	2.2	3.4	8.6	6
			CON	2015	V	56.4	FI3	3_10125864	3.2	54 - 57.5	3.1	4.7	11.5	3
			CON	2016	V	56.4	FI3	3_10125864	3.1	54 - 57.5	3.0	4.7	12.6	3
		17	CON	2015	UR	23.2	FI17	17_4280635	3.9	21.2 - 33.3	3.3	5.1	12.8	4
			TE	2015	UR	16.7	FI17	17_3063542	3.0	14.8 - 23.7	2.1	3.5	10.1	5
			TE	2016	V	31.0	FI17	17_4698550	2.8	31.0 - 45.8	2.1	3.4	10.8	2

Significant QTL are highlighted in red. Y yield, CN cluster number, CW cluster weight, FI fertility index, LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome - wide (CW) and genome - wide (GW) LOD threshold ($p < 0.05$), %var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal - Wallis significance level, given by the p - value (“1”: 0.1, “2”: 0.05, “3”: 0.01, “4”: 0.005, “5”: 0.001, “6”: 0.0005, “7”: 0.00001; “ - ”: no significance).

The stable QTL detected in LG17 for yield, cluster weight and fertility index traits and putative only in 2015 for cluster number (CN17) co-localizes with the regions found for berry length, berry diameter, berry weight, seed weight and ovary length (Supplementary material 3.4.6 b). This finding supports the correlations above mentioned (Table 3.4.3). Fanizza et al. (2005) and Doligez et al. (2010) also found QTL on LG17 related to cluster traits and fertility, respectively. For fertility index (FI) only the QTL located in LG3 (FI3) resulted stable and co-localizes with the stable QTL found for berry diameter and berry weight in G x T progeny (Supplementary material 3.4.6 a). Authors as Carreño-Ruiz (2012) and Grezskowiak et al. (2013) also found a QTL on LG3 related to fertility trait. QTL on LG18 for yield and cluster number, and in LG5 for cluster number had been reported in Doligez et al. 2010. These QTL do not co-localize with QTL for berry traits (Supplementary material 3.4.6 a). A reproducible QTL, not previously reported was identified in LG10, in Grenache and Consensus maps across two years (10 % and 12 % of the phenotypic variance, respectively). The variation between progenies for these parameters could be explained because cultivars, environmental factors, and training methods could be other factors that trigger the difference in fruitfulness (Sommer et al. 2000).

QTL detection for must composition

Must composition traits were analyzed in G x T population with data of four consecutive years 2014 - 2017 in UR plot and two years 2016 - 2017 in Varea plot. Unexpectedly, flower sex had an influence in these parameters, and a QTL for total acidity was found in LG2 in *Sex* locus region only in Tempranillo map. The number and stability of QTL for must composition was influenced by flower sex, as previously reported for productivity and flower traits.

Two significant QTL were found in LG1 and LG8 for must sugar content (expressed as Brix º) (Table 3.4.5 a). QTL on LG1 was stable across two years in Consensus and Grenache maps and it explained 16 % and 14% of the variance, respectively. QTL on LG8 resulted significant in Grenache and Consensus maps, explaining 17 % and 23 % of the variance, respectively. No stable QTL for pH were found, but two putative QTL were detected in LG3 (14 % variance explained, LOD = 3.4) and LG13 (14 % variance explained, LOD = 4.1) in Consensus and Tempranillo maps in one of the years (Supplementary material 3.4.4).

Although there are only a few references to the study of QTL in Total Soluble Solids, Chen et al. (2015) found a QTL on LG1 as reported here, and also Yang et al. (2016) for the ratio Soluble Solids / Malic Acid in their study in other interspecific grapevine hybrid family, and Bayo-Canha et al. (2019) for the ratio TSS/acidity. For the trait pH, Viana et al. (2013) also found a QTL on LG13 for pH, and the QTL on LG3 is not located in the same region as berry traits (Supplementary material 3.4.6 a), confirming that the relationship between berry size and pH is not evident.

Table 3.4.5 a. Summary of significant (LOD > GW) and stable (reproducible in at least two years/environments) QTL detected for must composition traits in G × T progeny (130 genotypes).

		LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var expl	KW
											CW	GW		
MUST COMPOSITION TRAITS	Brix°	1	CON	2016	V	29.9	BR1	1_5545137	3.7	16.4 - 31.2	3.4	4.8	16.5	4
			CON	2017	UR	17.1	BR1	1_2432068	3.6	16.9 - 24.8	3.3	4.7	14.3	3
			GAR	2016	V	25.6	BR1	1_3148212	2.9	20.6 - 26.2	2.0	3.2	12.9	5
			GAR	2017	UR	23.5	BR1	1_2719075	3.4	15.4 - 32.3	2.0	3.2	13.6	7
		8	CON	2015	UR	39.6	BR2	8_20417494	7.3	50.3 - 71.4	3.0	4.8	23.2	6
			GAR	2015	UR	65.3	BR2	8_20286709	5.2	53.1 - 75	1.9	3.2	17.1	6
	TA	2	TE	2016	UR	12	TA2_1	2_2896958	3.0	10.7 - 19.8	2.0	3.5	19.4	3
			TE	2016	V	15.2	TA2_1	2_3594627	2.1	12.3 - 19.3	2.0	3.4	10.6	4
			TE	2017	UR	41.7	TA2_2	2_5759917	2.8	38.0 - 43.4	2.0	3.8	11.4	6
			CON	2014	UR	47.2	TA2_2	2_8573837	4.8	44.7 - 59.0	3.2	4.7	15.8	3
		3	TE	2015	UR	13.8	TA3	3_1773674	2.0	12.4 - 18.3	2.3	3.2	7.0	3
			TE	2017	UR	20.2	TA3	3_3253543	2.5	12.7 - 25.1	2.4	3.8	10.4	4
			CON	2015	UR	14.7	TA3	3_1910501	3.2	13.4 - 161	3.1	4.7	10.9	2
		4	CON	2015	UR	20.2	TA4	4_3158756	6.0	11.2 - 30.8	3.2	4.7	19.4	2
			CON	2017	UR	11.8	TA4	4_1977333	3.1	12.6 - 36	3.0	4.8	12.6	2
			TE	2016	UR	24.2	TA4	4_4124240	2.5	12.6 - 36	2.0	3.5	16.3	7
		12	CON	2014	UR	79.3	TA12	12_19583519	4.2	72.8 - 89.3	3.5	4.7	14.1	4
			CON	2015	UR	83.3	TA12	12_21105311	4.8	72.8 - 89.3	3.2	4.7	16.1	5
			CON	2017	UR	83.3	TA12	12_21105311	4.8	72.8 - 89.3	3.6	5.8	18.8	7
			TE	2015	UR	48.9	TA12	12_19182259	3.4	45.2 - 54.7	1.8	3.2	11.7	6
			TE	2017	UR	48.9	TA12	12_19182259	2.6	47.4 - 61.4	2.0	3.8	10.6	5
		13	GAR	2015	UR	18.8	TA13	13_5921513	2.9	10.6 - 23.3	1.9	3.3	10.1	5
			GAR	2017	UR	18.8	TA13	13_5921513	2.0	17.1 - 22.5	1.8	3.1	8.2	4
			CON	2015	UR	32.2	TA13	13_5643108	3.2	30.8 - 36.4	3.1	4.7	11.0	4
			CON	2017	UR	36.1	TA13	13_6540900	4.1	30.6 - 36.9	1.8	3.1	16.3	4
		14	CON	2015	UR	82.4	TA14	14_28065742	3.0	77.8 - 83.3	3.1	4.7	10.0	2
			CON	2017	UR	77.8	TA14	14_27962249	3.9	76.8 - 79.8	3.2	4.8	15.6	5
			GAR	2015	UR	72.7	TA14	14_25556485	1.8	71.1 - 73.6	1.8	3.3	6.2	4
			GAR	2016	V	72.7	TA14	14_25556485	2.0	70.7 - 74.4	1.9	3.3	10.1	4
			GAR	2017	UR	81	TA14	14_27351673	2.9	65.8 - 86.6	2.1	3.1	11.9	6
		17	CON	2017	UR	32.1	TA17	17_5647891	4.5	19.8 - 43.2	3.1	5.8	17.9	7
			CON	2016	V	21.9	TA17	17_3063542	3.6	19.8 - 43.2	3.1	4.9	16.7	4
			GAR	2016	V	55.9	TA17	17_3161035	3.0	44.5 - 60.5	1.8	3.3	14.5	4
			GAR	2017	UR	44.1	TA17	17_5647891	4.5	30.3 - 55.4	1.7	3.1	17.8	7

Significant QTL were highlighted in red. TA Total acidity. LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome - wide (CW) and genome - wide (GW) LOD threshold (p < 0.05), %var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal - Wallis significance level, given by the p - value (“1”: 0.1, “2”: 0.05, “3”: 0.01, “4”: 0.005, “5”: 0.001, “6”: 0.0005, “7”: 0.00001; “ - ”: no significance).

Seven different QTL were detected for **total acidity (TA)** on LG2, LG3, LG4, LG12, LG13, LG14 and LG17. A major QTL was identified on LG17, reproducible in two different plots and years in Grenache and Consensus maps and explaining up to 30 % and 36 % of the phenotypic variance of the trait. QTL on LG4 and LG12 were also stable and detected in three years in Consensus map and 2 years in Tempranillo map, explaining up to 19 % of the variance. Other important QTL was significative in LG14, being detected in three years in Consensus and Grenache maps and explained 16 % of the variance in Consensus map and 10 % of Grenache map. QTL on LG13 was also stable during two years in Grenache and Consensus maps, and also covered 10 % and 15 % of the total variance. QTL on LG2 resulted located close to the *Sex* locus region in Tempranillo map, but is placed in colour region in Consensus map.

When the analysis was conducted without female genotypes less QTL were detected, and QTL on LG2 was not detectable in this new analysis. One significant QTL was found in LG8 (Table 3.4.5 b) and two putative QTL for pH were detected in LG4 and LG14 (Supplementary material 3.4.5). For total acidity and QTL on LG6, LG12 and LG17 were stable across two and three years depending on the map.

Table 3.4.5 b. Summary of significant (LOD > GW) and stable (reproducible in at least two years/environments) QTL for must composition traits detected in G × T hermaphrodite population (102 genotypes).

		LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var expl	KW
											CW	GW		
MUST COMPOSITION TRAITS	BRIX	8	CON	2015	UR	60.1	BR8	8_20286709	5.8	49.3-68.8	3.0	4.8	23.3	6
			GAR	2015	UR	65.3	BR8	8_20286709	5.0	53.1-75	1.9	3.2	20.5	7
	TA	6	CON	2016	UR	51.2	TA6	6_12610755	3.3	50.8-52.7	3.2	4.8	26.5	4
			CON	2017	UR	56	TA6	6_11610900	4.3	42.8-57.2	3.3	5.8	21.4	4
			GAR	2016	UR	50.9	TA6	6_12306821	1.8	49-52.9	1.8	3.3	15.4	3
			GAR	2017	UR	51.9	TA6	6_13135494	2.5	50.9-55.9	1.8	3.1	12.8	4
		12	TE	2014	UR	28.5	TA12_1	12_9713704	2.2	11.1-32.8	1.9	3.5	9.6	4
			TE	2016	V	15.3	TA12_1	12_1982474	3.0	11.1-32.8	1.8	3.2	17.4	4
			TE	2017	UR	21.9	TA12_1	12_1982474	2.5	11.1-32.8	2.0	3.8	13.1	2
			CON	2016	V	30.9	TA12_1	12_10230232	4.3	23.9-44.3	3.5	4.8	24.4	4
			GAR	2016	V	27.2	TA12_1	12_10230232	2.4	25-33.8	1.8	3.3	14.6	4
			CON	2014	UR	79.3	TA12_2	12_19583519	3.9	72.8-85	3.5	4.7	16.5	4
			CON	2017	UR	83.3	TA12_2	12_21105311	3.9	72.8-85	3.6	5.8	19.9	5
			TE	2017	UR	48.9	TA12_2	12_19182259	2.9	47-54.7	2.0	3.8	15.2	6
		17	GAR	2014	UR	12	TA17	17_2997781	2.5	11.6-38.3	1.7	3.2	10.9	5
			GAR	2015	UR	22.6	TA17	17_5647891	2.0	12-38.3	1.8	3.3	8.5	4
			GAR	2016	UR	4.9	TA17	17_2141301	3.8	2.9-19.9	1.9	3.3	29.4	6
			GAR	2016	V	10.8	TA17	17_3161035	2.4	12-38.3	1.8	3.3	14.3	4
			GAR	2017	UR	22.6	TA17	17_5647891	3.7	12-32.3	1.7	3.1	19.0	7
			CON	2017	UR	32.1	TA17	17_5647891	3.8	30.1-35.1	3.1	5.8	19.4	7

Significant QTL were highlighted in red. TA Total acidity. LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome - wide (CW) and genome - wide (GW) LOD threshold (p < 0.05), %var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal - Wallis significance level, given by the p - value ("1": 0.1, "2": 0.05, "3": 0.01, "4": 0.005, "5": 0.001, "6": 0.0005, "7": 0.00001; " - ": no significance).

Total acidity (TA) is probably the trait in which more stable QTL have been found in this work. Probably because it was one of the few traits in which more differences were found between the two parents of the study, Grenache and Tempranillo, and presumably, more variability was generated in the descendance. Seven QTL were found on LG2, LG3, LG4, LG12, LG13, LG14 and LG17. Comparing these results with the study of Song (2014), in T x G progeny LG12 and LG14 resulted significant in both populations explaining between 15 – 20 % of the phenotypic variance. QTL that resulted significative in LG2, was also found by Bayo-Canha et al. (2019), and here co-localizes with the region of *colour* locus in G x T progeny, presumably due to the higher acidity of white grapes comparing to red ones (Supplementary material 3.4.6 a). QTL on LG4 and LG17 were also detected in Bayo-Canha et al. (2019) associated to malic acid. Besides, Viana et al. (2013) also reported a QTL on LG13 associated to total acidity. The fact that the main QTL for acidity was found in LG17 in G x T progeny and it co-localizes with the QTL found for berry weight, yield, cluster number and cluster weight (Supplementary material 3.4.6 a) suggest that productivity and acidity could be related. This has been reported by other authors (Etaio et al. 2008, García-Muñoz et al. 2014) who cited that yield per vine is correlated to aggressive wines with green-character notes, maybe associated to more acidity wines.

As it happened with productivity traits, the analysis without female genotypes reduce the variability in the progeny and only three stable QTL were found in LG6, LG12 and LG17 for total acidity.

QTL associated to phenology stages

The influence of *Sex* locus in the QTL detection was confirmed as QTL for Flowering (F), Start Veraison (SV), Flowering - Start Veraison (F- SV), End Veraison (EV), Ripening (RD) dates were detected in LG2 when all the population was considered in the analysis (Table 3.4.6 a). Ramos et al. (2017) also reported differences in blossom and initial development stages according to flower sex. QTL on LG2 was also detected in other works for phenology traits (Constantini et al. 2008, Margueritt et al. 2009), and Costantini et al. (2008), suggested an association between the microsatellite VVIB23, phenology, and productivity traits.

Significant and putative QTL analysis removing female phenotypic data are presented in Table 3.4.6 b and Supplementary material 3.4.5, respectively. In contrast to productivity and must composition analysis, for phenology traits more stable QTL were detected for end veraison (EV), veraison length (VL), ripening date (R) and end veraison-ripening interval (EV-R), when only hermaphrodite plants were considered. A significant and reproducible QTL on LG2 was found in Consensus map for start veraison (SV2) (LOD = 5.1, 24 % of variance), and end veraison (EV2, LOD = 5.9, 24.6 %), being reproducible for ripening date (RD2, LOD = 4.1, 17 %) and putative for flowering–start veraison interval (F-SV) (LOD = 4.7, 28 %). This QTL presented in Consensus and Tempranillo maps seemed to be more related to colour region rather than *Sex* locus due to the region where is detected, but also another QTL on LG2 close to *Sex* locus region was detected in Grenache map for start veraison (SV), and end veraison (EV) traits.

Table 3.4.6 a. Summary of significant (LOD > GW) and stable QT (reproducible in at least two years/environments) detected for phenological stages in G × T progeny (130 genotypes).

		LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var expl	KW
											CW	GW		
PHENOLOGICAL STAGES	SV	2	GAR	2017	UR	14.4	SV2_1	-1_31650356	3.5	9.3-40.7	1.7	3.3	15.4	5
			GAR	2017	V	24.9	SV2_1	2_6111245	2.0	23.8-25.2	1.8	3.3	11.3	4
			CON	2015	UR	47.2	SV2_2	2_8573837	4.4	38.2-59.1	3.6	4.8	18.3	4
			CON	2017	UR	35.1	SV2_2	-1_41916789	5.1	30.2-44.1	3.3	4.9	23.8	4
			TE	2015	UR	54.9	SV2_2	2_8125744	2.0	54.4-55.4	2.0	3.5	8.6	-
		11	CON	2015	UR	12.8	SV11_1	11_1569201	3.3	7.6-36.6	3.1	4.8	14.1	2
			CON	2017	UR	38.2	SV11_1	11_4929080	4.5	11.1-49.2	3.2	4.9	19.5	-
			GAR	2017	UR	47.9	SV11_1	11_4447089	3.6	41.7-52.8	1.7	3.3	15.8	6
			CON	2016	UR	43	SV11_2	11_5758657	4.1	38.2-50.1	3.1	4.8	17.6	2
			GAR	2015	UR	17.4	SV11_2	11_15732731	2.4	5.8-24.5	1.7	3.4	10.5	4
		17	CON	2016	V	64.3	SV17	17_13466470	7.2	45.7-62.5	3.2	4.8	25.8	-
			GAR	2016	UR	44.1	SV17	17_5647891	3.6	29.2-51.3	1.8	3.1	15.5	7
			GAR	2014	V	34.4	SV17	17_7211570	3.2	29.6-44.7	2.0	3.2	20.1	5
		19	CON	2016	UR	34.5	SV19	19_6279096	4.2	15.2-46.4	3.4	4.8	19.1	4
			TE	2016	UR	30.7	SV19	19_5201829	3.6	22.7-36.5	2.0	3.5	15.7	6
	EV	2	CON	2016	UR	47.2	EV2_1	2_8573837	3.7	43.5-50.3	3.5	4.8	16.0	4
			CON	2017	UR	47.2	EV2_1	2_8573837	5.9	44.4-58.9	3.8	4.8	24.6	3
			GAR	2017	V	24.9	EV2_2	2_6111245	2.2	22.8-25.3	1.7	3.2	12.7	4
		17	CON	2014	UR	24.9	EV17	17_4038712	3.4	23.2-27.6	3.2	4.2	14.9	5
			CON	2017	V	27.2	EV17	17_5014430	4.4	25.2-31.5	3.2	4.7	24.2	4
			GAR	2014	UR	55.9	EV17	17_3161035	3.1	34.5-63.8	1.8	3.3	13.6	4
			GAR	2014	V	36.5	EV17	17_6994088	2.3	30.8-44.5	1.9	3.3	14.7	4
			GAR	2016	UR	34.4	EV17	17_7211570	2.0	30.8-44.5	1.7	3.2	9.3	4
			GAR	2017	V	55.9	EV17	17_3161035	2.8	41.3-62.8	1.8	3.2	16.1	4
		18	CON	2016	V	44.4	EV18	18_9105388	4.4	33.4-53.2	3.3	4.8	21.8	4
			GAR	2016	V	28.7	EV18	-1_1130317	4.3	24.9-51.2	1.9	3.2	21.4	7
			TE	2015	V	21.3	EV18	18_12257588	2.2	18.1-22.4	2.1	3.6	12.9	4
		19	CON	2016	UR	3.9	EV19_1	19_151519	3.1	0.0-10.3	3.1	5.0	14.0	2
			GAR	2014	UR	44.2	EV19_1	19_5156867	1.9	43.8-47	1.8	3.3	8.5	4
			GAR	2015	UR	47	EV19_1	19_3914840	2.0	44.2-49.6	1.8	3.2	9.7	4
			TE	2014	UR	24.9	EV19_1	19_3503099	2.0	12.7-25.8	2.0	3.4	8.8	4
			TE	2015	V	15.3	EV19_1	19_2808222	2.5	14.4-19.1	2.4	3.6	14.5	4
	RD	2	CON	2014	UR	55.1	RD2	2_14981293	3.2	46.2-58.9	3.4	4.8	11.0	-
			CON	2016	V	47.2	RD2	2_8573837	4.1	37.2-59	3.1	4.7	16.9	3
			CON	2017	UR	47.2	RD2	2_8573837	4.0	44.1-57.5	3.3	5.0	15.2	2
			GAR	2017	V	32.9	RD2	2_7999163	2.1	32.2-33.3	1.8	3.3	12.8	2
			TE	2016	V	60.7	RD2	2_14822674	2.1	48.6-63.5	1.9	3.4	9.0	3
			TE	2017	UR	53.5	RD2	2_8742134	2.2	46.3-54.1	2.1	3.3	8.6	3
		6	CON	2016	V	17.1	RD6	6_2704384	4.9	8.3-32.1	3.2	4.7	19.8	6
			TE	2016	V	30.1	RD6	6_3980171	2.9	24.2-35.5	1.9	3.4	12.3	6
		8	CON	2015	UR	39.6	RD8	8_20417494	7.2	18.2-52.8	2.9	4.7	22.4	5
			GAR	2015	UR	65.3	RD8	8_20286709	5.2	48.3-76.6	2.0	3.2	16.7	7
			TE	2015	UR	58.8	RD8	8_17906169	2.5	48.8-58.9	2.1	3.4	8.3	4
		11	CON	2015	UR	66.2	RD11	11_14564138	3.6	53.7-70.4	3.3	4.7	12.0	5
			GAR	2016	UR	22.1	RD11	11_7720179	2.3	19.7-26.1	1.8	3.3	14.7	-
			TE	2015	UR	71	RD11	11_11228153	3.7	53.2-77.8	2.1	3.4	12.2	6
	EV-R	13	CON	2014	V	39.6	EVR13	-1_17242391	3.5	33.7-42.2	3.2	4.6	15.5	4
			CON	2015	V	9.4	EVR13	-1_23028641	3.6	8.8-45.3	3.2	4.7	17.0	3
			GAR	2017	UR	12.5	EVR13	13_4550603	2.9	0.0-21.3	2.0	3.2	10	4

Significant QTL were highlighted in red. SV start veraison date, EV end veraison date, RD ripening, EV-R end veraison- ripening interval, LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome - wide (CW) and genome - wide (GW) LOD threshold ($p < 0.05$), %var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal - Wallis significance level, given by the p - value ("1": 0.1, "2": 0.05, "3": 0.01, "4": 0.005, "5": 0.001, "6": 0.0005, "7": 0.00001; " - ": no significance).

In relation to **sprouting dates**, only putative QTL were detected, maybe because only 2016 data was available, although in two environments. A putative QTL on LG10 was found for start sprouting (SS10), explaining 12 % and 10 % of the phenotypic variance respectively. A putative QTL was detected in LG14 for End Sprouting (ES14) and Sprouting Length (SL14) on Consensus and Tempranillo maps, explaining 13 % and 12% of the phenotypic variance, respectively (Supplementary material 3.4.4).

For **flowering date** (F) two putative QTL were detected in LG10 and LG15 (Supplementary material 3.4.4). QTL F10 co-localizes with the one found for start sprouting (SS10) and explained up to 17% of the phenotypic variance in Consensus map, being also detected in Grenache and Tempranillo maps. QTL detected in LG15 for flowering (F15), explained 13 % and 15 % of the variance in Grenache and Consensus maps, respectively. QTL for flowering date in LG10 and LG15 has been also reported in the literature by Fechter et al. (2014) and Carreño-Ruiz. (2012) respectively. Besides other QTL regions for flowering time have been found on chromosome 1 (Fecther et al. 2014, Kamal et al. 2019) and chromosome 14 (Duchene et al. 2012, Kamal et al. 2019), supporting the hypothesis of a complex trait with numerous genes involved in flower development and the date of flowering (Fechter et al. 2014). These regions are associated to VvFT and CONSTANS-like genes.

For **veraison dates**, significant/stable QTL were detected in LG17 and LG19 for start veraison and end veraison dates. Besides, two significant QTL were detected for start veraison on LG11 (SV11_1, SV11_2) and in LG18 for end veraison date (EV18) (Table 3.4.7 a). The QTL found in LG17 for end veraison (EV) and start veraison (SV) dates were detected in Grenache and Consensus maps in at least two years: SV17 (20% and 25% of the variance explained) and EV17 (24% and 15% of the variance). A QTL region in LG17 was also reported associated to veraison by other authors (Carreño-Ruiz 2012, Grzeskowiak et al. 2013). Interestingly, QTL on LG17 co-localizes with stable QTL found for yield, fertility index, cluster weight and total acidity and berry weight (Supplementary material 3.4.6 b). In LG11, two QTL were found for start veraison (SV11_1, SV11_2), reproducible in Consensus and Grenache maps and explaining up to 19% and 16% of the variance. On LG19, two different QTL was were found for start veraison (SV19) and end veraison dates (EV19), being SV19 significant and explaining 19 % of the phenotypic variance, and EV19 stable during two years in Grenache and Tempranillo maps, explaining up to 15 % of the phenotypic variance in Tempranillo map (Table 3.4.7 a). A significant QTL on LG18 was also found for end veraison date (EV18) explaining up to 20 % of the phenotypic variance, in agreement with Duchene et al. (2012), who also reported a QTL on LG18 for veraison date.

In relation to **ripening date (RD)**, significant QTL were detected on LG6, LG8 and LG11 (Table 3.4.7 a). QTL located on LG6 (RD6, LOD = 4.9, 20% of the variance) and LG8, (RD8, LOD = 7.2, 22 % variance), were found in Tempranillo and Consensus maps. RD8 was also found as putative for end veraison-ripening interval (EV-R) (Supplementary material 3.4.4), supporting the high correlation found between these traits (0.9, $p < 0.01$, Table 3.4.4). A QTL region in LG8 was previously reported by Fisher et al. (2004), and Song, (2014) also found a QTL on LG8 for veraison length. QTL on LG11 (RD11), explained 15 % of the variance in Consensus map and was also detected in Tempranillo and Grenache maps, but was not located in the same region than SV11, QTL found for start veraison date. Besides, several putative QTL were detected on LG4, LG6, LG9, LG13 and LG14 (Supplementary material 3.4.5). The putative QTL detected on LG14 (RD14) explained 13 % of the variance in Grenache and Consensus maps, and co – localizes with QTL ES14 and SL14 found for End Sprouting (ES) and Sprouting Length (SL), respectively.

Related to phenological periods, only a few and putative QTL were detected. A stable QTL on LG13 for end veraison-ripening interval (EV-R) co-localizes with putative QTL for start veraison (SV), ripening date (RD), flowering- start veraison interval (F-SV).

When the analysis was performed in the hermaphrodite G x T progeny, additional / stable QTL were found. The significant / stable QTL on LG2 found for start veraison (SV), end veraison (EV) and ripening date (RD) traits in G x T population (Table 3.4.6 b) was not detected in the analysis in the hermaphrodite population and only a putative QTL on LG2 in the region of colour was detected for ripening date (RD) (Supplementary material 3.4.5). That confirms the effect of *Sex* locus on phenological traits and also the effect of colour, as was shown in Chapter 3.1, where differences were reported between female and hermaphrodite plants in some stages and between white and red genotypes in ripening date.

In summary, whilst in productivity and must parameters traits fewer and weaker effects were found in the analysis conducted with hermaphrodite progeny, in the case of phenological stages, more and stronger QTL were found in the new analysis. The influence of *Sex* and *colour* locus were confirmed in all the parameters studied except to TSS, pH, sprouting date (maybe because only data for one year was evaluated), veraison length, and F-SV and EV-R periods. Several genes potentially affecting fertility and the timing of fruit development were proposed, based on their position and putative function, what is in agreement with the co-localizations found in LG2 and LG17 in this work between fertility index and phenological traits as veraison dates (Supplementary material 3.4.6 a and b).

The detection of a large number of QTL with small effect could be due to a large influence of environmental variation. Phenological stages are complex traits particularly difficult to analyze because they are controlled by many loci such as regulation of flowering time (Costantini et al. 2008). Considering the 4 years of the study, weather conditions were extremely different, being 2014 a low productivity year due to the impact of powdery-mildew, 2016 a really productive one, and 2017 a really warm year. Alternate bearing has not been reported in grapevine, but in the light of the results of these years, it could be a plausible hypothesis, that affected the detection of more stable QTL.

Table 3.4.6 b. Summary of significant (LOD > GW) and stable (reproducible in at least two years/environments) QTL for phenological stages detected in G × T hermaphrodite population (102 genotypes).

		LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var expl	KW
											CW	GW		
PHENOLOGICAL STAGES	SS	10	CON	2016	UR	53.2	SS10_1	10_6374751	6.8	53.1-56.8	3.3	4.7	26.5	7
			GAR	2016	UR	36.3	SS10_1	10_5178597	3.3	34.4-40.5	2.0	3.3	13.7	6
			CON	2016	UR	64.5	SS10_2	10_9919995	4.2	59.2-64.4	3.3	4.7	17.1	7
			GAR	2016	UR	21.9	SS10_2	10_10176689	4.1	20.7-26.8	2.0	3.3	16.8	7
	SV	11	CON	2017	UR	38.2	SV11	11_4929080	4.4	35.7-41.5	3.3	4.8	24.0	2
			GAR	2017	UR	47.9	SV11	11_4447089	3.8	45.1-50.9	2.2	3.5	21.0	7
		17	CON	2014	V	51.5	SV17_2	17_8987545	4.4	37.1-53.2	3.3	4.8	34.6	6
			GAR	2014	V	34.4	SV17_2	17_7211570	4.5	22.1-37.6	2.1	3.4	34.8	7
	EV	14	CON	2016	UR	77.8	EV14	14_27962249	3.4	76.8-80.4	3.2	4.7	19.3	6
			TE	2016	UR	62	EV14	14_25784210	2.1	56.2-65.5	1.9	3.2	12.5	4
			GAR	2015	V	52.5	EV14	14_22118591	2.8	49.6-70.7	1.9	3.2	20.7	5
			GAR	2016	V	54.7	EV14	14_23084906	2.9	48.7-60.4	2.1	3.3	19.9	6
		17	GAR	2016	UR	31.3	EV17	17_6884962	1.7	19.9-32.3	1.9	3.2	10.5	5
			GAR	2014	V	25.3	EV17	17_6473195	2.5	19.9-32.3	2.1	3.3	20.8	6
			GAR	2014	UR	20.9	EV17	17_5398742	2.0	19.9-32.3	1.9	3.3	13.8	4
			CON	2014	V	36.8	EV17	17_6573650	3.2	34.4-39.5	3.0	4.7	25.6	5
			CON	2014	UR	24.5	EV17	17_4051401	3.1	21.9-28.6	3.0	4.7	16.9	4
			CON	2017	V	21.9	EV17	17_3063542	4.2	19.8-33.5	3.2	4.7	29.9	3
		18	TE	2014	UR	6.4	EV18_1	18_10007101	2.2	4.2-10.3	2.1	3.4	12.8	3
			TE	2016	V	3.8	EV18_1	18_8563287	2.9	2.6-6.5	2.0	3.4	19.4	6
			GAR	2016	V	45.9	EV18_1	18_10851690	2.9	41.9-50.4	2.1	3.3	19.7	6
			CON	2016	V	48.5	EV18_1	18_10207857	5.0	42.6-53.5	3.2	4.7	31.5	4
	VL	8	CON	2014	UR	26.8	VL8	8_12139306	3.8	22.5-30.5	3.1	4.7	21.2	2
			CON	2015	UR	22.8	VL8	8_11215864	3.8	19.1-29.1	3.3	4.7	23.4	5
			GAR	2014	UR	33.4	VL8	8_12031076	1.9	26.5-34.1	1.8	3.2	10.2	3
			TE	2014	UR	21.1	VL8	8_12263719	2.1	19.9-24.5	2.0	3.3	12.6	4
		15	CON	2016	UR	89.8	VL15	15_17998243	4.6	77.1-90.1	3.3	4.8	25.6	2
			CON	2016	V	86.6	VL15	15_17652929	3.4	80.1-86.7	3.1	4.7	22.4	2
			GAR	2016	UR	63.6	VL15	15_18646059	2.2	62.0-64.7	1.9	3.2	13.0	4
			TE	2016	UR	59.1	VL15	15_19195249	1.7	59.0-59.9	1.9	3.1	10.1	2
	RD	8	CON	2015	UR	60	RD8	8_19227329	6.9	50.3-70.4	3.2	4.7	26.8	7
			GAR	2015	UR	65.3	RD8	8_20286709	4.7	64.7-72.5	2.1	3.3	19.1	7
			TE	2015	UR	58.8	RD8	8_17906169	2.3	56.5-59.2	2.0	3.4	9.9	-
		13	CON	2015	UR	33.4	RD13_1	13_6503443	3.0	26.6-35.4	3.0	4.7	12.5	3
			CON	2017	V	27.9	RD13_1	13_5132831	3.4	23.5-29.3	3.1	4.7	24.6	3
			TE	2017	V	32.2	RD13_1	13_6925489	2.1	31.3-32.7	1.9	3.2	15.9	3
		17	CON	2014	UR	18.7	RD17	17_2012240	3.5	17.5-26.6	3.2	4.7	14.7	4
			CON	2017	V	25.2	RD17	17_4156201	3.8	18.5-26.8	3.2	4.8	27.3	2
			GAR	2014	UR	12	RD17	17_2997781	2.3	10.8-14.6	2.1	3.3	9.9	4
	EV-R	8	CON	2015	UR	59.3	EVR8	8_19698160	3.2	58.3-69.8	3.1	4.7	19.6	4
			CON	2017	UR	68.2	EVR8	8_21059244	3.7	59.4-71.4	3.3	4.8	23.2	4
			GAR	2015	UR	67.4	EVR8	8_21241515	2.0	64.3-72.8	1.8	3.2	11.3	4
			GAR	2017	UR	71.3	EVR8	8_21620320	2.8	64.5-76.5	1.9	3.4	18.0	5

Significant QTL were highlighted in red. SS start sprouting date, SV start veraison date, EV end veraison date, VL veraison length, RD ripening date. LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome - wide (CW) and genome - wide (GW) LOD threshold ($p < 0.05$), %var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal - Wallis significance level, given by the p - value (“1”: 0.1, “2”: 0.05, “3”: 0.01, “4”: 0.005, “5”: 0.001, “6”: 0.0005, “7”: 0.00001; “ - ”: no significance).

Pre - selection of improved genotypes for climate change adaptation

A principal component analyses was conducted with the aim of identifying the variables that best described the phenotypic variability present in the F_1 progeny. Two independent PCA were done to assess the variability for red and white genotypes (Figure 3.4.3 a and b). The two first dimensions explained 40.1 % and 20.6 % and 48.7 % and 22.1 % of the total observed variability, respectively in red and white genotypes representation. In both groups the first component was strongly associated with ripening date and other phenology stages and negatively correlated with must parameters as º Brix or pH. The second component resulted negatively correlated with productivity traits, particularly cluster number.

The genotypes were selected based on the average values for the most relevant criteria for wine grape breeders: production, must parameters and ripening time, in years 2014, 2015, 2016 and 2017. In the current global warming context, the purpose was to preselect hybrids with low berry weight, moderate production, high acidity and late ripening date.

Acidity is crucial in wine stability inhibiting spoilage and is also a key factor in sensory analysis. An excessive acidity conducts to aggressive wines, and deficient acidity to flat taste wines (Conde et al. 2007, Bayo-Canha et al. 2012), being acidities between 5 - 7 g / L tartaric acid considered suitable for red genotypes and between 5 - 8 g / L in white cultivars. Remarkedly, acidity together with Brix degree are the most influenced parameters by the temperature rise associated to climate change.

The groups obtained based on the classification of red and white genotypes are shown in Figure 3.4.4. For red-berry, 98 genotypes were grouped into two main clusters and eight subgroups, and the 32 white genotypes were classified into two main clusters and four subgroups considering the above selection criteria. Regarding red genotypes, cluster 1 includes 59 genotypes that presented low berry weight (BW ≤ 1.6g) moderate yield (2.1 kg/vine as group average), cluster weight (130 g in average), and total acidity (4.7 g / L) compared to cluster 2 with higher berry weight (BW ≥ 1.7 g), more productive vines (Y = 4.4 kg / vine and CW = 280 g in average), and higher total acidity (5.4 g / L in average). The first subgroup of cluster 1 is formed by 20 genotypes with small berry weight (BW ≤ 1.6 g), moderate yield (1.6 ± 0.7) and quite late ripening date, but cluster weight is too low for selection. Interestingly, most of the clones selected are among subgroups IV and V constituted by early ripening and late ripening genotypes, and hence with the desirable plasticity for the different scenarios of climate change. Subgroup IV is characterized by early ripening date, moderate - high yield, and moderate cluster weight, whilst subgroup V consists of genotypes with moderate total acidity, late - ripening date, high productivity and cluster weight, suitable for global warming scenario (Table 3.4.7). Besides, pre-selected genotypes 147 and 193 are placed in Subgroup 3, also characterized by late ripening, but with low Brix degree (21.6 º), indicating an even later ripening date.

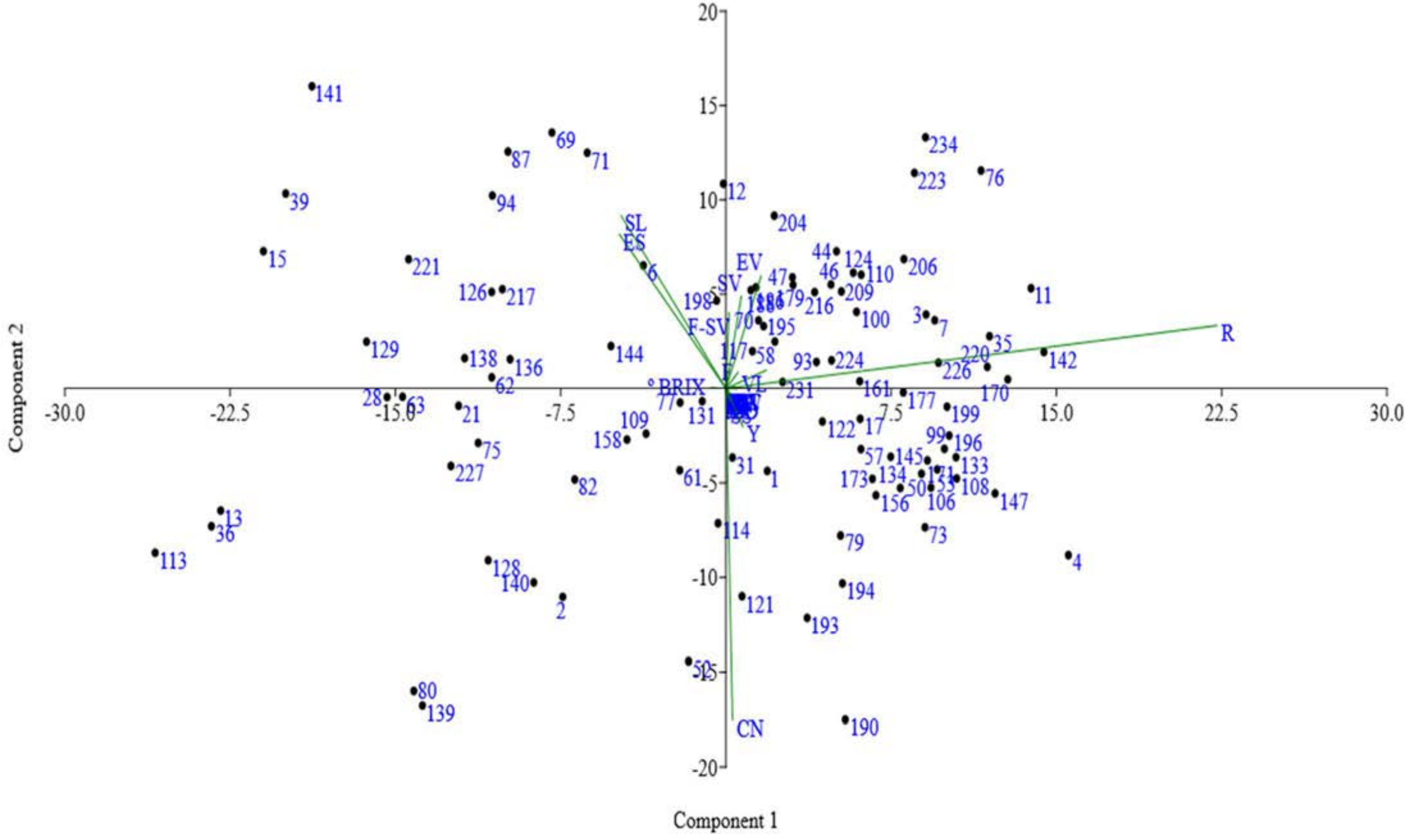
Concerning white genotypes, cluster 2 includes 8 genotypes, highly productive with a BW = 2 g, Y = 4.6 Kg and CW = 300 g in average. In contrast, in cluster 1, vines were grouped by a lower berry weight BW ≤ 1.7 g, a moderate yield and cluster weight, and higher values of total acidity and Brix degree (Table 3.4.8). Pre-selected genotypes, belong to Subgroups I and II, cluster I and cluster II, III genotypes with higher values of berry weight and productivity.

Contrary to red wine varieties, in white cultivars larger berries are associated to higher quality wines due to better sensory properties like in Muscat Hamburg (Rolle et al. 2015), higher total acidity in Riesling (Friedel et al. 2016), and higher concentration of varietal aromatic

compounds as methoxypirazines in Sauvignon blanc (Suklje et al. 2012). The reason being that in white-wine fermentation skin contact is limited, and therefore the contribution of skin compounds to wine composition is not as relevant. Thus, white genotypes 18, 41, 91, 125, 151 and 232 with berry weights larger than 1.6 g were selected based on their potential: better acidity, ripening date or productivity traits.

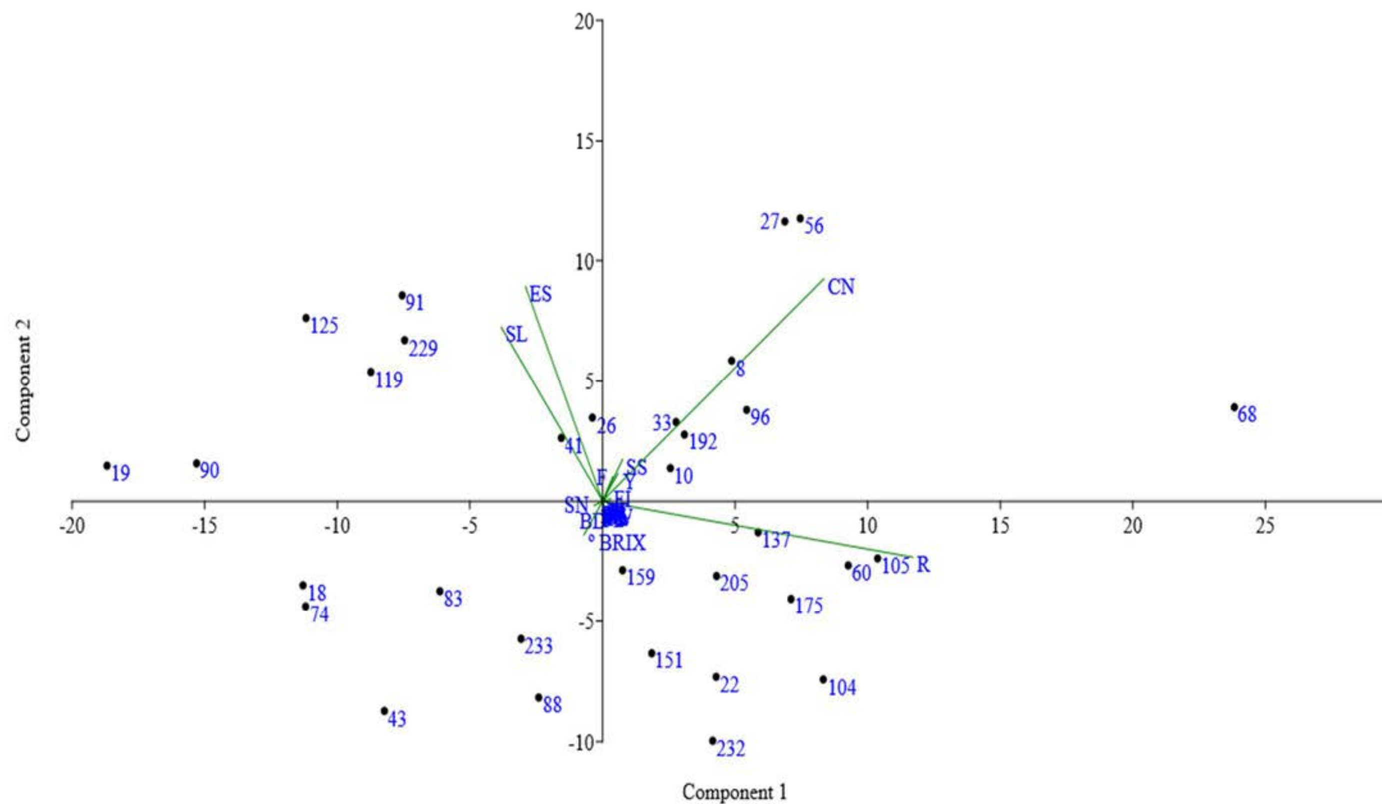
In summary, eleven red and 11 white genotypes were pre - selected from the 130 F1 progeny obtained from Grenache x Tempranillo cross. All the red genotypes (Table 3.4.7) presented BW ≤ 1.6 g, yield > 1.8 kg / vine, CW > 150 g and a total acidity > 5 g / L. Genotypes 7, 139, 147, 198 and 220 are especially interesting being able to reach maturity even though their late - ripening behaviour (TSS < 23.4 Brix º). Genotypes 196 and 198 have enological potential due to their high total acidity and small berry weight (Table 3.4.8 a). Among the pre - selected white genotypes (Table 3.4.8 b), all of them present a CW > 150 g, yield > 1.5 kg / vine, and a minimum total acidity of ≈ 5 g / L. Five pre - selections presented a berry weight ≤ 1.6 g, and six a berry weight higher than 1.6 g since berry size influence in white wine quality is still debated. Genotypes 18, 41, 91 and 232 are especially promising due to their total acidity (around 6 g / L) while genotypes 68 and 125 stand out as late ripening reaching only 20.1 Brixº.

Figure 3.4.3 a. PCA and plot distribution of red genotypes.



Legend: CN Cluster number, Y Yield, R Ripening date, SL Sprouting length, EV End veraison, F-SV flowering - start veraison interval.

Figure 3.4.3 b. PCA and plot distribution of white genotypes.



Legend: CN Cluster number, ES End Sprouting date, SL Sprouting length, R Ripening date, SN seed number, BD Berry diameter.

Figure 3.4.4. Cluster analysis of red genotypes (left) and white (right) of G x T progeny.

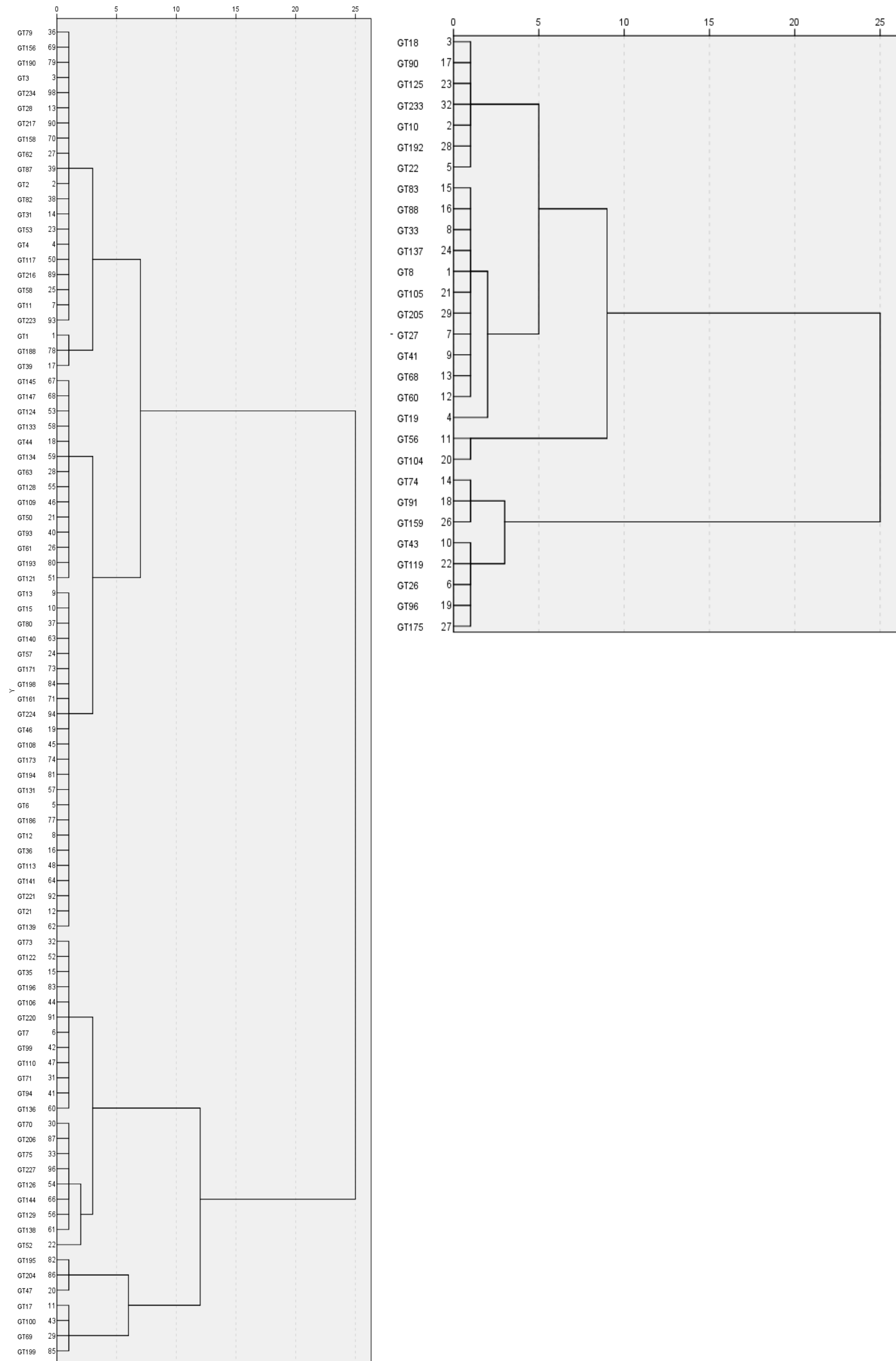


Table 3.4.7. Groupings of red and white genotypes from squared Euclidean distance combined with the average linkage clustering methods.

C	SG	Red Genotypes	N	BW	R	Y	CN	CW	AT	BRIX
I	I	GT2,GT3,GT4,GT11,GT28,GT31,GT53,GT58,GT62,GT79,GT82,GT87,GT117,GT156 ,GT158,GT190,GT216,GT223,GT234	20	1.6±0.4	183±8	1.6±0.7	18±8	87±13	4.7±1.2	22.5±1.4
	II	GT1, GT39, GT188	3	1.4±0.6	178±8	0.6±0.2	14±5	40±4	4.6±1.25	23.2±0.9
	III	GT44, GT50, GT61, GT63, GT93, GT109, GT121, GT124, GT128, GT133, GT145, **GT147**, **GT193**	13	1.6±0.4	184±8	3.7±1.0	21±6	177±8	4.6±0.9	21.6±1.9
	IV	GT6, GT12, GT13, GT15, GT21, GT36, GT46, **GT57**, GT80, GT108, GT113, GT131, **GT139, GT140**, GT141, GT161, **GT171**, GT173, GT186, GT194, **GT198**, GT221, GT224	23	1.6±0.4	177±11	2.6±0.9	19±6	135±13	4.9±0.9	23.4±1.6
II	V	GT7, GT35, GT71, GT73, **GT94**, GT99, GT106, GT110, GT122, GT134, GT136, **GT196, GT220**	13	1.8±0.3	186±7	3.8±1.1	17±5	221±12	5.5±0.9	23.4±1.5
	VI	GT70, GT75, GT126, GT129, GT144, GT206, GT227	7	1.9±0.4	175±8	4.4±1.3	17±4	258±9	5.2±0.7	23.1±1.6
	VII	GT47, GT195, GT204	3	1.7±0.2	184±2	5.1±1.1	14±3	370±5	5.1±0.4	22.5±1.1
	VIII	GT17, GT52, GT69, GT100, GT138, GT199	6	1.7±0.3	181±9	5.1±2.7	16±9	299±15	5.6±0.9	23.4±1.4

C	SG	White Genotypes	N	BW	R	Y	CN	CW	TA	ºBRIX
I	I	GT10, GT18, **GT22, GT90, GT125, GT192,** GT233	7	1.7±0.1	176±7	2.9±0.9	16±5	200±4	4.4±0.8	22.9±1.4
	II	GT8, GT19, GT27, **GT33**, GT41, **GT60, GT68**, GT83, **GT88**, GT105, GT137, GT205	12	1.6±0.4	180±9	2.9±1.0	22±7	133±18	4.5±0.6	22.5±2.0
	III	GT56, GT104	2	1.5±0.1	187±2	1.4±0.8	23±7	65±10	4.3±1.2	22.5±0.0
II	IV	GT26, GT43, **GT74**, GT91, **GT96**, GT119, **GT159**, GT175	8	2.0±0.2	178±7	4.6±1.8	15±6	306±25	4.2±0.3	22.0±1.6

Bold numbers correspong to pre - selected material. C Cluster, SG Subgroup, BW (berry weight), R (Ripening days from 1st March) Y (Yield) CN (Cluster number) CW (Cluster weight), TA (Total acidity), ºB (Brix º).

Table 3.4.8 a. Pre - selected red genotypes with the mean values for the selection criteria.

Red GT	BW	RD	Brix°	TA	Yield	CN	CW
GT07	1.63±0.09	**217.3±11.0**	22.9±0.4	4.67±0.88	3.07±1.34	17.25±5.85	185.4±82.53
GT57	1.63±0.21	210.8±20.3	23.6±1.8	4.68±0.65	2.42±1.45	16.67±9.07	153.14±12.72
GT94	1.53±0.35	203.0±19.1	24.2±1.1	5.00±1.13	1.85±0.73	10.0±2.0	184.12±53.82
GT139	1.63±0.27	**217.0±10.1**	**22.3±1.3**	4.71±0.67	2.78±0.75	17.67±9.87	173.28±46.4
GT140	1.59±0.72	206.5±4.7	23.8±0.5	4.60±0.50	3.76±1.15	23±11.36	181.64±54.93
GT147	1.57±0.34	**217.4±17.9**	**22.0±2.0**	5.51±0.45	3.05±1.85	16.67±7.09	169.52±57.83
GT171	1.61±0.30	215.6±17.4	23.2±1.6	**5.74±0.72**	2.69±0.97	17.0±4.58	156.66±39.36
GT193	1.57±0.28	207.2±16.6	24.2±2.0	4.81±0.41	3.94±1.12	23.0±8.19	187.07±82.76
GT196	1.30±0.20	214.7±13.8	23.2±0.9	**6.47±0.82**	3.15±0.82	15.75±8.66	239.01±96.21
GT198	1.46±0.45	**213.0±22.5**	**22.0±1.4**	**6.91±1.24**	2.61±0.52	17.0±4.58	166.1±73.75
GT220	1.38±0.34	**216.8±19.3**	**22.1±1.2**	5.00±2.15	2.98±0.65	19.33±5.86	163.87±59.23

BW berry weight, R Ripening date, TA Total acidity, CN Cluster number, CW Cluster weight.

Table 3.4.8 b. Pre - selected white genotypes with the mean values for the selection criteria.

White GT	BW	R	Brix°	TA	Y	CN	CW
GT18	1.86±0.57	204.2±13.9	23.2±1.1	**5.8±0.8**	1.5±0.46	8.0±2.16	162.9±76.77
GT33	1.20±0.34	200.8±15.07	23.5±1.3	4.7±0.7	3.4±0.69	23.8±2.22	147.7±38.76
GT41	1.88±0.25	195.2±15.8	24.0±1.4	**5.9±1.3**	2.41±1.43	16.33±8.14	148.6±28.22
GT60	1.54±0.41	210.5±19.05	23.2±2.2	5.5±0.54	4.3±1.3	21.5±0.71	198.2±54.02
GT68	1.62±0.35	**220.3±12.09**	**20.5±3.2**	5.1±0.23	3.3±2.27	16.5±7.68	146.1±13.29
GT88	1.16±0.14	198.2±12.91	22.7±1.1	4.6±0.5	3.1±1.45	19±5.57	157.9±49.78
GT91	2.12±0.09	203.4±14.5	22.9±1.3	**5.8±0.5**	4.13±2.49	16±6.16	264.2±138.2
GT125	1.80±0.08	**221.7±9.29**	**20.0±2.9**	4.6±0.21	2.8±1.06	13.3±4.51	211.3±32.01
GT151	2.28±0.34	207.8±18.5	23.2±0.5	6.0±0.5	4.54±2.87	13.25±3.1	233.8±68.7
GT192	1.54±0.1	207±19.03	22.3±1.4	4.9±0.67	2.2±1.51	14±8.37	146.7±81.25
GT232	2.08±0.19	207±20.9	21.0±2.0	**6.4±1.6**	3.97±1.71	10.67±2.52	251.7±49.17

BW berry weight, R Ripening date, TA Total acidity, CN Cluster number, CW Cluster weight.

Conclusions

Genetic determinants of must parameters, productivity and phenology were identified in a wine grape progeny. For productivity traits, QTL on LG3, LG10, LG10, LG17 and LG18 were found associated to the traits studied, being the QTL on LG17 the most reproducible across years.

A major QTL was detected on LG1 for TSS as well as minor QTL for pH on LG 3 and LG13. For total acidity, for which Grenache and Tempranillo, presented large differences, several significant QTL were detected in LG12, 13, 14 and 17, being reproducible in at least three years.

QTL on LG 7 and 10 were identified for traits as sprouting time and flowering date, being unstable along the years of the study. Other QTL on LG 8, 13, 14 and 17 were found to control several traits related to veraison and ripening time. As previously reported for flower, seed and berry related traits, the influence of *Sex* locus in productivity and phenological traits related to veraison has been confirmed, being *colour* locus also linked to the detection of QTL in total acidity and ripening date. Co-localization in LG2 and LG17 of factors controlling fertility index and phenological traits points out that genes potentially affecting fertility could be also responsible of the timing of fruit development.

The results obtained for productivity, must and phenological traits may be useful in the current search of suitable cultivar in global warming conditions and in assisting breeding programs to identify candidate genes for further functional studies. This research confirms the potential for selection of improved genotypes in an intra - specific cross of premium varieties already adapted in order to face climate change.

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Supplementary material

Supplementary material 3.4.1. Plot effect for the traits that were studied in both environments.

	Year	UR				Varea			
		N	Mean SD	MIN	MAX	N	Mean SD	MIN	MAX
FI	2014	128	1.4 ± 1.0**a	0.11	10	87	0.8 ± 0.4 b	0.14	2.0
	2015	130	1.14 ± 0.41 **a	0.29	2.88	101	0.85 ± 0.56 b	0.13	2.33
	2016	130	0.89 ± 0.28 ns	0.18	1.54	113	0.94 ± 0.46 ns	0.08	2.25
	2017	130	1.05 ± 0.39**a	0.25	1.94	106	0.89 ± 0.55 b	0.08	3.8
Brix	2016	67	12.1 ± 0.99 ns	10	14.1	96	12.1 ± 1.12 ns	6	14.25
	2017	107	12.86 ± 0.74**a	11.1	15.2	68	12.51 ± 0.93 b	10.6	14.8
pH	2016	65	3.26 ± 0.44*a	1.65	3.92	90	3.42 ± 0.38 b	1.69	4.25
	2017	106	3.29 ± 0.15**a	2.93	3.71	68	3.43 ± 0.18 b	3.02	3.88
TA	2016	64	4.79 ± 1.15 *a	2.17	7.03	90	5.35 ± 1.52 b	1.73	9.19
	2017	106	5.44 ± 1.4 ns	2.83	11.69	68	5.43 ± 1.29 ns	2.72	9.15
SV	2014	97	152.37 ± 6.35 **a	119	166	65	157.02 ± 4.92 b	146	166
	2015	101	144.23 ± 5.96	133	166	71	145.04 ± 9.36	123	162
	2016	97	158.69 ± 3.09	151	170	84	158.01 ± 6.72	153	213
	2017	95	143.82 ± 4.4	139	154	75	142.99 ± 4.05	138	152
EV	2014	97	178.4 ± 6.13 **a	166	193	66	173.8 ± 6.48	163	188
	2015	88	172.44 ± 3.3 *a	166	176	74	169.08 ± 5.95	152	181
	2016	96	175.68 ± 5.32	164	185	83	174.87 ± 4.8	164	185
	2017	96	159.48 ± 7.76	131	173	74	157.96 ± 7.46	131	173
VL	2014	97	25.93 ± 7.92 **a	12	59	65	16.83 ± 5.19	7	31
	2015	86	28.79 ± 5.43 **a	16	40	71	23.83 ± 8.24	9	47
	2016	96	17 ± 4.13	7	25	83	16.84 ± 8.45	7	29
	2017	95	15.77 ± 6.6	7	34	72	15.24 ± 5.78	7	31
RD	2016	67	219.1 ± 9.44 **a	203	231	101	212.86 ± 12.59	191	230
	2017	110	199.48 ± 10.1 **a	184	222	69	192.59 ± 13.23	177	222

FI fertility index, TA Total acidity, SV start veraison date, EV end veraison date, VL veraison length, RD ripening date.

Supplementary material 3.4.2. MANOVA results for progeny Grenache x Tempranillo.

Factor	Trait	F	Sig	Factor	Trait	F	Sig	Factor	Trait	F	Sig
Year	**Y**	17.11	0.00	Plot	**FI**	0.09	0.77	Year*Plot	**FI**	0.75	0.39
	CN	52.91	0.00		**Brixº**	3.00	0.08		**Brixº**	1.54	0.21
	FI	11.60	0.00		**pH**	24.9	0.00		**pH**	0.12	0.73
	Brixº	23.55	0.00		**TA**	3.88	0.05		**TA**	3.91	0.05
	pH	23.94	0.00		**SV**	1.86	0.17		**SV**	0.01	0.93
	TA	5.15	0.00		**EV**	0.50	0.48		**EV**	0.46	0.49
	SV	213.1	0.00		**VL**	0.00	0.98		**VL**	0.04	0.83
	EV	222.2	0.00		**RD**	98.3	0.00		**RD**	14.13	0.00
	VL	74.42	0.00								
	RD	120.2	0.00								

Y yield, CN cluster number, CW cluster weight, FI fertility index, TA Total acidity, ES end Sprouting date, F flowering date, SV start veraison date, EV end veraison date, VL veraison length, RD ripening.

Supplementary material 3.4.3. MANOVA for productivity, must composition and phenological traits with parent and vintage as factors.

	Parent		Vintage		Parent*Vintage	
Trait	**F**	**Sig**	**F**	**Sig**	**F**	**Sig**
Y	0.00	0.99	4.10	0.03	0.43	0.66
CN	0.06	0.81	0.35	0.79	0.12	0.89
CW	0.37	0.55	12.07	0.00	7.24	0.01
FI	2.98	0.13	0.63	0.45	0.27	0.62
Brix º	1.00	0.34	4.33	0.03	0.47	0.64
pH	13.22	0.00	1.63	0.23	0.31	0.74
TA	4.61	0.05	14.92	0.00	4.96	0.03
F	3.90	0.00	117.97	0.00	0.07	0.62
SV	159.58	0.00	120.86	0.00	0.44	0.83
EV	213.35	0.00	1.75	0.61	7.02	0.31
VL	0.62	0.79	93.49	0.01	10.97	0.27
RD	159.58	0.01	477.63	0.00	193.42	0.01
F-SV	532.24	0.00	368.40	0.00	136.12	0.03
EV-R	741.97	0.00	421.49	0.00	126.75	0.04

Y yield, CN cluster number, CW cluster weight, FI fertility index, TA Total acidity, ES end Sprouting date, F flowering date, SV start veraison date, EV end veraison date, VL veraison length, RD ripening, F-SV flowering-start veraison interval, EV-R end veraison-ripening interval.

Supplementary material 3.4.4. Summary of putative (LOD > CW) QTL in G × T progeny (130 genotypes).

		LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var	KW
											CW	GW		
PRODUCTIVITY TRAITS	Y	2	**GAR**	2014	UR	25.8	Y2	2_4863957	1.9	25.8-28.4	1.8	3.2	6.5	2
			CON	2014	UR	26.9	Y2	2_4863957	3.4	26.9-29.7	3.4	4.8	11.4	3
		7	**GAR**	2016	UR	7.2	Y7_1	7_729864	3.1	6.4-10.6	2	3.3	19.0	6
			CON	2014	UR	16.4	Y7_1	7_628816	3.2	12.9-27.1	3.2	4.8	10.9	6
			GAR	2016	UR	30.5	Y7_2	7_4508601	3.1	27.2-30.8	2	3.3	19.3	-
			CON	2016	UR	9.6	Y7_2	7_1109825	3.1	9.2-12.9	3	4.7	17.9	-
		18	**CON**	2017	UR	87.7	Y18	18_27984510	4.0	84.4-93.6	3.4	4.8	15.5	2
			GAR	2017	UR	77.0	Y18	18_26048189	2.3	68.2-78.4	2.0	3.1	9.1	3
			TE	2015	UR	38.3	Y18	18_24809321	2.4	36.6-38.9	2.0	3.4	8.0	-
	CN	17	**CON**	2015	UR	25.2	CN17_1	17_4156201	2.6	24.9-26.5	3.0	4.8	8.6	4
			TE	2015	UR	19.1	CN17_1	17_3304673	1.9	16.7-21.9	2.0	3.5	6.4	2
			CON	2015	UR	64.2	CN17_2	17_11084580	3.2	61.1-66.0	3.0	4.8	10.6	3
			GAR	2015	UR	63.8	CN17_2	17_15591403	2.1	49.5-64.8	1.8	3.3	7.0	4
	FI	2	**TE**	2017	UR	14.4	FI2	2_3043378	2.7	9.1-24.3	2.0	3.4	9.1	2
			CON	2017	UR	7.6	FI2	2_1347733	3.7	4.1-13.1	3.4	4.7	12.2	5
		9	**TE**	2017	UR	43.9	FI9	9_4555301	2.5	41.7-49.7	1.8	3.4	8.6	4
			CON	2017	UR	39.1	FI9	9_4555301	3.0	37.8-47.7	2.9	4.7	10.1	4
MUST TRAITS	pH	3	TE	2017	UR	10.3	pH3	3_1639001	3.1	3.2-16.5	2.2	3.3	12.4	**5**
			CON	2017	UR	6.2	pH3	3_989303	3.4	2-17.3	3.0	4.8	13.7	**5**
		13	TE	2016	UR	14.0	pH13	13_4448129	2.2	11.2-19.4	2.1	4.5	14.3	**-**
			CON	2015	UR	40.7	pH13	13_6816148	4.1	34.9-46.2	3.2	4.7	13.7	**3**
	TA	6	**GAR**	2017	UR	51.9	TA6	6_13135494	2.4	50.8-55.9	1.8	3.1	9.7	**4**
			CON	2017	UR	46.2	TA6	6_8056677	4.2	40.3-62.7	3.3	5.8	16.5	**5**

Continue

		LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var	KW
											CW	GW		
PHENOGICAL STAGES	SS	10	CON	2016	UR	53.2	SS10	10_6374751	3.7	41.3-56.6	3.2	4.7	12.3	3
			GAR	2016	UR	21.9	SS10	10_10176689	2.8	19.2-30.2	1.8	3.2	9.6	4
	SL	14	CON	2016	V	14.1	SL14	14_964807	3.7	0-18.1	3.4	4.8	13.1	6
			TE	2016	V	1.8	SL14	14_579397	3.3	0-7.3	2.0	3.4	12.0	6
	ES	14	CON	2016	V	14.1	ES14	14_964807	3.7	0-24.5	3.2	4.8	13.0	6
			TE	2016	V	1.8	ES14	14_579397	3.3	0-7.3	2.0	3.4	11.5	6
	F	10	CON	2016	V	42.7	F10	10_2524063	4.5	30-51.8	3.1	4.9	16.6	6
			TE	2016	V	23.9	F10	10_1618757	2.5	20.4-29	1.7	3.5	9.3	7
			GAR	2016	V	44.7	F10	10_2524063	2.1	41.4-44.7	1.8	3.1	8.1	6
		15	GAR	2016	V	57.2	F15	15_11318578	3.0	48.6-64.8	1.9	3.1	13.1	7
			CON	2016	V	3.8	F15	-1_39397398	4.1	0-34.2	3.4	4.9	15.1	3
	SV	13	CON	2015	UR	48.1	SV13	-1_358137	4.3	40.7-54.7	3.2	4.8	17.7	4
			GAR	2015	UR	40.1	SV13	13_16059853	2.4	32.3-44.4	1.9	3.4	10.3	5
	EV	7	CON	2015	UR	71.1	EV7	7_17147459	3.2	62.9-76.1	3.2	4.9	15.6	-
			TE	2015	UR	78.5	EV7	-1_32721093	2.6	77.9-79.6	2.1	3.4	12.8	-
		15	CON	2016	UR	74.7	EV15	15_15435998	3.7	67.5-88.7	3.3	4.8	16.1	3
			GAR	2017	V	29.7	EV15	15_15269478	1.9	28.9-37.7	1.8	3.2	11.2	-
		19	CON	2014	UR	64.1	EV19_2	19_22905959	4.0	52.7-69.9	3.2	4.2	17.2	4
			TE	2016	UR	60.6	EV19_2	-1_33982878	2.4	58.8-61.5	2.3	3.5	10.6	4
	VL	13	TE	2014	V	32.2	VL13	13_6925489	2.6	20.3-47.8	2.0	3.4	16.9	2
			CON	2014	V	9.1	VL13	13_1371790	3.4	5.3-20.4	3.3	5.0	21.3	2
		15	CON	2016	UR	74.7	VL15	15_15435998	4.2	69.4-98.2	3.3	4.8	18.3	3
			GAR	2016	UR	7.1	VL15	15_18646059	2.3	3.1-12.1	1.9	3.2	10.5	4
			TE	2016	UR	50.3	VL15	15_16698906	1.8	41.5-52.2	1.9	3.5	8.4	3
	RD	13	CON	2015	UR	33.7	RD13	13_6024941	3.6	47.6-66.8	3.3	4.7	12.1	4
			GAR	2015	UR	65.1	RD13	-1_3191380	2.1	34.9-62.9	1.8	3.2	7.3	-
		14	CON	2016	UR	20.3	RD14	14_3057627	3.3	16.4-20.4	3.1	4.8	20.4	4
			TE	2016	UR	28.9	RD14	14_8810733	2.2	18.9-29.2	2.3	3.4	14.1	4
	F-SV	2	GAR	2014	V	43	FSV2	2_17798068	2.2	41.7-45.1	1.7	3.0	14.6	-
			CON	2014	UR	45.6	FSV2	2_9003102	4.7	42.1-47.0	4.6	6.5	28.2	-
		13	GAR	2015	UR	62.3	FSV13	-1_3165962	2.2	61-65.1	1.9	3.3	10.0	4
			CON	2015	UR	48.1	FSV13	-1_358137	4.0	41.1-54.9	3.1	4.8	17.3	3
	EV-R	1	CON	2017	V	43	EVR1	1_20813560	4.6	33.4-49.6	3.1	4.7	21.2	3
			GAR	2017	V	81	EVR1	1_19838155	2.1	80.2-83.9	1.9	2.3	11	4
		8	CON	2015	V	37.2	EVR8	8_19844946	3.2	21.3-44.7	2.9	4.7	15.4	4
			GAR	2015	UR	67.4	EVR8	8_21241515	2.3	56.7-67.7	1.9	3.2	11.2	5
			TE	2017	UR	56.2	EVR8	8_17664677	2.0	50.1-58.6	1.9	3.4	9.8	-
		11	CON	2016	UR	82	EVR11	11_18278532	3.1	79.5-83.1	3.0	4.7	13	-
			GAR	2016	UR	38.5	EVR11	11_16378753	3.0	34.2-42.2	2.0	3.2	14.9	4
			TE	2015	UR	71	EVR11	11_11228153	2.9	68.4-73.8	2.1	3.4	13.9	6

Y yield, CN cluster number, FI fertility index, TA Total acidity SS start sprouting , SL sprouting length, ES end sprouting date, F flowering date, SV start veraison date, EV end veraison date, VL veraison length, RD ripening date, F-SV flowering-start veraison interval, EV-R end veraison-ripening interval. LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome - wide (CW) and genome - wide (GW) LOD threshold ($p < 0.05$), %var. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal - Wallis significance level, given by the p - value (“1”: 0.1, “2”: 0.05, “3”: 0.01, “4”: 0.005, “5”: 0.001, “6”: 0.0005, “7”: 0.00001; “ - ”: no significance).

Supplementary material 3.4.5. Summary of putative (LOD > CW) QTL in G × T Hf progeny (102 genotypes).

		LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var	KW
											CW	GW		
PRODUCTIVITY TRAITS	Y	17	CON	2015	UR	31.2	Y17	17_5296971	3.5	30.4-32.2	3.3	4.8	14.7	6
			TE	2015	UR	31	Y17	17_4698550	3.1	25.8-43.2	2.1	3.5	13.0	6
	CN	17	CON	2015	UR	64	CN17	17_10954821	3.5	63.7-65.5	3.2	5.0	14.5	4
			TE	2014	UR	58.1	CN17	17_11859049	1.9	53.9-58.5	2.1	3.4	8.4	2
		18	CON	2017	UR	87.7	CN18	18_27984510	3.6	86.5-89.7	3.4	4.6	17.7	3
			GAR	2017	UR	72.3	CN18	18_24968109	1.9	69.9-74.2	1.7	3.2	9.7	1
	CW	9	CON	2014	UR	13.3	CW9	9_2721760	3.8	9.6-27.7	3.4	5.0	16.1	4
			TE	2014	UR	11.6	CW9	9_2455719	2.2	10.4-24	2.1	3.5	9.8	3
		10	CON	2015	UR	64.5	CW10	10_9919995	3.8	58.5-65.5	3.3	4.8	15.7	6
			GAR	2015	UR	15.3	CW10	10_8053703	3.4	10.6-19.7	1.9	3.4	14.8	1
	FI	2	CON	2016	UR	54.4	FI2	2_7999157	3.1	53.5-55.2	3.1	4.7	13.2	1
			TE	2017	UR	44.2	FI2	2_3043378	2.3	43.7-44.5	2.0	3.4	10.0	4
		3	GAR	2014	V	3.8	FI3	3_2022904	2.8	3.2-8.9	1.7	3.3	17.8	3
			CON	2014	V	19.2	FI3	3_2208990	3.4	18.3-22.6	3.1	5.1	20.6	3
		9	CON	2017	UR	24.5	FI9	9_4555301	3.6	21.7-25.2	3.2	4.8	14.9	2
			TE	2017	UR	22	FI9	9_4555301	3.5	19.2-23.2	2.1	3.5	14.8	5
		12	CON	2017	UR	4.8	FI12	12_845353	4.0	0.0-13.8	3.3	4.8	16.4	6
			TE	2017	UR	11.1	FI12	12_1982474	2.3	0.0-12.8	2.0	3.4	9.8	4
MUST	TA	4	CON	2015	UR	25.3	TA4	4_3158756	4.2	11.8-28.4	3.2	4.7	17.3	2
			TE	2015	UR	22.6	TA4	4_3818194	2.6	19.4-26	2.0	3.2	11.1	6
		14	CON	2017	UR	78.8	TA14	14_27846297	4.4	77.8-82.4	3.5	5.8	22.0	6
			GAR	2017	UR	82.3	TA14	14_27453581	3.1	72.7-88.6	2.1	3.1	16.2	6
PHENOLOGICAL STAGES	SS	7	CON	2016	V	67.4	SS7	7_15794912	3.3	67.1-70.1	3.2	4.8	14.0	2
			TE	2016	V	79.6	SS7	7_15665813	2.0	78.1-79.9	1.9	3.4	8.9	4
		12	CON	2016	V	52.9	SS12	12_10230232	3.6	50.3-54.9	3.2	4.8	15.4	6
			GAR	2016	V	36.3	SS12	12_10230232	3.3	36.8-40.4	2.1	3.3	13.9	6
	ES	16	CON	2016	V	24.7	ES16	16_14568037	3.4	21.1-26.3	3.2	4.7	15.1	5
			GAR	2016	V	31.4	ES16	16_14959074	2.8	28.6-32.5	2.1	3.3	12.4	6
	SL	18	CON	2016	V	86.5	SL18	18_28122721	3.1	85.6-86.8	3.0	4.7	14.1	3
			TE	2016	V	42.3	SL18	18_28668179	2.2	42.2-48.5	2.0	3.3	10.0	2
	F	7	CON	2014	UR	46.3	F7	- 1_1108704	3.2	45.9-50.8	3.0	4.6	18.3	2
			TE	2014	UR	48.5	F7	-1_19613096	2.2	43.9-48.4	2.1	3.4	14.4	2
		10	CON	2016	V	42.7	F10	10_2524063	4.3	38.8-55.5	3.3	4.8	20.1	4
			GAR	2016	V	44.7	F10	10_2524063	2.2	43.8-45.2	1.9	3.2	10.9	4
			TE	2016	V	23.9	F10	10_1618757	2.1	22.5-27.7	2.0	3.3	10.0	4
	SV	13	CON	2015	UR	48.1	SV13	-1_358137	4.4	46.5-50.7	3.3	4.8	22.9	4
			GAR	2015	UR	40.1	SV13	13_16059853	2.1	39.7-47.6	1.9	3.2	12.0	4
		17	CON	2016	UR	32.1	SV17_1	17_5647891	3.7	30.4-34.4	3.2	4.7	20.8	6
			GAR	2016	UR	22.6	SV17_1	17_5647891	3.2	19.9-32.3	2.1	3.4	18.3	7

Continue

		LG	Map	Year	Plot	Pos (cM)	QTL	Marker	LOD peak	1-LOD interval	LOD 0.95		% Var	KW
											CW	GW		
PHENOLOGICAL STAGES	EV	1	CON	2016	UR	68.8	EV1	1_18912078	3.5	67.3-70.4	3.3	4.8	20.0	2
			GAR	2016	UR	73.3	EV1	1_17552218	2.9	71.3-79.4	2.1	3.3	16.9	4
		3	CON	2014	V	14.7	EV3	3_1910501	3.4	12.5-14.7	3.2	4.7	27.1	1
			GAR	2014	V	11.4	EV3	3_2672708	3.1	8.9-14.6	2.1	3.3	24.9	2
		7	CON	2015	UR	5.6	EV7	7_1274079	3.6	5.3-7.6	3.2	4.7	21.6	4
			GAR	2015	UR	8.4	EV7	-1_7415004	2.3	5.6-11.1	1.9	3.2	14.4	4
		18	CON	2016	V	70.5	EV18_2	18_20265299	4.3	67.5-74.3	3.2	4.7	27.6	6
			TE	2016	V	34.7	EV18_2	18_22255285	2.1	34.2-35.5	2.0	3.4	14.6	4
		19	CON	2014	V	18.9	EV19	19_2823652	3.6	16.8-20.7	3.2	4.7	28.4	2
			TE	2014	V	15.6	EV19	19_2702989	2.2	9.6-19.1	2.0	3.4	18.5	3
	VL	1	CON	2016	UR	68.6	VL1	1_18752133	3.2	66.7-69.9	3.1	4.7	18.2	4
			GAR	2016	UR	73.3	VL1	1_17552218	2.6	71.3-77.4	2.1	3.4	15.5	6
		3	CON	2014	V	14.7	VL3	3_1910501	3.3	10.5-15.5	3.1	4.7	27.4	2
			GAR	2014	V	11.4	VL3	3_2672708	2.8	8.9-14.1	2.1	3.3	23.6	4
		13	CON	2014	V	42.2	VL13	13_6925489	3.4	42.1-44.4	3.3	4.8	27.8	4
			TE	2014	V	32.2	VL13	13_6925489	2.6	29.9-34.5	2.2	3.4	22.2	4
	RD	2	CON	2016	V	53	RD2	2_14565191	4.6	52.4-54.9	3.4	4.9	23.4	5
			GAR	2017	V	48.6	RD2	2_7999163	2.9	47.5-59.8	2.2	3.3	21.7	4
		4	CON	2014	UR	57.2	RD4	4_2705125	3.9	52.7-57.2	3.2	4.7	16.5	2
			TE	2017	UR	14	RD4	4_1783736	1.9	13.0-15.9	1.9	3.3	8.7	3
			GAR	2014	UR	10.9	RD4	4_937393	2.2	10.1-22.4	1.9	3.3	9.4	3
		6	CON	2016	V	17.1	RD6	6_2704384	3.6	16.5-19.8	3.2	4.7	18.9	5
			TE	2016	V	46	RD6	6_2230799	2.3	42.8-51.1	1.9	3.3	12.5	5
		9	CON	2017	V	43.8	RD9	9_3896426	3.2	43.1-49.1	3.0	4.7	23.4	4
			TE	2017	V	39.9	RD9	9_4897617	1.9	37.5-41.9	1.9	3.3	14.4	4
		13	GAR	2017	UR	65.1	RD13_2	-1_3191380	1.9	64.6-66.3	1.9	3.3	9.8	4
			CON	2017	UR	62	RD13_2	13_21066207	3.1	60.4-62.2	3.0	4.7	15.5	2
		14	CON	2015	UR	81.4	RD14	14_27709216	3.1	77.9-81.7	3.0	4.7	12.9	4
			GAR	2015	UR	54.7	RD14	14_23084906	2.9	54.3-64.1	2.1	3.3	12.4	5
	F-SV	4	CON	2014	UR	68.8	FSV4	4_13198221	3.5	68.7-77.0	3.1	4.7	28.4	2
			GAR	2014	UR	67	FSV4	4_18395179	2.0	62.1-67.0	1.9	3.2	16.6	2
	EV-R	14	CON	2015	UR	51	EVR14	14_21336665	3.3	49.8-52.9	3.1	4.7	20.1	6
			TE	2015	UR	63	EVR14	14_25628382	2.0	43.8-63.0	1.9	3.4	12.5	4
		19	CON	2014	UR	7.3	EVR19	19_1593892	3.2	6.2-7.9	3.2	4.8	18.5	4
			TE	2014	UR	7.5	EVR19	19_1593892	2.0	6.7-9.3	2.0	3.3	11.9	4

Y yield, CN cluster number, CW cluster weight, FI fertility index, TA Total acidity, SS start sprouting date, ES end sprouting date, F flowering date, SV start veraison date, EV end veraison date, VL veraison length, RD ripening, F-SV flowering-start veraison interval, EV-R end veraison-ripening interval. LG Linkage Group, Marker Nearest marker to the QTL position, Pos (cM) QTL position on LG. LOD peak LOD value at QTL position, LOD threshold chromosome - wide (CW) and genome - wide (GW) LOD threshold ($p < 0.05$), %var Expl. Proportion of the total phenotypic variance explained by the QTL. KW = Kruskal - Wallis significance level, given by the p - value (“1”: 0.1, “2”: 0.05, “3”: 0.01, “4”: 0.005, “5”: 0.001, “6”: 0.0005, “7”: 0.00001; “ - ”: no significance).

Supplementary material 3.4.6 a. Co - localization matrix explaining the QTL regions found in G×T population (LG1 to LG9).

	LG1		LG2	LG3			LG4	LG6	LG7		LG8				LG9
	16-30	53-75	15-30	0-16	20-26	47-63	10-30	38-50	10-20	50-70	5-20	20-35	35-52	55-70	43-60
BW						3									
Y															
CN			2												
CW			2												
FI						2									
ºB	**2**														
pH							2								
TA															
SS															
FD															
SV															
EV															
VL												2			
RD															
EV-R														2	

Colour legend: LOD 3-3.5 light green, LOD 3.5-4 dark green, LOD 4-4.5 orange, LOD 4.5-6 red, LOD 6-8 light blue, LOD > 8 dark blue and LOD > 10 purple. The number inside the cells indicate the number of years in which the QTL was found. BW berry weight, Y yield, CN cluster number, CW cluster weight, FI fertility index, ºB Brixº, TA total acidity, SS start sprouting date, F flowering date, SV start veraison date, EV end veraison date, R ripening, VL veraison length, S-F sprouting flowering period, EV-R veraison ripening period.

Supplementary material 3.4.6 b. Co - localization matrix explaining the QTL regions found in G × T population (LG10 to LG19).

	LG10		LG11			LG12		LG13			15	14	16	LG17		LG18		LG19
	6-15	40-60	10-25	35-42	50-65	0 -15	69-85	0-15	30-36	45-60	70-90	62-85	75-80	14-40	43-50	30-50	84-95	10-20
BW																2		
Y														2				
CN																		
CW		2												2				
FI														2				
ºB																		
pH																		
TA							3		2			4		2				
SS																		
SL																		
FD																		
SV															2			
EV												2		2		2		
VL											2							
RD									2	2				2				
F-SV																		

Colour legend: LOD 3-3.5 light green, LOD 3.5-4 dark green, LOD 4-4.5 orange, LOD 4.5-6 red, LOD 6-8 light blue, LOD > 8 dark blue and LOD > 10 purple. The number inside the cells indicate the number of years in which the QTL was found. BW berry weight, Y yield, CN cluster number, CW cluster weight, FI fertility index, ºB Brixº, TA total acidity, SS start sprouting date, F flowering date, SV start veraison date, EV end veraison date, R ripening, VL veraison length, S-F sprouting flowering period, F-SV flowering veraison period.

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CHAPTER 4.

EVALUATION OF WINES DERIVED FROM TEMPRANILLO INTRASPECIFIC HYBRIDS AND PINOT NOIR CLONES

4. Evaluation of wines derived from Tempranillo intraspecific hybrids and Pinot Noir clones

4.1. Wine quality and berry size: A case of study with Tempranillo progenies

Abstract

Small berry size is normally associated with quality wine production. However, the contribution of grapevine variety and environment on sensory quality has not been well established. Herein, genotypes from two intra-specific hybrid populations were categorized by size according to berry diameter and weight: small (< 13.5 mm, < 1.5g), and large (> 16 mm, > 2g). Chemical and sensory attributes of wines produced in two consecutive vintages (2017 and 2018) from each size category were characterized. Wine quality was evaluated by a panel of experts. Consistently, wines obtained from small berry genotypes presented higher proportions of phenolic compounds, deeper colour and had higher quality scores regardless genetic background and vintage. Quality was positively correlated with anthocyanin and phenolic content. Wines presented high sensory variability differing in nine and seven attributes in each vintage. Small berry size genotypes produced sweeter, fruitier wines with greater astringency; whereas wines from larger berries were perceived as more alcoholic and with lower positive aroma intensities. Berry size influenced colour and phenolic compounds more than genotype or environment. In summary, small berry size genotypes produced higher quality wines in both years, thus providing a predictor of wine categories in order to meet different market demands.

Introduction

Wine grapes are considered to be one of the world's most valuable crops (FAO 2017). Wine grape value is closely tied to the quality of the wines produced; currently reaching a production of 250 million hL (OIV 2019). Grape berries are rich in secondary metabolites such as anthocyanins, flavonols, norisoprenoids, terpenoids, and tannins which affect wine quality by determining colour, aroma, and flavor (Wong et al. 2016).

Skin-to-flesh ratio influences grape composition and quality with higher concentrations of phenolic compounds in small berries (Gil et al. 2015). However, the direct relationship between berry size and wine quality is still highly debated (Friedel et al. 2016, Xie et al. 2018). Several studies reported that berry size had no influence on grape and wine quality, while viticulture practices such as pruning (Holt et al. 2008, Roby & Matthews 2004), and environmental conditions (Van Leeuwen & Ollat 2017) are major drivers in vine metabolism, hence grape composition (Dai et al. 2011) not berry size per se (Xie et al. 2018).

One of the limitations in the study of berry size and composition is variability. Mean and range values of both parameters are the result of complex interactions among genotype, environmental factors, such as temperature or light, their interactions, and cultural practices (Keller 2010). Variability is present within berries, among berries within a cluster, among clusters on a vine, and among vines within a vineyard (Dai et al. 2011). Sink competition at the tip of a cluster produces lower weight berries than in the centre or shoulder (Tarter & Keuter 2005). Berry

weight shows high genetic diversity within the *Vitis* genus, ranging from < 0.5 to > 10 g (Houel et al. 2013).

Cultivar is a key factor in berry size and composition (Barbagallo et al. 2011). Genetic variability and plasticity allow the adaptation of existing cultivars to specific growing regions to produce distinct wine styles from one cultivar (Dai et al. 2011). However, the wine industry is based on very tight genotype × environment interactions, with a limited number of *V. vinifera* cultivars. Thus, only 12 varieties constitute 70 – 90% of hectares in many countries; representing 1 % of total diversity (Wolkovich et al. 2018). Recently, potential wine quality has been evaluated from intraspecific (Manso-Martínez et al. 2020) as well as interspecific crosses (Biasoto et al. 2014, Lago-Vanzela et al. 2013) in order to broaden the sensory and agronomic variability to adapt to new market preferences and environmental scenarios.

Thus, the objective of this study was to assess the effect of berry size, genetic background, and environment on the chemical and sensory quality of wines in two different vintages. Two genotype groups differing in average berry size were selected from two segregating hybrid populations: Grenache × Tempranillo and Cabernet Sauvignon × Tempranillo. The chemical and sensory attributes of wines derived from both categories were evaluated. We hypothesized that small berry genotypes would produce higher quality wines, based on sensory analysis, regardless of vintage or genetic background.

Materials and methods

Plant material and agronomic evaluation

Twenty and twenty-six hybrids were selected according to their berry size among two and one wine-grape populations in 2017 and 2018, respectively. Both F_1 populations were obtained from controlled crosses between Tempranillo (male parent) and Grenache (female parent) with 130 plants and between Tempranillo (male parent) and Cabernet Sauvignon (female parent) with 80 plants. Since 2003, the hybrids (one plant per genotype) had been grown on their own roots; first flowering and fruiting in 2007, as described in Song et al. (2014). Both populations had been previously genotyped for SSRs and SNP markers in order to discard individuals resulting from self-pollinations and foreign pollen sources.

Ripening date was set at technological maturity (23.4 ºBrix) by measuring 10 berries randomly taken from both sides of the vine. Mean berry weight (BW, g) of each genotype was calculated at harvest by sampling 200 berries from representative clusters. A set of 110 berries were squeezed and Total Soluble Solids (TSS) expressed as Brix degree were determined with an Atago Master-Baume refractometer (Atago, Tokio, Japan); pH and total acidity (g / L tartaric acid) were measured with a TitroMatic 1S-1B (Crison, Barcelona, Spain). Three sets of 30 berries per plant were frozen at - 20 ºC to determine berry morphology. Berry length (BL, mm) and berry diameter (BD, mm) were measured with a Mitutuyo digital calibre. Berry shape coefficient (BS) was calculated as the ratio between length and diameter (Houel et al. 2013).

Selection of genotypes was based on data from three previous vintages. Genotypes with berry weights less than 1.5 g with diameters and lengths less than 14 mm constituted the small berry size. Large berry size was characterized by weights greater than 2 g with diameters and lengths greater than 16 mm. As a result, in 2017, 11 genotypes were selected as small berry size from the pool of both populations with 4 from a Grenache × Tempranillo progeny and 7 from the

Cabernet × Tempranillo population, whereas 9 large berry genotypes were all selected from Grenache × Tempranillo progeny. In 2018 the analysis was performed only in the Grenache × Tempranillo offspring because it was the worst-case scenario being that the average berry size was larger. Fourteen and 12 genotypes matched the criteria of small and large berry sizes, respectively both within the Grenache × Tempranillo population.

Vinifications

In two consecutive vintages, microvinifications of each category, small (SMB) and large (LGB), were elaborated in duplicate; Tempranillo and Grenache in triplicate. Grapes from each sample (10 kg for each hybrid group, 25 kg for parents) were destemmed, crushed, and vinified in the Instituto de Ciencias de la Vid y del Vino experimental winery (Logroño, Spain) and vinifications were performed as detailed in Manso-Martínez et al. (2020).

Physicochemical characterization of wines

Official OIV practices (OIV, 2003) were used to assess oenological traits. By an accredited laboratory, in accordance with standard UNE-EN ISO/IEC 17025 (Estación Enológica de Haro, La Rioja, Spain), reducing sugars (RS, g / L), malic acid (MA, g / L), free dioxide sulphur (Free SO2, mg / L), volatile acidity (VA, g / L of acetic acid), % ethanol (% Eth, v / v), pH, total acidity (TA, g / L of tartaric acid), anthocyanin content (ANT, mg / L), total polyphenolic index (TPI), colour intensity (CI), and CIELAB coordinates whose values correspond to the degree of wine lightness (L_{10}*) and the degree of red (when $a_{10}* > 0$), green (when $a_{10}* < 0$), yellow (when $b_{10}* > 0$), and blue colour (when $b_{10}* < 0$)(Ayala et al. 1997) were analyzed.

Sensory characterization of wines

In random and distinct arrangements, panellists were given twenty millilitres of each sample covered with plastic Petri dishes (labelled with 3-digit random codes) in clear glasses to evaluate quality; in black glasses for descriptive analysis. Evaluations, recorded on paper, were carried out by unpaid panellists in individual tasting booths in a ventilated, air-conditioned, tasting room. Samples were served at room temperature (approximately 20 ºC). Panellists rinsed with water and pectin solution (1 g / L) between samples to minimize carry over effects as described by Colonna et al (2010).

Quality evaluation

Twenty winemakers from La Rioja (Spain) (11 women, average age of 45 years, 5 - 35 years of experience in wine tasting) participated in the study. Each participant evaluated the overall intrinsic quality of 10 wines each year in one session (average 50 min). They were instructed to place the samples in a 15 cm-non-structured continuous scale according to their global quality perception based on visual, olfactory, and in-mouth cues. They tasted all samples and identified two samples representing the extremes in the sample set (highest and lowest quality). The relative degrees of quality of the remaining samples were ranked and scored with distances from the extremes.

Descriptive analysis

Seventeen participants (12 women, average age of 24 years) were selected to carry out the final descriptive session of wines based on their performance during training. They attended 5 sessions, 1.5 hours each, throughout a three-week period in February 2018 and 2019. The training consisted of four training sessions and one session to describe the wines using Rate-all-that-apply (RATA) methodology (Ares et al. 2014) as fully detailed in Manso-Martínez et al. (2020).

Data analysis

One-way ANOVAs were calculated on variables to evaluate differences among SMB and LGB categories and parents. To find discriminant sensory attributes for the wines, two-way ANOVAs (panellists as random and wines as fixed factors) were calculated for the 28 terms and the four wines (Tempranillo, Grenache, SMB, LGB). To evaluate the differences between SMB and LGB two-way ANOVAs (panellists as random and wines as fixed factors) were calculated with the 28 terms and these two groups. Pair-wise comparisons (Fischer test) were applied (5% risk) to the discriminant terms found in at least one of the vintages to detect significant effects.

Two principal Component Analyses (PCA), one for each vintage, were calculated with mean ratings (averaged across panellists) of the significant sensory descriptors for all the samples.

The effect of vintage and wines was evaluated with a three-way ANOVA (participants as random, wines and vintage as fixed factors and second order interactions) followed by a Student–Newman–Keuls post-hoc pairwise comparison (95%) test. All analyses were carried out with SPSS 25, XLSTAT and SPAD software (version 5.5, CISIA-CESRESTA, France).

Results and discussion

Berry morphology and grape juice characterization

The influence of berry size on wine chemical and sensory parameters was evaluated for parents and small (SMB) and large berry size (LGB) groups, as previously described. Notably SMB and LGB did not share the same genotypes in either year. Only one genotype was collected in both years for SMB; whilst for LGB, 50% of the samples were common to both vintages. In 2018 the study was carried out only with genotypes from the Grenache × Tempranillo population. SMB consisted of a greater size due to the Grenache parent (1.24 ± 0.36 g in 2017 and 1.55 ± 0.35 g in 2018). This approach was taken in order to assess the effect of the worst-case scenario in terms of berry size; therefore, enhancing the relevance of berry size selection per se, regardless genetic background.

In both years berry parameters were significantly different among categories with the exception of berry shape (Table 4.1.1). Parental cvs. showed intermediate values compared to both categories with the exception of berry shape; Grenache presented the most elongated berries while Tempranillo and SMB genotypes had the roundest. Berry weight from SMB was significantly different from Grenache, Tempranillo, and LGB. Given that the Cabernet Sauvignon parent could not be evaluated and 7 out of the 11 small berry size genotypes proceeded from a Cabernet Sauvignon × Tempranillo progeny in 2017, values obtained from the literature were used for comparison. Gil et al. (2015), reported mean berry weight values of 1.4 g for Cabernet

Sauvignon (CS) grapes, lower berry weight than either Grenache or Tempranillo; similar to the SMB category in the present study.

For must parameters in 2018 smaller berries retained more acidity than larger ones which was similar to that found by Gil et al. (2015) and Barbagallo et al. (2011). In both years, Grenache presented higher total acidity than Tempranillo, whilst Holt et al. (2008) reported similar pH (3.4 - 3.6) and TA (4.5 - 5.2 g / L) values in Cabernet Sauvignon compared to Tempranillo. Genetic background seems to influence traits related with must total acidity since differences were obtained between years.

Table 4.1.1. Berry and must parameters of selections with small (SMB) and large (LB) berry size in 2017 and 2018 vintages.

	Vintage 2017								Vintage 2018							
	SMB17		LGB17		GRE		TE		SMB18		LGB18		GRE		TE	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
BL	11	13.3 ± 1.1**a	9	16.1 ± 1.2b	3	17.12 ± 0.23b	4	15.4 ± 0.61b	14	13.92 ± 1.26**a	12	15.7 ± 0.7bc	3	15.64 ± 1.68b	4	14.08 ± 1.18ab
BD	11	13.2 ± 1.2*a	9	15.8 ± 1.0b	3	15.62 ±0 .21b	4	15.19 ± 0.47b	14	14.18 ± 1.3**a	12	15.79 ± 0.79b	3	14.47 ± 1.28ab	4	14.47 ± 0.73a
BS	11	1.01 ± 0.04*a	9	1.02 ± 0.02a	3	1.10 ± 0.00b	4	1.01 ± 0.01a	14	0.98 ± 0.04*b	12	1.00 ± 0.03b	3	1.08 ± 0.03a	4	0.97 ± 0.04b
BW	11	1.24 ± 0.4**a	9	2.04 ± 0.36b	3	2.01 ±0.29b	4	1.72 ± 0.35b	14	1.55 ± 0.35**a	12	2.06 ± 0.26b	3	1.79 ± 0.37ab	4	1.62 ± 0.3a
º Brix	11	22.96 ± 2.62	9	23.11 ± 1.90	3	24.43 ±1.45	4	24.16 ± 2.23	14	22.67 ± 1.62*a	12	23.09 ± 1.95a	3	24.33 ± 1.39b	4	23.52 ± 2.05ab
pH	11	3.18 ± 0.71ab	9	3.32 ± 0.7ab	3	3.27 ±0.08b	4	3.55 ± 0.23a	14	3.25 ± 0.62*a	12	3.3 ± 0.62a	3	3.26 ± 0.09a	4	3.58 ± 0.19b
TA	11	4.98 ± 1.69ab	9	4.77 ± 1.5ab	3	6.17 ±1.0b	4	4.84 ± 0.76a	14	4.83 ± 1.56*a	12	5.34 ± 1.31b	3	5.36 ± 1.6ab	4	4.30 ± 0.93a

** reflect statistical differences at 0.01 level, * at 0.05. BL berry length, BD berry diameter, BS berry shape, BW berry weight, TA Total acidity (g / L of tartaric acid).

Physicochemical characteristics

Wine composition parameters for both vintages are shown in Table 4.1.2. Reproducibility of replicated tanks was confirmed based on physicochemical variables (Supplementary material 4.1.1), therefore averaged data are presented. In both years, LGB generated wines with consistently higher malic acid content similar to that of Friedel et al. (2016). Smaller berries present inherently less malic acid due to higher malic respiration during maturation. Larger differences were detected in 2017; likely due to Cabernet Sauvignon background influence. Total acidity (TA) was higher in larger berries wines like Tempranillo, in 2017. This result contradicts reports by Gil et al. (2015) regarding higher TA in smaller berries wines. In this study SMB samples exhibited higher TA only for grape juices in one year; presumably because tartaric acid is accumulated mainly in flesh while content in skin is negligible. Consequently, larger berries should have higher content even though a dilution effect may also be present (Melo et al. 2015). LGB wines also presented lower levels of reducing sugars and ethanol content than the small-berry wines, which is similar to that found by Friedel et al. (2016), Melo et al. (2015), in which smaller berries had higher sugar content yielding wines with more ethanol. Traits such as tartaric acid, malic acid, reducing sugars and ethanol content seem to be more influenced by genetic factors or by genotype × environment interaction than by berry size, since differences between berry size categories were not consistent among years.

In both vintages SMB wines presented higher TPI, anthocyanin content, and deeper colour than LGB wines. Tempranillo showed higher TPI, CI and anthocyanin content than Grenache, whose values were lower than in both SMB and LGB categories (Table 4.1.2). A significant correlation between anthocyanin content and berry size has been widely reported (Melo et al. 2016, Mirás-Avalos et al. 2019) due to higher skin/pulp ratios of smaller berries, hence, higher accumulation of phenolic compounds. Holt et al. (2008) and Gil et al. (2015) found Cabernet Sauvignon to present total phenolic index values of 50 - 60 and anthocyanin contents of 400 - 600 mg / L, similar to the values found for Tempranillo in the present research. Thus, the values for Tempranillo varied greatly between both years (Table 4.1.2).

Grenache wines seem to be less influenced by weather conditions regarding TPI and anthocyanin content. The later was higher in 2017 for LGB and especially in Tempranillo wines possibly due to the fact that 2017 was warmer and drier than 2018. This was similar to findings from Ferrer et al. (2014) who reported moderate deficit irrigation and high temperature to promote higher levels of anthocyanin content. Anthocyanins are responsible for young wine colour (He et al. 2010). Herein CI increased when berry size decreased and the Hue value (calculated as the ratio of absorbance at 420 nm-yellow to 520 nm-red) was higher in wines derived from larger berries, indicating a higher contribution of the yellow component to the CI in this category as reported by Gil et al. (2015); Melo et al. (2016). High significant correlations ($r \geq 0.89$, $p < 0.01$) were found between quality scores and CI, TPI, and anthocyanin content in both vintages.

Table 4.1.2. Composition and colour of wines made from small berry (SMB), large berry (LGB) hybrid genotypes and Grenache (GRE) and Tempranillo (TE) parents for 2017 and 2018 vintages. Means ± SD (calculated for duplicate tanks in hybrids and triplicate in parents) and ranges for chemical and colour parameters. Spearman correlation coefficient with the sensorial quality.

	Vintage 2017						Vintage 2018					
	SMB17	LGB17	GRE	TE	Range	R-Quality	SMB18	LGB18	GRE	TE	Range	R-Quality
RS	2.9 ± 0.1b	2.1 ± 0.1a	2.7 ± 0.2b	2.9 ± 0.1b	2.1-2.9	0.15	2.0 ± 0.0	2.3 ± 0.3	2.1 ± 0.0	2.5 ± 0.3	2.0-2.5	0.29
MA (g/L)	1.3 ± 1.3a	3.3 ± 0.0b	2.4 ± 0.0ab	3.3 ± 0.1b	1.3-3.3	0.73**	3.2 ± 0.0b	3.4 ± 0.1c	2.8 ± 0.1a	3.4 ± 0.1c	2.8-3.4	0.82**
FSO_2 (mg/L)	34.5 ± 2.1c	27 ± 1.4b	21.3 ± 1.5a	21 ± 1.7a	21.0-34.5	0.65**	29.5 ± 5.0b	24.0 ± 1.4b	10.3 ± 0.6a	22.7 ± 1.5b	10.3-29.5	0.78**
VA (g/L)[a]	0.3 ± 0.1a	0.4 ± 0.0b	0.2 ± 0.0a	0.3 ± 0.0a	0.2-0.4	-0.43*	0.2 ± 0.0a	0.3 ± 0.0a	0.8 ± 0.1c	0.5 ± 0.1b	0.2-0.8	-0.73*
% ETH(v/v)	13.5 ± 0.2b	13.0 ± 0.1a	13.4 ± 0.0b	15.0 ± 0.1c	13.0-15.0	-0.77*	12.3 ± 0.0a	12.8 ± 0.3b	14.2 ± 0.1c	13.0 ± 0.1b	12.3-14.2	-0.75*
pH	4.05 ± 0.04a	4.06 ± 0.04a	3.99 ± 0.06a	4.18 ± 0.03b	3.99-4.18	0.45	3.80 ± 0.01	3.84 ± 0.00	3.85 ± 0.02	3.83 ± 0.06	3.80-3.85	-0.32
TA (g/L)[b]	5.1 ± 0.7a	6.0 ± 0.0b	4.6 ± 0.1a	5.7 ± 0.1b	4.6-6.0	-0.22*	6.7 ± 0.0	6.7 ± 0.1	7.0 ± 0.1	7.0 ± 0.2	6.7-7.0	-0.42
ANT(mg/L)	438 ± 21c	374 ± 19.8b	191.7 ± 17a	744.7 ± 31d	192-745	0.93**	442.5 ± 7.8c	300.5 ± 5.0b	193.3 ± 3.1a	490.7 ± 29c	19-491	0.91**
TPI	47.0 ± 0.3c	37 ± 1.3b	24.3 ± 1.9a	57.2 ± 0.9d	24.3-57.2	0.89**	48.2 ± 1.6c	38.5 ± 0.2b	27.6 ± 1.2a	42.8 ± 3.9bc	27.6-48.2	0.92**
HUE	0.8 ± 0.0b	0.8 ± 0.0b	1.0 ± 0.0c	0.7 ± 0.0a	0.7-1.0	-0.92**	0.7 ± 0.0b	0.8 ± 0.0c	0.9 ± 0.0d	0.6 ± 0.0a	0.6-0.9	-0.91**
CI	5.0 ± 0.0b	4.4 ± 0.2b	1.8 ± 0.1a	12.2 ± 1.1c	1.8-12.2	0.92**	6.4 ± 0.1b	4.0 ± 0.1a	3.1 ± 0.1a	8.0 ± 1.1c	3.1-8.0	0.90**
L*	27.8 ± 0.1b	31.7 ± 1.8c	61.7 ± 2.dc	8.5 ± 1.7a	8.5-61.7	-0.93**	22.4 ± 0.0a	34.4 ± 0.4b	44.0 ± 0.8c	19.2 ± 3.0a	19.2-44.0	-0.94**
a_{10}^*/b_{10}^*	1.9 ± 0.0a	2.1 ± 0.0ab	2.0 ± 0.2a	2.7 ± 0.3b	1.9-2.7	0.32*	1.7 ± 0.0a	2.5 ± 0.1b	1.7 ± 0.1a	1.7 ± 0.1a	1.7-2.5	-0.12

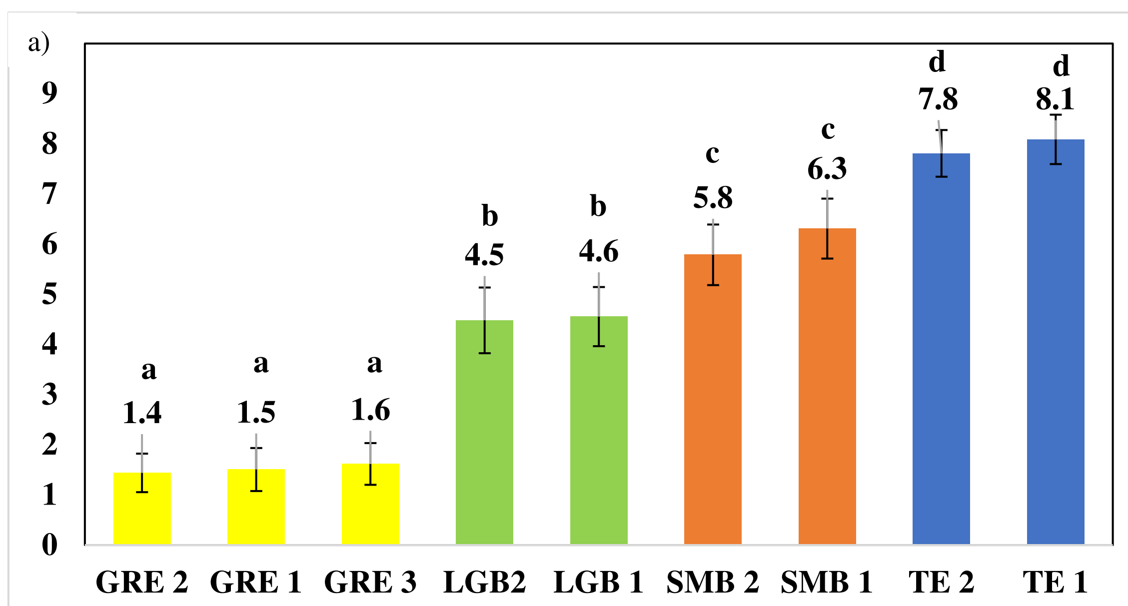
Data expressed as means ± SD (n = 2 and n = 3 for GRE and TE) and ranges. Means followed by different letters in the same column differ by LSD test ($p < 0.05$). MA: malic acid, FSO_2: free dioxide sulfur, VA: volatile acidity[a] expressed as g / L acetic acid, %ETH: % ethanol, TA: total acidity[b] expressed as g / L tartaric acid, ANT: Anthocyanin content, TPI: Total Polyphenolic Index, CI: colour intensity, L*: Lightness, and a^*_{10}/b^*_{10}: red/yellow.

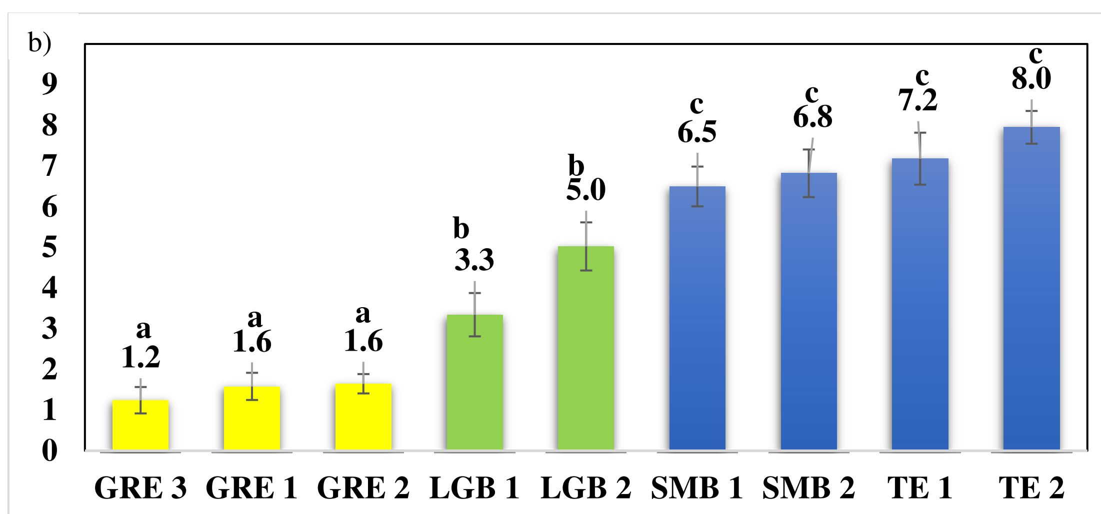
Sensory characterization of wines

Wine quality assessment

The expert panel quality scores for wines elaborated in 2017 and 2018 are presented in Figure 4.1.1. Quality ranges of very low/low (1.2 ± 0.1) correspond to Grenache while high/very high quality (8.1 ± 0.3) was achieved by Tempranillo in both vintages. Wines made from large berry genotypes (LGB) presented lower perceived quality in both vintages compared to wines made from smaller berries (SMB) (Figure 4.1.1). The consistency between years reflects the correlation between berry size and wine quality independent of weather conditions or genetic background. The LGB genotypes in 2018 were selected from the Grenache × Tempranillo population with lower genetic variability for berry size. Tempranillo cv. presented the highest quality scores in both years. Due to the fact that Cabernet Sauvignon wines were not available, we could not assess how they would have affected the sensory evaluation of SMB wines in 2017 which, without them, received a higher quality score.

Figure 4.1.1. Mean sensory quality scores in a) 2017 and b) 2018, being: small berry size (SMB) and large berry size (LGB) groups, Grenache (GRE) and Tempranillo (TE). Error bars are calculated as sd / (number of panellists)$^{0.5}$. Numbers 1 and 2 indicate replicate tanks (n = 2 for each category, n = 3 for GRE and TE).





**Letters indicate statistical differences with LSD test in quality scores at 0.05 level.

<u>Descriptive analysis</u>

From the sensory descriptions of the trained panel, nine and six sensory attributes differed statistically among the Tempranillo, Grenache, SMB and LGB wines ($p < 0.05$) between 2017 and 2018, respectively (Table 4.1.3). According to ANOVA these attributes were “cooked vegetables”, “fresh grass”, “alcoholic aroma”, “white fruit”, “roasted”, “vegetal”, “oxidation”, “liquorice” and “astringency” in 2017, and “liquorice”, “dried grass”, “alcoholic aroma”, “reduction”, “astringency” and “sweetness” in 2018. Attributes that differed among the wines (Table 4.1.3) were represented in a PCA for each year (Figure 4.1.2 a and b) together with quality scores.

Figure 4.1.2 a contains the sensory profile of the 2017 wines. Total variance of 78 % was explained by the first two principal components. The four groups of samples were separately projected highlighting their distinct sensory profiles. Tempranillo was mainly characterized by its fruity character (“white fruit”) and high a*/b* ratio, which suggests that the colour of Tempranillo wines was mainly red with low yellow nuances. Grenache samples presented high L*, thus high lightness (or low darkness in terms of colour), and in general presented low scores in all aroma descriptors. The high and low-quality scores for Tempranillo and Grenache samples, respectively, could be due to colour properties, because Spanish experts, in absence of evident aroma defaults, consider colour to be an important cue driving wine quality. High a* / b* ratios and low L* have already been related to high quality perception of young red wines (Sáenz-Navajas et al. 2016). The LGB wines were described with terms such as “cooked vegetables”, “fresh grass”, “vegetal” and “alcoholic”, which are generally considered to be defect nuances. The SMB wines were projected on the opposite side of the plot and linked to positive liquorice aroma and higher astringency, which has already been linked to high quality exemplars by wine experts (Sáenz-Navajas et al. 2013).

The PCA results of 2018 wines (Figure 4.1.2 b) show that the two first principal components accounted for 92 % of the total variance, and PC1 distinguished Grenache from the rest of the samples. Grenache presented high lightness (L*), as was observed in 2017, together with reductive notes, which most likely determined their low-quality score. In the upper side of the plot, LGB wines

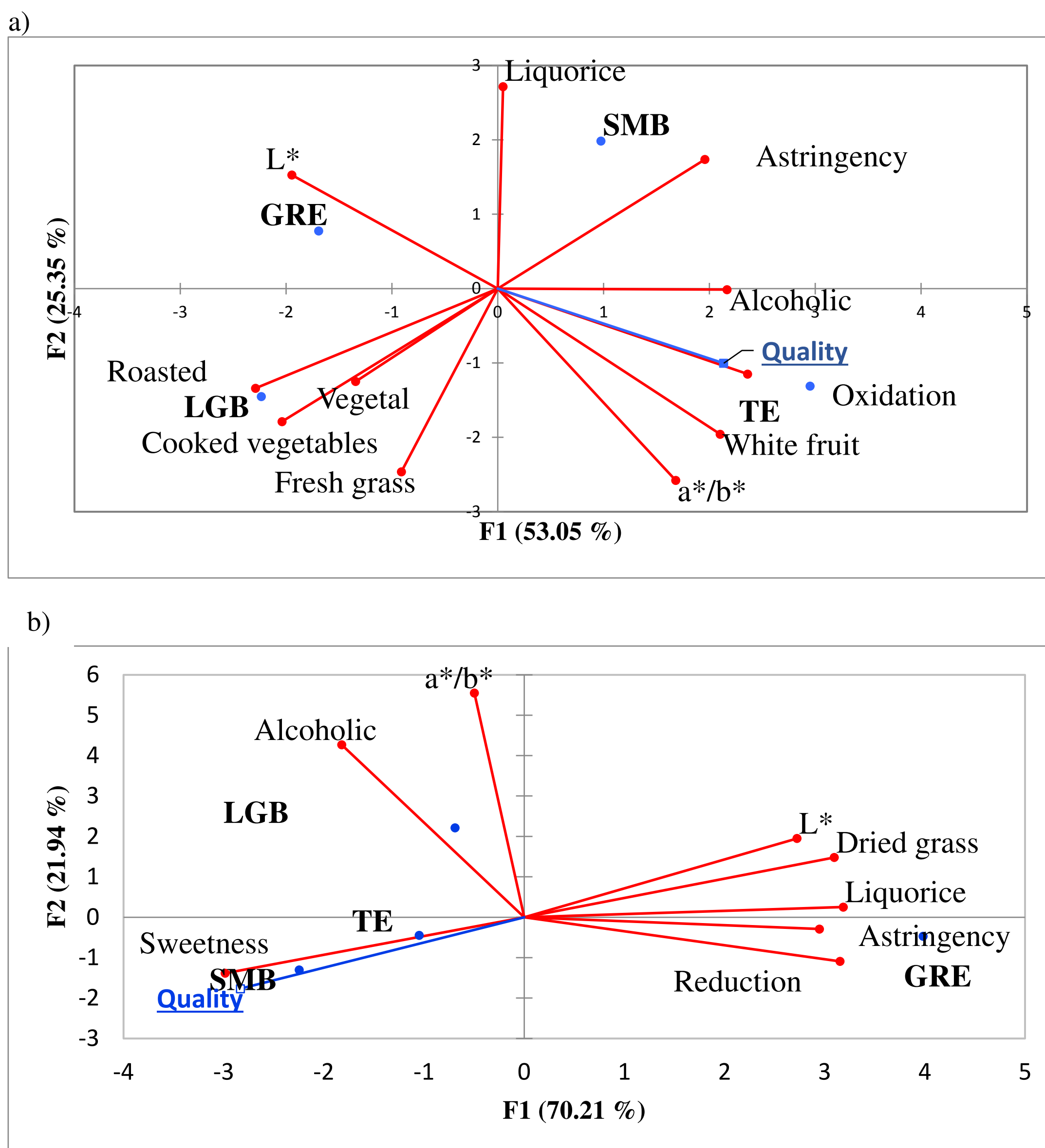
Table 4.1.3. Two-way ANOVAs (panellists as random factor and wines as fix factors) calculated on the 28 sensory attributes of wines elaborated in 2017 and 2018 vintages (F, F-ratios; p, p-values; Sig., significance: *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant).

	2017			2018		
	F	**p**	**Sig.**	**F**	**p**	**Sig.**
Cooked vegetables	12.81	< 0.0001	***	0.544	0.653	ns
Fresh grass	4.36	0.01	**	1.018	0.386	ns
Floral	0.77	0.51	ns	1.352	0.260	ns
Reduction	0.10	0.90	ns	8.371	< 0.0001	***
Alcoholic	3.90	0.01	**	4.997	0.002	**
White fruit	3.31	0.02	*	1.154	0.330	ns
Citric	1.34	0.26	ns	1.282	0.282	ns
Smoked	0.82	0.48	ns	0.367	0.777	ns
Dried fruit	2.54	0.06	ns	0.504	0.680	ns
Red fruit	0.10	0.90	ns	2.275	0.082	ns
Roasted	2.70	0.05	*	1.093	0.354	ns
Spiced	0.61	0.61	ns	0.672	0.570	ns
Vegetal	11.93	< 0.0001	***	0.636	0.593	ns
Tropical fruit	1.79	0.15	ns	1.541	0.206	ns
Leather	1.60	0.19	ns	2.509	0.061	ns
Black fruit	0.10	0.90	ns	1.376	0.252	ns
Dried grass	1.81	0.15	ns	2.612	0.050	*
Balsamic	2.23	0.09	ns	0.529	0.663	ns
Oxidation	3.94	0.01	**	0.259	0.855	ns
Mushroom	2.36	0.07	ns	0.746	0.527	ns
Vanilla	0.92	0.43	ns	1.192	0.315	ns
Liquorice	3.15	0.03	*	11.117	< 0.0001	***
Astringency	29.51	< 0.0001	***	3.548	0.016	*
Sourness	1.64	0.18	ns	0.279	0.840	ns
Alcoholic	1.64	0.18	ns	0.808	0.491	ns
Body	0.10	0.90	ns	0.634	0.594	ns
Bitterness	0.03	0.99	ns	0.500	0.683	ns
Sweetness	0.79	0.50	ns	11.522	< 0.0001	***

were mainly associated with alcoholic aroma nuances and presented low scores for the rest of attributes, which suggests that even though these wines presented no aroma default, they were scored low in quality due to their lack of positive attributes. Distinctly, TE and SMB samples were projected close together with higher sweetness and quality scores. Figure 4.1.3 illustrates the sensory profiles of both SMB and LGB wines. Interestingly SMB wines presented significantly higher positive aroma nuances scores and mouthfeel sensations related to “red fruits” ($F = 10.91$, $p < 0.01$) and “astringency” ($F = 42.90$, $p < 0.001$) in year 2017 (Figure 4.1.3 a), and to “white fruits” ($F = 3.51$, $p < 0.1$) and “sweetness” ($F = 5.06$, $p < 0.05$) in the 2018 vintage (Figure 4.1.3 b).

In the PCA, the quality arrow is located in both vintages opposite to lightness (L*), confirming the results of the correlation analysis (Table 4.1.2). Thus, Lightness, % ethanol, volatile acidity were negatively correlated ($r = -0.9$, $p < 0.01$) to quality scores, while anthocyanin content, colour index, and TPI were positively correlated ($r = 0.9$, $p < 0.01$), similar to previous studies (Sáenz-Navajas et al. 2016).

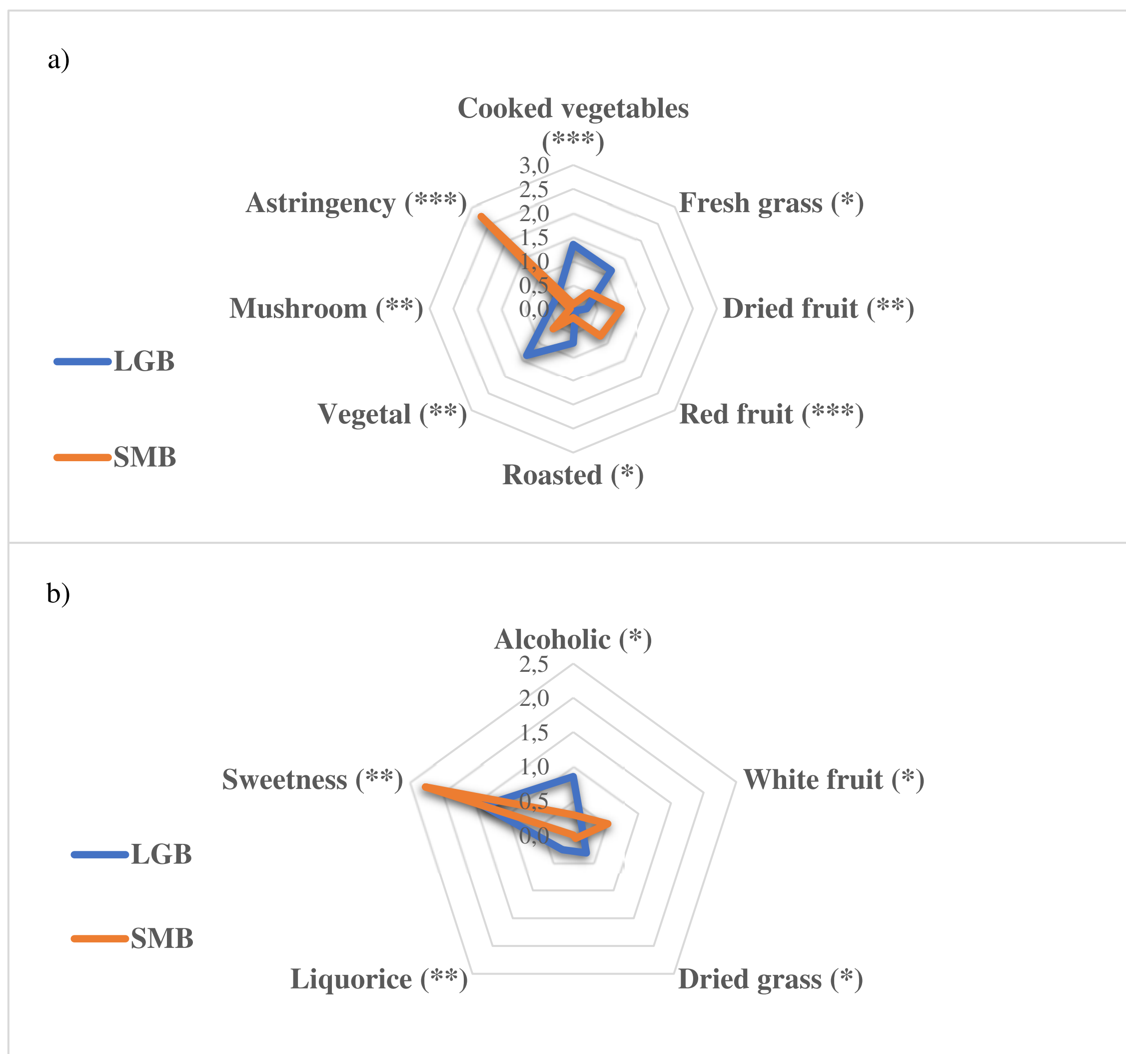
Figures 4.1.2 a) and 4.1.2 b). Principal component analysis biplot with sensory attributes that differed between samples for each year and colour (L* and a/b) as active variables and quality scores as supplementary variable in 2017 a) and 2018 b).



Sensory data revealed that SMB and Tempranillo wines presented similar characteristics in both vintages; sharing adequate "astringency" and "dried fruit" notes in 2017 and "sweetness" and fruity notes in 2018. The LGB wines were characterized by "fresh grass", "cooked vegetables" and vegetal notes in 2017 (Figure 4.1.3 a) and were alcoholic in nose in 2018 (Figure 4.1.3 b). "Cooked vegetables" is considered to be an off-flavour present in oxidized wines (Escudero et al. 2000) which can trigger aroma deterioration, loss of citric and fresh aromas among others (Bueno et al. 2016). The LGB wines were perceived to be more alcoholic due to the absence of other aromas. Compared to the SMB wines, the lower phenolic content present in both vintages of LGB made these wines more susceptible to oxidation (Gambuti et al. 2017), and could be related, among other reasons to the higher yield presented by these vines in 2017 (data not shown). "Vegetal aromas" are commonly related to high productivity values (García-Muñoz et al. 2014)

which could have promoted the low-quality scores obtained for LGB wines. Melo et al. (2015) found that Syrah wines made from larger berries were described as watery; similar to the higher alcoholic perception due to dilution of aroma compounds detected in the present study.

Figure 4.1.3. Average values of the sensory profile of wines made from SMB and LGB in a) 2017 and b) 2018.



Significant differences according to two-way ANOVA (panellists as random and wines as fix factors) at 0.01 ***, 0.05 ** and 0.1 *.

Interactions between vintage and wine samples

Table 4.1.4 shows the vintage, wine, and vintage * wine interactions. Grenache varied in “liquorice” and “reduction” notes (Supplementary material 4.1.2), whilst Tempranillo differed among years in “sweetness”, “astringency”, “alcoholic”, “oxidation” and “white fruit” aromas. An interaction of vintage × wine was found for “liquorice” and “sweetness” in Grenache with higher values in 2018; Tempranillo in “sweetness”, “astringency” and “oxidation” with those perceptions higher in the 2017 vintage (Supplementary material 4.1.3). Reductive note intensities

were highest in Grenache regardless of the vintage (Supplementary material 4.1.2). Generally, they are attributed to a lower polyphenolic content, thus a higher tendency to generate Strecker aldehydes linked to oxidation nuances in wine (Bueno et al. 2016).

Table 4.1.4. Two-way ANOVA calculated with wine and year as fixed factors and their interaction for descriptors with significant effect in at least one vintage.

	Wine		Year		Wine * Year	
	F	p	F	p	F	p
Cooked vegetables	5.445	0.001	0.135	0.713	5.747	0.001
Fresh grass	0.571	0.634	0.776	0.379	3.287	0.021
Dried grass	0.950	0.330	0.273	0.845	3.258	0.022
Vegetal	5.793	0.001	1.797	0.181	6.027	0.001
Reduction	18.061	< 0.0001	1.728	0.190	0.837	0.474
Alcoholic	4.285	0.006	13.898	0.000	1.806	0.146
Oxidation	3.455	0.017	2.799	0.095	2.987	0.031
White fruit	1.196	0.312	8.808	0.003	1.501	0.214
Liquorice	4.614	0.004	4.014	0.046	9.188	< 0.0001
Astringency	15.566	< 0.0001	73.600	< 0.0001	26.212	< 0.0001
Sweetness	8.564	< 0.0001	99.407	< 0.0001	9.353	< 0.0001

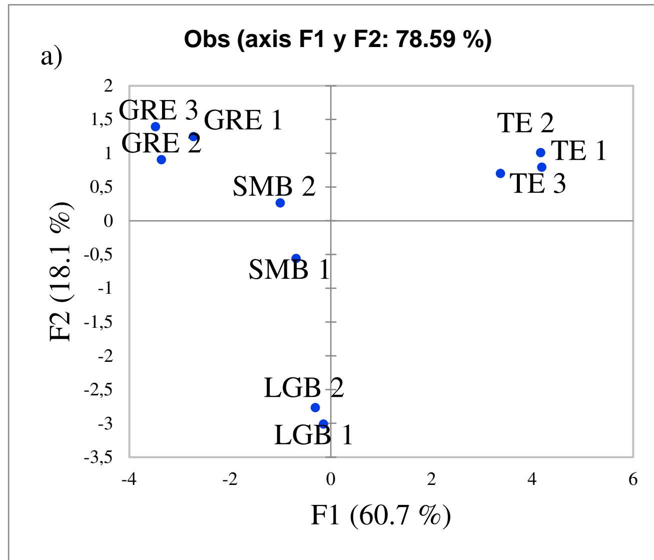
The performance of SMB and LGB groups could not be assessed for vintage since samples integrating each group varied with years. An important effect of vintage on sensory properties of wines was observed; 2017 wines resulted more alcoholic in nose due to warmer weather conditions, while “white fruit” perception (normally associated to Tempranillo variety), was higher in 2018 (Supplementary material 4.1.3). This reduction of varietal aromas as a consequence of higher temperatures found in 2017 had been mentioned previously (Mozell & Thachn 2014).

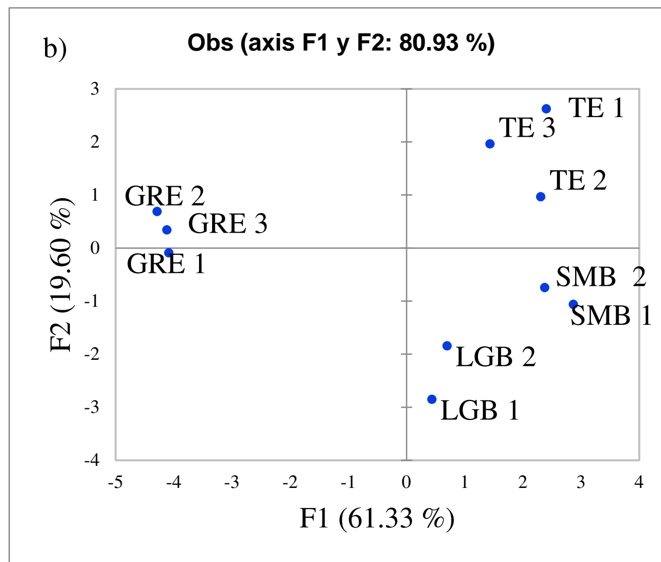
Conclusions

This is the first study addressing the influence of berry size on wine quality by comparing wines derived from intraspecific hybrids differing in berry weight. Results confirm our initial hypothesis that wines obtained from smaller berry size genotypes reached higher quality than the larger size group independently of the vintage, environmental conditions, and genetic backgrounds. SMB wines consistently reached higher phenolic and anthocyanin contents, deeper colour, and higher sensory scores. Despite differences in genetic background, all SMB wines were characterized with higher “sweetness”, “astringency”, and “fruity” notes compared to LGB wines, which were perceived as more “alcoholic” and to contain some off-flavours such as “cooked vegetables” notes in the sensorial analysis. The fact that the two berry-size categories originated from different hybrids in both vintages strengthened the conclusion of the study. Even within the worst-case scenario, when selection was made among Grenache offspring, being the larger-berry sized parental compared to Cabernet-Sauvignon, SMB wines were perceived as higher quality exemplars. These results could be useful to design selection strategies in the vineyard in order to diversify wine styles.

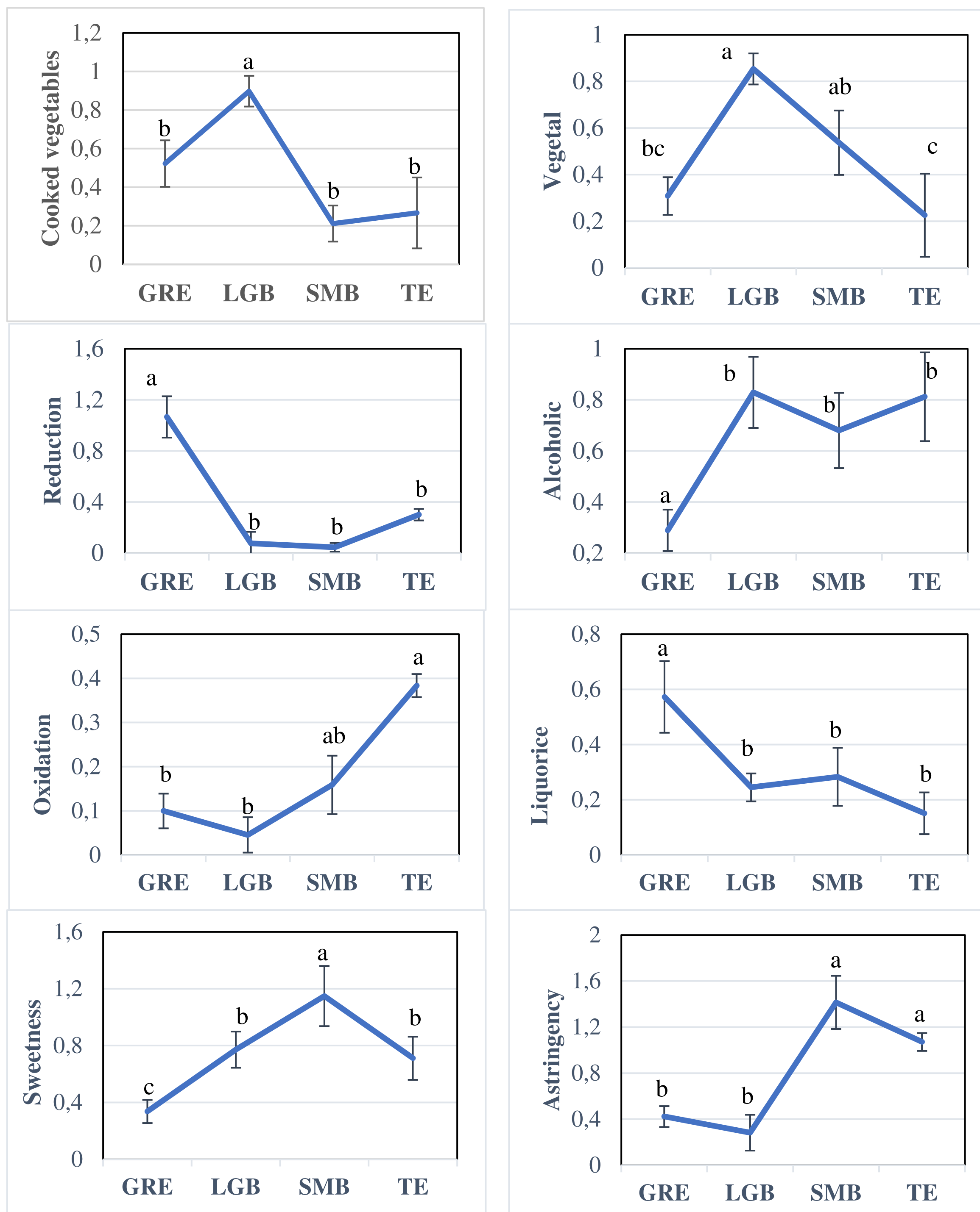
Supplementary material

Supplementary material 4.1.1. Projection of samples on the first two components of PCA calculated with chemical data as active variables for year a) 2017 and b) 2018.

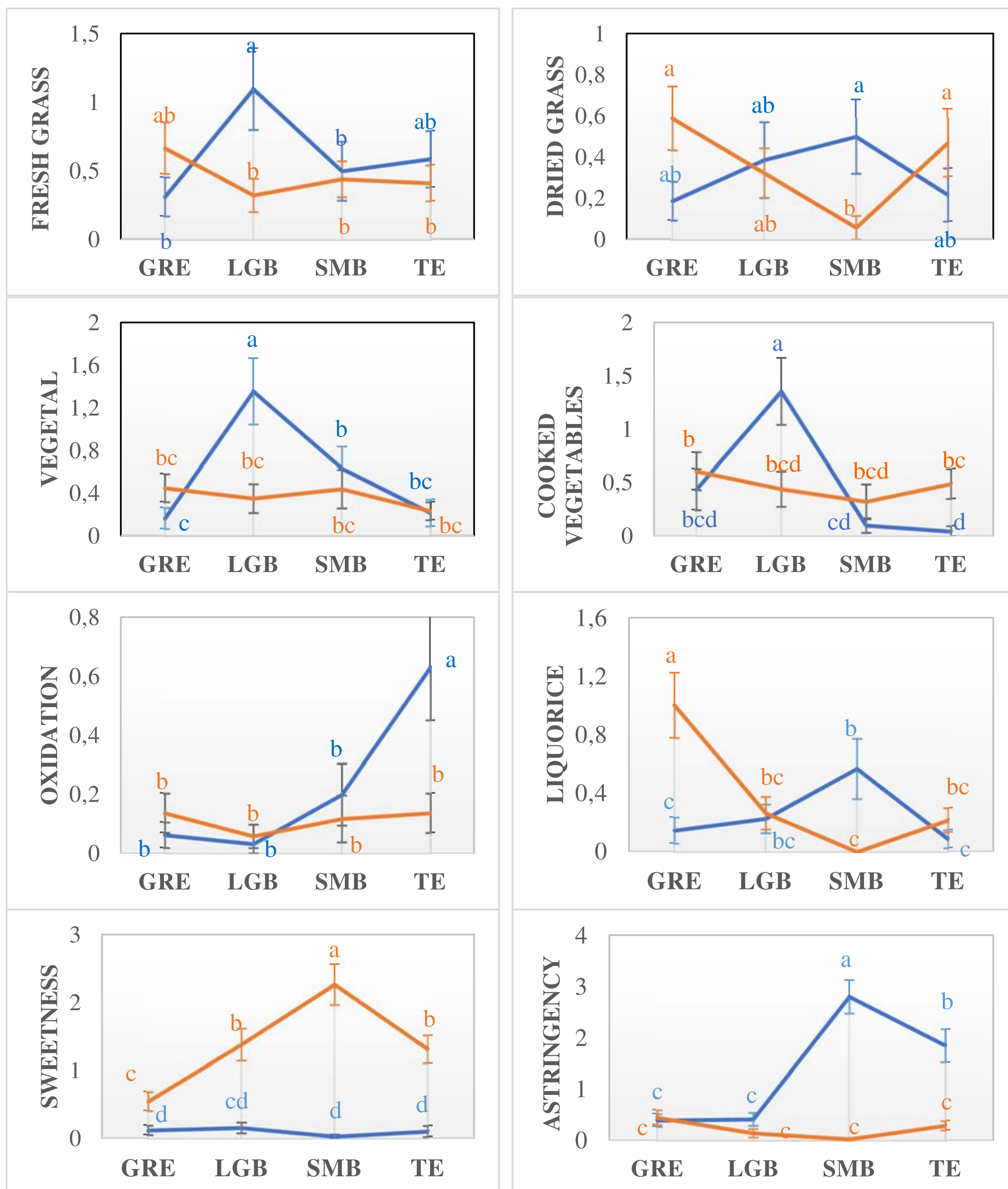




Supplementary material 4.1.2. Attributes significantly different according to wine sample between Grenache (GRE), Tempranillo (TE), SMB, LGB size categories.



Supplementary material 4.1.3. Significant wine* vintage interactions in sensory differing descriptors of the wines in at least one vintage. Vintage 2017 is represented with a blue line and 2018 in orange.



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4.2. Sensory profiling and quality assessment of wines derived from Graciano x Tempranillo selections

Abstract

Wine production is a dynamic process that must be adapted to changes such as global warming and new consumer interests. Obtaining new cultivars by hybridization of traditional varieties is a promising approach with great potential to produce wines that are able to preserve regional typicity, together with adaptability to both evolving market preferences and distinct environmental scenarios.

In this research, wines from twelve Graciano x Tempranillo selections were analyzed in two consecutive years. Sensory properties and quality were evaluated by a trained panel and a group of wine experts, respectively. Quality was positively correlated with anthocyanin and phenolic content ($r = 0.8$, $r = 0.7$, $p < 0.01$, respectively). Wines presented high sensory variability differing in eight attributes in each vintage. Two high quality selections, TG8 and TG63 consistently improved Tempranillo and Graciano specimens, presenting high colour intensity, acidity, and positive aroma related to red fruit. Furthermore, TG129 a late-ripening genotype with high polyphenol content and fruity aroma, and other selections with roasted or dried fruit aroma notes appear as potential cultivars suitable to satisfy distinct consumer demands in the context of global warming.

Introduction

Grape is considered to be one of the major fruit crops in the world based on hectares cultivated (7.4 Mha), and the economic value of its most valuable product, wine, reaching global wine production 292 MhL (OIV 2019). In recent years a shift in traditional winemaking patterns has appeared in order to provide solutions to new consumer preferences and new viticulture scenarios due to the influence of global warming in vine phenology, grape composition, wine microbiology and chemistry, and sensory aspects.

These warmer conditions promote an advancement in plant growth periods, with significantly earlier veraison and harvest dates reported (Petropoulos et al. 2017). Berry ripening occurs earlier in summer under higher temperatures, leading to incomplete phenolic maturity (Resco et al. 2016). The reduction in anthocyanin content, colour and aroma expression, as well as the appearance of polyphenols yielding unpleasant sensory properties (Van Leeuwen & Ollat 2017), could lead to what are called “flabby” wines (Mozell & Thachn 2014) characterized by high alcohol content, low total acidity and less aroma.

Obtaining plant material resilient to new climatic conditions, is a promising approach to confront climate change (Duchene 2016). New cultivars should have either a longer vegetative cycle with late maturation or the ability to complete grape phenolic and technological maturity (related to sugar and acidity content) earlier. These contexts would compensate for the shorter growing period induced by the effect of global warming.

The most famous European wine cultivars are the result of intraspecific hybridizations being Cabernet Sauvignon, Chardonnay, or Merlot descendants of other known varieties (Duchene 2016). Nowadays these varieties dominate the winemaking culture causing a loss of

biodiversity and accordingly a limited offer in the wine market (Lago-Vanzela et al. 2013) and therefore more variability in terms of cultivars is required.

Recently, quality of wines obtained from minor grapevine varieties (García-Muñoz et al. 2014; Pérez-Navarro et al. 2019, Petropoulos et al. 2017) or new interspecific hybrid varieties (De Castilhos et al. 2016, Lago-Vanzela et al. 2013) has been evaluated seeking to identify new better adapted products linked to regional identity. However, although interspecific hybrids show an increased resistance to climatic changes and pathogenic fungal infections, they produce wines with lower quality, in comparison to noble varieties (Socha et al. 2015). This difficult acceptance of non-traditional or interspecific material could be eased by the introduction of intraspecific hybrids between traditional varieties that are better adapted to future climate change scenarios, maintain regional typicity, and are appreciated by winegrowers and consumers.

With climate change in mind, Song et al. (2014), identified a set of genotypes from a segregating population obtained from two relevant Spanish wine varieties, Tempranillo and Graciano. These cultivars present complementary characteristics being Tempranillo an early ripening cultivar with low acidity, while Graciano provides higher colour intensity, acidity and aroma to the mixture (Escudero-Gilete et al. 2010). Graciano is typically less productive with lower berry weight and has a late-ripening period.

Our approach relies on the hypothesis that hybrids obtained from these Spanish cultivars would be able to produce high quality wines with diverse flavor properties, broadening the sensorial variability associated to Tempranillo and Graciano, and therefore adapt to the new market preferences and environmental scenarios. In addition, earlier or later ripening hybrids could produce wines with less alcohol, which are better adapted to new environmental scenarios.

The aim of the present study was to evaluate the oenological potential of 12 pre-selected superior agronomic Graciano x Tempranillo hybrids, and the physicochemical and sensory profile of the young red wines derived from them, as a tool for winegrowers to face the challenges associated with climate change.

Materials and methods

Plant material and agronomic evaluation

Twenty genotypes were selected among a Graciano x Tempranillo population characterized by 27 agronomic, phenological and enological traits over 3 years (2008 – 2010) and located in Varea, La Rioja, Spain (Song et al. 2014). Four traits were considered in order to select improved plants for a climate change scenario: yield per vine (> 1.5 kg per vine), mean cluster weight (> 150 g) anthocyanin content (> 1.8 mg / g berry skin) and mean berry weight (< 1.6 g).

Five to 20 plants per genotype were vegetatively propagated over Richter 110. They first flowered and fruited in 2014. Based on agronomical evaluations between 2014 and 2017, twelve of the twenty previously selected genotypes were chosen to perform the microvinifications evaluated in the present research.

Phenology and fertility related traits were recorded between 2014-2017 for Tempranillo, Graciano and the twelve selections as described in Song et al. (2014). Ripening date was established when three berries randomly picked from the top, medium, and bottom regions on

both sides of three clusters from at least 3 plants (in average 50 berries) reached 23.4 ºBrix using an Atago Master refractometer (Atago, Tokyo, Japan). The flowering, veraison and ripening dates were calculated as the number of days from April first. The interval from flowering to start of veraison (F - SV), the veraison period (VP), and the interval from end of veraison to ripening (EV - R) were calculated as described by Costantini et al. (2008).

Yield per vine (kg / vine), number of clusters per vine, average cluster weight (g) and mean berry weight (g) were recorded at harvest for each genotype. Berry skin anthocyanin content was measured as previously described (Song et al. 2014).

Vinifications

Fifty-four wines were elaborated (12 hybrids in 2017 and 9 hybrids in 2018 in duplicate; Tempranillo and Graciano in triplicate) in two consecutive vintages. Wines from TG44, TG62 and TG107 were only elaborated in 2017 due to insufficient productivity in 2018. Grapes from each sample (10 kg for hybrids, 25 kg for parents) were destemmed, crushed, and vinified in the experimental winery of the Instituto de Ciencias de la Vid y del Vino (Logroño, Spain). Vinifications were performed at room temperature; potassium metabisulfite was added to the samples for a final total SO_2 concentration of 40 m g/ L. Musts were inoculated with the commercial *Saccharomyces cerevisiae* strain Uvaferm VRB (Lallemand, St Simon, France) (20 g / hL). Caps were punched down daily, and fermentation activity was monitored by determining must temperature and Brix degree. Once the alcoholic fermentation was completed (glucose and fructose < 2 g / L) wines were pressed and total SO_2 was adjusted to 50 mg / L in order to avoid malolactic fermentation. Wines were cold stabilized at 10 ºC for 2 months. Thereafter, the wines were racked off to remove lees and bottled after adjusting free SO_2 to 40 mg / L. Bottles were stored at 12 - 15 ºC until chemical and sensory analyses were performed (1 and 2 months later, respectively).

Physicochemical characterization of wines

Conventional wine oenological parameters such as malic acid (MA, g / L), free dioxide sulfur (FreeSO_2, mg / L), volatile acidity (VA, g / L of acetic acid), % ethanol (% ETH, v / v), pH, total acidity (TA, g / L of tartaric acid), anthocyanin content (ANT, mg / L), total polyphenolic index (TPI), colour intensity (CI), and CIELAB coordinates were determined according to the methodology established by OIV (2019).

Sensory characterization of wines

Twenty millilitres of each sample (labelled with 3-digit random codes) were presented to all participants in clear glasses for quality evaluation and black glasses for descriptive analysis covered with plastic Petri dishes in a random arrangement which was distinct for each participant. All assessments were conducted in individual tasting booths and results were collected in paper ballots. The wine samples were served at room temperature and evaluated in a ventilated, air-conditioned tasting room (approximately 20 ºC). Participants were instructed to rinse between samples with water and pectin solution (1 g / L) to minimize carry over effects as described elsewhere (Colonna et al. 2010). They were not paid for their participation.

Quality evaluation

Twenty winemakers from Rioja area (Spain) (11 women, average age of 45 years, 5-35 years of experience in oenology and wine tasting) participated in the study. Each participant evaluated the overall intrinsic quality of 30 and 24 wines in year 2018 and 2019, respectively, in

one session (average 50 min) by a categorization task as described in the literature (Sáenz-Navajas et al. 2013). They had to sort the samples into five different quality categories (“very low”, “low”, “average”, “high” or “very high”) according to their global quality perception (based on visual, olfactory and in-mouth cues).

Descriptive analysis

A total of 17 participants (12 women, average age of 24 years) were selected to carry out the final descriptive session of wines based on their performance during training. They attended a total of 6 sessions (1.5 hours per each session) throughout a three-week period in February 2018 and 2019. The training consisted of four training sessions and two sessions to describe the wines of the study. They were trained to identify and score the intensity of 28 aroma, taste and mouthfeel terms (Supplementary material 4.2.1) as described in Supplementary material 4.2.2.

Each group of samples was described in two sessions (replicates presented in different sessions) with a 10-minute mandatory break every 5 samples. Participants had to taste and exclusively rate the intensity of those terms (out of 28) that applied to the sample on a seven-point scale according to Rate-all-that-apply (RATA) methodology (Ares et al. 2014).

Data analysis

One-way ANOVAs were calculated on variables to evaluate differences among parents and the hybrids. Spearman correlation coefficient was calculated between physicochemical variables and sensory parameters. In order to find discriminant sensory attributes for the wines, a two-way ANOVA (panelists as random and wines as fixed factors) was calculated for each of the 28 terms of the list. Pair-wise comparisons (Fischer test) were applied (5% risk) to the discriminant terms found in at least one of the vintages to detect significant effects. The number of times each wine was classified by participants in each of the five quality groups was counted. Data were encoded in a wine × quality level (5) contingency table, in which each cell represented the frequency of the categorization of a wine in one category level. Correspondence analysis (CA) was performed on the contingency table. Hierarchical cluster analysis (HCA) with the Ward criteria was applied to all the factors derived from CA. The quality category best defining the resulting clusters were identified by computing their probability of characterizing a cluster.

Two principal Component Analyses (PCA), one for each vintage, were calculated with mean ratings (averaged across panelists) of the significant sensory descriptors for all the samples. HCA with the Ward criteria was applied to all PCAs. To evaluate sensory differences among clusters, one-way ANOVAs for each of the sensory attributes with clusters as fixed factors were calculated. The effect of vintage and wines was evaluated with a three-way ANOVA (participants as random, wines and vintage as fixed factors and second order interactions) followed by a Student–Newman–Keuls post-hoc pairwise comparison (95%) test. All analyses were carried out with SPSS 25, XLSTAT (2018) and SPAD software (version 5.5, CISIA-CESRESTA, St Mandé, France).

Results and discussion

Agronomic characterization of hybrids

Characterization of phenology periods

Seven of the hybrids were early ripening (TG107, TG63, TG128, TG44, TG43, TG62, TG8) and five late ripening (TG147, TG129, TG35, TG17, TG146) in comparison to Tempranillo, the reference variety (Supplementary material 4.2.3). The difference in number of days between the earliest and the latest ripening hybrid was 26 days on average; TG8 was the earliest and TG147 the latest. Differences among genotypes seem critical especially for EV-R period, reaching up to 21 days between TG62 and TG147, with the early ripening plants characterized by a shorter interval between veraison and maturity.

Meteorological differences may explain the two-week flowering advancement date of 2017 compared to 2018. In 2017, spring was dry (4 L / m^2), and hot (28 ºC max), while spring 2018 was wet (65 L / m^2), and cool (22 ºC max). Moreover, both accumulated radiation and temperatures were higher (643 MJ / m^2, 38 ºC max) and rainfall (37 L / m^2) was lower between flowering and veraison in 2017, representing a typical global warming scenario compared to 2018 (576 MJ / m^2, 27 ºC max, 55 L / m^2).

Variability found in this study is in agreement with Coombe & Hale (2008); who reported phenology periods varying greatly with grapevine variety, climate, and geographical location. On the one hand, interest in late ripening genotypes relies on their ability to complete phenolic maturity, which is the main challenge when elaborating high quality wines in a global warming context. On the other hand, early ripening hybrids are suitable for cold regions that are initiating viticulture activity due to climate change (Socha et al. 2015), or in wine-growing regions at high latitudes where reaching the correct ripeness is the limitation (Van Leeuwen et al. 2017). In warmer climates, early ripening allows genotypes to achieve phenolic and technological maturity before higher temperatures are reached.

Productivity of hybrids and berry properties

Table 4.2.1 contains the 2014 - 2017 data for the productivity and berry traits for all genotypes. In terms of productivity, TG8 and TG129 followed by TG128, TG44 and TG107 presented the lowest yield per vine, and TG8 produced clusters with the lowest weight, maybe because TG8 was the only female plant selected. On the contrary, TG62 and TG147 were amongst the most productive hybrids; even more than Tempranillo.

Berry weight is also considered a relevant trait contributing to grape quality, mainly due to its relationship with the concentration of polyphenols. Most hybrids produced lower berry weights than Tempranillo; similar to Graciano, with TG107 producing the smallest berries. Grape anthocyanin content varied greatly among genotypes: TG129 showed the highest content, even higher than Graciano; followed by TG107 or TG8, while TG35, TG146 and TG147 showed the lowest. Interestingly, the latter had the highest productivity and number of clusters, suggesting that a deficient ripeness due to high productivity could have led to lower phenolic compound synthesis. This is notable since phenolic composition at maturity, especially the amount and profile of the anthocyanins present in red grapes, are largely dependent on cultivar (Dai et al. 2011).

Characterization of wines

Reproducibility of replicated tanks was demonstrated based on both wine physicochemical variables and sensory properties (Supplementary material 4.2.4). Therefore, averaged data of replicated tanks are further presented.

Wine quality assessment

Based on CA-HCA analysis, three main quality groups of wines could be identified in both years included in one of the following quality categories: "low/very low", "average" or "high/very high" (Table 4.2.2). Quality categorization was mainly consistent in both years, except for Graciano, Tempranillo, and TG129. The latter performed distinctly in both vintages; maybe due to the distinct weather conditions previously mentioned.

One of the most important results of the study is that wines obtained from the early ripening hybrids TG8 and TG63, both belonging to "high/very high" category in both years, were perceived significantly higher in quality than their parental varieties, Tempranillo and Graciano. This finding is particularly relevant since 2017 and 2018 vintages were very different in terms of climatic conditions suggesting that both hybrids are interesting candidates to produce high quality wines.

Interestingly, wines obtained from the late ripening hybrids TG146 and TG147 were consistently classified in the lowest quality category in both years. This suggests that these hybrids would not be adequate to generate quality wines according to Rioja winemakers. Furthermore, TG62, only evaluated in 2017, and hybrids TG35, TG17, TG128, TG43 and TG129 in 2018, were perceived as higher-quality exemplars than Graciano and Tempranillo.

These results suggest that five early (TG8, TG62, TG63, TG128, TG43) and three late (TG35, TG17 and TG129) ripening hybrids have the potential to produce quality wines, perceived to be higher in quality than their parental varieties.

Table 4.2.1. Agronomic traits from 2014-2017 for Graciano, Tempranillo and 12 Graciano x Tempranillo genotypes.

	Yield (kg/vine)	Cluster number	Cluster weight (g)	Fertility index	Berry weight (g)	Brix degree	Anthocyanin content (mg/g)
GRA	2.5 ± 0.7abc	15 ± 1ab	162 ± 17abc	0.9 ± 0.2ab	1.5 ± 0.1ab	23 .1 ± 2.0a	2.3 ± 0.2ab
TE	3.6 ± 0.7ab	18 ± 3ab	218 ± 44bc	1.6 ± 0.2a	2.1 ± 0.0a	25.1 ± 1.9a	1.4 ± 0.1c-g
TG8	1.7 ± 0.7c	15 ± 4ab	92 ± 44c	1.1 ± 0.4ab	1.3 ± 0.2ab	25.1 ± 0.7a	2.1 ± 0.6a-d
TG17	2.9 ± 0.8b	13 ± 4ab	200 ± 38abc	1.0 ± 0.4ab	1.5 ± 0.2ab	23.1 ± 0.8a	1.8 ± 0.3a-f
TG35	3.8 ± 0.2a	13 ± 4ab	267 ± 35a	1.2 ± 0.2ab	1.6 ± 0.2ab	25.9 ± 2.9a	1.1 ± 0.1fg
TG43	3.1 ± 0.2abc	16 ± 3ab	191 ± 23abc	1.2 ± 0.5ab	1.3 ± 0.1ab	25.6 ± 2.5a	1.8 ± 0.2a-f
TG44	2.4 ± 0.3abc	16 ± 5ab	186 ± 21abc	1.1 ± 0.3ab	1.6 ± 0.2ab	24.7 ± 0.2a	2.1 ± 0.2abc
TG62	4.7 ± 0.8ab	14 ± 7ab	224 ± 20ab	1.2 ± 0.2ab	1.6 ± 0.3ab	22.9 ± 0.3a	2.0 ± 0.4b-e
TG63	3.0 ± 1.0ab	11 ± 3ab	245 ± 37ab	0.9 ± 0.1ab	1.5 ± 0.1ab	25.1 ± 3.1a	1.5 ± 0.5b-g
TG107	2.6 ± 0.2abc	11 ± 9b	149 ± 66ab	0.8 ± 0.3b	1.1 ± 0.1b	26.1 ± 0.1a	2.2 ± 0.3abc
TG128	2.2 ± 1.7bc	10 ± 6ab	136 ± 21bc	0.9 ± 0.3ab	1.3 ± 0.3ab	24.7 ± 0.3a	1.3 ± 0.0d-g
TG129	2.1 ± 0.5bc	16 ± 3ab	135 ± 84bc	1.2 ± 0.3ab	1.3 ± 0.1ab	23.1 ± 0.3a	2.5 ± 0.4a
TG146	4.0 ± 2.2abc	19 ± 8a	174 ± 19abc	1.1 ± 0.2ab	1.5 ± 0.2ab	25.0 ± 3.6a	1.2 ± 0.2efg
TG147	4.5 ± 2.8ab	16 ± 4ab	215 ± 129ab	1.2 ± 0.3ab	1.5 ± 0.3ab	22.5 ± 1.2a	0.9 ± 0.1g
Range	1.7 - 4.7	10 - 19	92 - 267	0.8 - 1.6	1.1 - 2.1	22.5 - 26.1	0.9 - 2.5

Data expressed as means ± SD (n = 12)

Means in the same column showing common letters are not significantly different ($p < 0.05$)

Table 4.2.2. Sensory quality categories for each hybrid and parental varieties in 2017 and 2018 vintages based on CA-HCA results.

	Vintage 2017	Vintage 2018
HIGH / VERY HIGH QUALITY	TG63 TG8 TG62	TG8 TG63 TG128 TG129 TG43
AVERAGE QUALITY	GRA TE TG128 TG43 TG35 TG44	TG17 TE TG146 TG35
VERY LOW / LOW QUALITY	TG146 TG 17 TG 147 TG129	TG147 GRA

Wine physicochemical and chromatic characteristics

Tables 4.2.3 and 4.2.4 contain conventional oenological parameters as well as chromatic characteristics of studied wines. Concerning total acidity (TA), TG8 and TG129 presented high total acidity values in both years, whereas TG17 and TG146 had the lowest. Tempranillo wines in 2018 presented abnormally high TA levels (7 g / L) compared to Pérez-Navarro et al. (2019). However, Garijo et al. (2017) encountered a similar value in what they call a "difficult vintage", since it was wet and cool, like 2018 conditions. Sadras et al. (2013) claimed that temperature effects on total acidity and pH levels are variety specific since Cabernet Franc suffers a great shift in pH and total acidity whereas Shiraz suffers little changes. In the present study, while Graciano experienced residual changes in TA (6.4 and 6.5 g / L in 2017 and 2018, respectively) and pH (3.55 and 3.39 in 2017 and 2018, respectively); Tempranillo presented important variability (Tables 4.2.3 and 4.2.4), which may account for its lowest perceived quality in 2018.

High temperatures caused a 3-week early harvest in 2017 and some wines presented approximately 15% ethanol (Table 4.2.3); unusual in Rioja wines. However, the 2018 harvest was not as hot, thus producing moderate alcohol values. Remarkably, wines produced from TG62, and three late-ripening hybrids TG129, TG147 and TG17 presented lower ethanol content, a very valuable result in light of climate change.

Regarding anthocyanin and polyphenolic content, hybrids TG8, TG63, TG107 reached values of 800 mg / g of anthocyanins in 2017 (Table 4.2.3) and higher total polyphenol indexes than Graciano and Tempranillo in both years. In general, values were higher in 2017, a year characterized by water deficiency, which could have led to higher polyphenolic concentration (Petropoulos et al. 2017). Anthocyanins and total

Table 4.2.3. Chemical, colour parameters, harvest dates and Spearman correlation coefficients calculated between wine variables and quality scores (Quality) for Tempranillo (TE), Graciano (GRA) and the 12 genotypes selected in 2017.

	MA g/L	Free SO_2 mg/L	MA g/L[a]	%ETH v/v	pH	TA g/L[b]	ANT mg/L	TPI	CI	L*	a^*_{10}/b^*_{10}	HD
GRA	1.8 ± 0.0abc	34.6 ± 2.5bc	0.3 ± 0.0b-e	13.7 ± 0.1de	3.55 ± 0.03i	6.4 ± 0.1a	726.1 ± 20.8b	52.8 ± 2.1ab	14.0 ± 1.1cd	12.6 ± 1.1cde	2.1 ± 0.1d-g	Sep-13
TE	3.3 ± 0.1ab	21.0 ± 1.7cd	0.3 ± 0.0b-e	15.1 ± 0.1b	4.21 ± 0.06ab	5.7 ± 0.1ab	744.7 ± 30.9b	57.2 ± 0.9ab	12.2 ± 1.1d	8.5 ± 1.7e-f	2.7 ± 0.3c-e	Sep-09
TG 8	2.6 ± 0.1abc	34.5 ± 10.6bc	0.4 ± 0.2ab	14.5 ± 0.1bc	3.97 ± 0.03d-g	5.8 ± 0.0ab	949.5 ± 6.4a	57.6 ± 16.0a	22.0 ± 2.6a	2.01 ± 0.7i	4.1 ± 0.0a	Aug-24
TG 17	1.2 ± 0.0ac	35.5 ± 7.8bc	0.3 ± 0.1b-e	12.9 ± 0.2fg	4.16 ± 0.05abc	4.9 ± 0.3b	764.5 ± 10.6b	62.5 ± 0.4a	10.7 ± 0.1de	11.1 ± 0.1c-f	2.2 ± 0.1d-g	Sep-14
TG 35	1.9 ± 0.1abc	13.5 ± 2.1d	0.3 ± 0.0b-e	15.9 ± 0.0a	4.03 ± 0.01cde	5.6 ± 0.1ab	491.5 ± 33.2c	57.6 ± 3.7ab	10.4 ± 0.0de	12.7 ± 0.1cde	2.0 ± 0.1efg	Sep-14
TG 43	3.5 ± 0.1a	35.0 ± 5.7bc	0.4 ± 0.0bcd	13.6 ± 0.1ef	4.07 ± 0.02bcd	5.9 ± 0.1ab	512.0 ± 5.7c	42.3 ± 1.9bc	7.7 ± 0.4ef	15.3 ± 0.3g	1.9 ± 0.0fg	Aug-24
TG 44	1.3 ± 0.2c	36.5 ± 9.2bc	0.3 ± 0.0b-e	14.1 ± 0.1cde	3.99 ± 0.03def	4.9 ± 0.1b	674.0 ± 41.0b	55.9 ± 5.1ab	13.0 ± 1.7d	7.5 ± 1.9fgh	2.9 ± 0.5cd	Aug-28
TG 62	1.5 ± 0.0bc	27.2 ± 4.2cd	0.3 ± 0.0 b-e	12.8 ± 0.0g	3.77 ± 0.03h	5.6 ± 0.3ab	770.0 ± 12.7b	59.3 ± 3.2ab	17.5 ± 0.9bc	5.9 ± 0.8ghi	3.3 ± 0.3bc	Aug-28
TG 63	1.5 ± 0.1bc	35.2 ± 1.4bc	0.2 ± 0.0cde	14.6 ± 0.3bc	4.01 ± 0.01def	4.9 ± 0.1b	914.5 ± 50.2a	69.6 ± 1.9a	18.6 ± 1.0ab	4.4 ± 0.6hi	3.8 ± 0.2ab	Aug-28
TG 107	1.5 ± 0.1bc	50.5 ± 0.7ab	0.4 ± 0.0abc	14.3 ± 0.1bcd	3.92 ± 0.02efg	6.4 ± 0.1a	894.0 ± 5.7a	69.3 ± 0.2a	19.4 ± 0.6d	4.1 ± 0.2hi	3.9 ± 0.1ab	Sep-04
TG 128	3.2 ± 0.1ab	22.1 ± 1.4cd	0.6 ± 0.0ae	15.8 ± 0.2a	4.27 ± 0.01a	5.7 ± 0.1ab	498.0 ± 1.4c	54.2 ± 1.6ab	10.9 ± 0.3de	9.2 ± 0.6d-g	2.5 ± 0.1def	Aug-24
TG 129	2.0 ± 0.7abc	60.0 ± 0.1a	0.1 ± 0.0e	12.8 ± 0.0g	3.86 ± 0.08gh	5.8 ± 1.0ab	753.0 ± 22.6b	52.4 ± 0.6ab	11.7 ± 0.4d	13.5 ± 0.1cd	2.0 ± 0.0efg	Sep-13
TG 146	1.5 ± 0.2bc	36.0 ± 8.5bc	0.3 ± 0.0b-e	13.7 ± 0.6de	4.00 ± 0.03def	4.9 ± 0.1b	327.5 ± 47.4d	41.5 ± 4.6bc	5.4 ± 0.4fg	24.6 ± 2.6b	1.6 ± 0.1g	Aug-28
TG 147	1.9 ± 0.8abc	27.0 ± 1.4cd	0.2 ± 0.0de	12.4 ± 0.0g	3.92 ± 0.09fgh	4.9 ± 0.6b	229.5 ± 12.0d	29.6 ± 1.3c	2.1 ± 0.1g	57.0 ± 1.6a	1.8 ± 0.0fg	Sep-13
Range	1.2 - 3.5	21.1 - 60.1	0.1 - 0.6	12.5 - 15.8	3.55 - 4.27	4.6 - 6.4	229.5 - 949.5	29.6 - 69.6	2.1 - 22.0	2.0 - 55.1	1.6 - 4.1	A24-S13
Quality	0.21	-0.14	0.32	0.35	-0.02	0.34	0.80**	0.70*	0.85**	-0.71*	0.94**	

Data expressed as means ± SD (n = 2 and n = 3 for GRA and TE) and ranges. Means followed by different letters in the same column differ by LSD test ($p < 0.05$)
MA: malic acid, Free SO2: free dioxide sulfur, VA: volatile acidity [a] expressed as g/L acetic acid, %ETH: % ethanol, TA: total acidity [b] expressed as g/L tartaric acid, ANT: Anthocyanin content, TPI: Total Polyphenolic Index, CI: colour intensity, L*: Lightness, and a*10/b*10: red/yellow, HD: Harvest date.

Table 4.2.4. Chemical, colour parameters, harvest dates and Spearman correlation coefficients calculated between wine variables and quality scores (Quality) for Tempranillo (TE), Graciano (GRA) and the 9 genotypes selected in 2018.

	MA g/L	Free SO_2 mg/L	VA g/L[a]	%ETH v/v	pH	TA g/L[b]	ANT mg/L	TPI	CI	L*	a^*_{10}/b^*_{10}	HD
GRA	1.5 ± 0.1e	35.1 ± 12.5abc	0.3 ± 0.1bc	11.9 ± 0.1efg	3.39 ± 0.06g	6.5 ± 0.3a-d	541.0 ± 43.3bc	45.5 ± 3.5b-e	10.5 ± 2.2bc	16.5 ± 3.0def	1.8 ± 0.1cde	Oct-11
TE	3.4 ± 0.1a	22.7 ± 1.5bc	0.5 ± 0.1a	13.0 ± 0.1ab	3.83 ± 0.06def	7.0 ± 0.2ab	490.7 ± 28.9c	42.8 ± 3.9de	8.0 ± 1.1cde	19.2 ± 3.0b-e	1.7 ± 0.1de	Sep-27
TG 8	3.0 ± 0.1b	40.1 ± 12.7abc	0.4 ± 0.0b	12.6 ± 0.3bcd	3.89 ± 0.04cd	6.6 ± 0.0abc	895.1 ± 86.3a	71.8 ± 5.7a	20.5 ± 0.1a	3.8 ± 0.2g	4.1 ± 0.1a	Sep-21
TG 17	3.0 ± 0.0b	38.1 ± 4.2abc	0.3 ± 0.0bc	11.8 ± 0.0fg	4.09 ± 0.04a	5.6 ± 0.9de	536.5 ± 23.3c	54.5 ± 0.6b	10.1 ± 0.1bc	13.3 ± 0.1ef	2.0 ± 0.0c	Oct-04
TG 35	2.6 ± 0.0c	24.5 ± 3.5abc	0.3 ± 0.0c	13.6 ± 0.3a	3.86 ± 0.02de	5.8 ± 0.2cde	369.0 ± 15.6d	43.6 ± 2.6de	6.6 ± 0.0de	24.4 ± 1.4bc	1.5 ± 0.1e	Oct-10
TG 43	3.4 ± 0.1a	42.1 ± 2.0ab	0.3 ± 0.0bc	13.1 ± 0.2abc	4.02 ± 0.04abc	5.9 ± 0.1cde	552.3 ± 43bc	43.3 ± 2.0de	6.5 ± 0.9de	21.4 ± 3.2bcd	1.8 ± 0.0cd	Sep-21
TG 63	2.2 ± 0.0d	32.3 ± 3.2abc	0.2 ± 0.0c	12.4 ± 0.1cde	3.72 ± 0.01ef	5.7 ± 0.1de	492.1 ± 10.8c	44.5 ± 1.9cde	8.2 ± 0.7cde	17.5 ± 1.0c-f	1.7 ± 0.0cde	Oct-02
TG 128	3.0 ± 0.0b	44.5 ± 2.1a	0.3 ± 0.0bc	13.1 ± 0.2ab	4.05 ± 0.08ab	6.0 ± 0.2cde	570.1 ± 21.2bc	52.6 ± 0.3bc	9.4 ± 0.7cd	14.2 ± 1.0ef	1.9 ± 0.1cd	Sep-21
TG 129	3.4 ± 0.1a	29.5 ± 2.1abc	0.3 ± 0.0bc	12.3 ± 0.1def	3.71 ± 0.01ef	7.4 ± 0.1a	654.5 ± 13.4b	50.0 ± 1.1def	13.0 ± 0.1b	10.7 ± 0.1fg	2.3 ± 0.0b	Oct-10
TG 146	2.4 ± 0.1cd	27.3 ± 2.5abc	0.3 ± 0.0bc	12.2 ± 0.1def	3.71 ± 0.01bcd	5.3 ± 0.1e	363.3 ± 8.5d	40.5 ± 0.1e	5.4 ± 0.2e	26.1 ± 0.9b	1.8 ± 0.1cde	Oct-04
TG 147	2.9 ± 0.1b	21.0 ± 2.8c	0.3 ± 0.0bc	11.5 ± 0.2g	3.71 ± 0.02f	6.3 ± 0.2bcd	149.1 ± 19.8e	21.4 ± 0.8f	1.9 ± 0.1f	60.3 ± 1.1a	2.0 ± 0.2cd	Oct-11
Range	1.5 - 3.4	21.0 - 44.5	0.2 - 0.5	11.5 - 13.6	3.39 - 4.09	5.3 - 7.4	149.1 - 895.1	21.4 - 71.8	1.9 - 20.5	3.8 - 60.3	1.5 - 4.1	S21-O11
Quality	0.22	0.71**	-0.02	0.27	0.26	0.05	0.76**	0.70**	0.62**	-0.65**	0.49*	

Data expressed as means ± SD (n = 2 and n = 3 for GRA and TE) and ranges. Means followed by different letters in the same column differ by LSD test ($p < 0.05$)

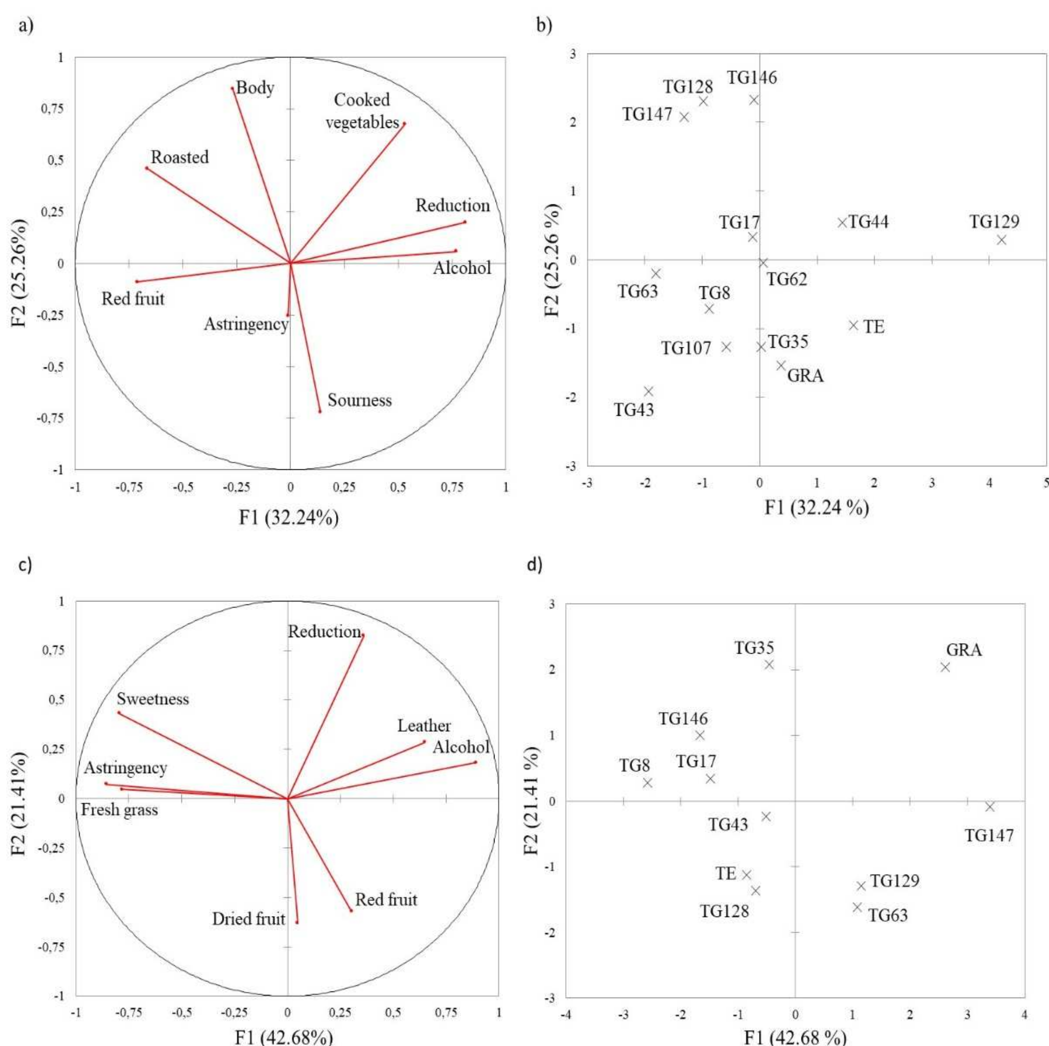
MA: malic acid, Free SO_2: free dioxide sulfur, VA: volatile acidity [a] expressed as g/L acetic acid, %ETH: % ethanol, TA: total acidity [b] expressed as g/L tartaric acid, ANT: Anthocyanin content, TPI: Total Polyphenolic Index, CI: colour intensity, L*: Lightness, and a^*_{10}/b^*_{10}: red/yellow, HD: Harvest date.

polyphenolic contents seem to be important quality drivers in this research, since samples TG146 and TG147 with the lowest polyphenolic content, colour intensity, a_{10}^* / b_{10}^* ratio (i.e. red to yellow colour) and highest lightness (L*) were classified in the lowest quality categories. Accordingly, in both years, quality was positively correlated to anthocyanin content ($r = 0.8$, $p < 0.01$), total polyphenolic index ($r = 0.7$, $p < 0.01$), colour intensity ($r = 0.6$ and $r = 0.8$, $p < 0.01$), a_{10}^* / b_{10}^* ratio ($r = 0.9$, $p < 0.01$, $r = 0.5$, $p < 0.05$), whereas Lightness was negatively correlated (- 0.6, $p < 0.01$; - 0.7, $p < 0.05$). These correlations have been previously observed (Lago-Vanzela et al. 2013, Niimi et al. 2018). Interestingly, a_{10}^* / b_{10}^* ratio has been reported to play an important role in predicting wine quality, as an indicator of the degree of oxidative aging in wines (Sáenz-Navajas et al. 2011).

Sensory characterization of wines

According to ANOVAs, the effect of wine was significant for eight attributes in each vintage (Supplementary material 4.2.1). Fig. 4.2.1 a-d show the PCAs with significant sensory attributes.

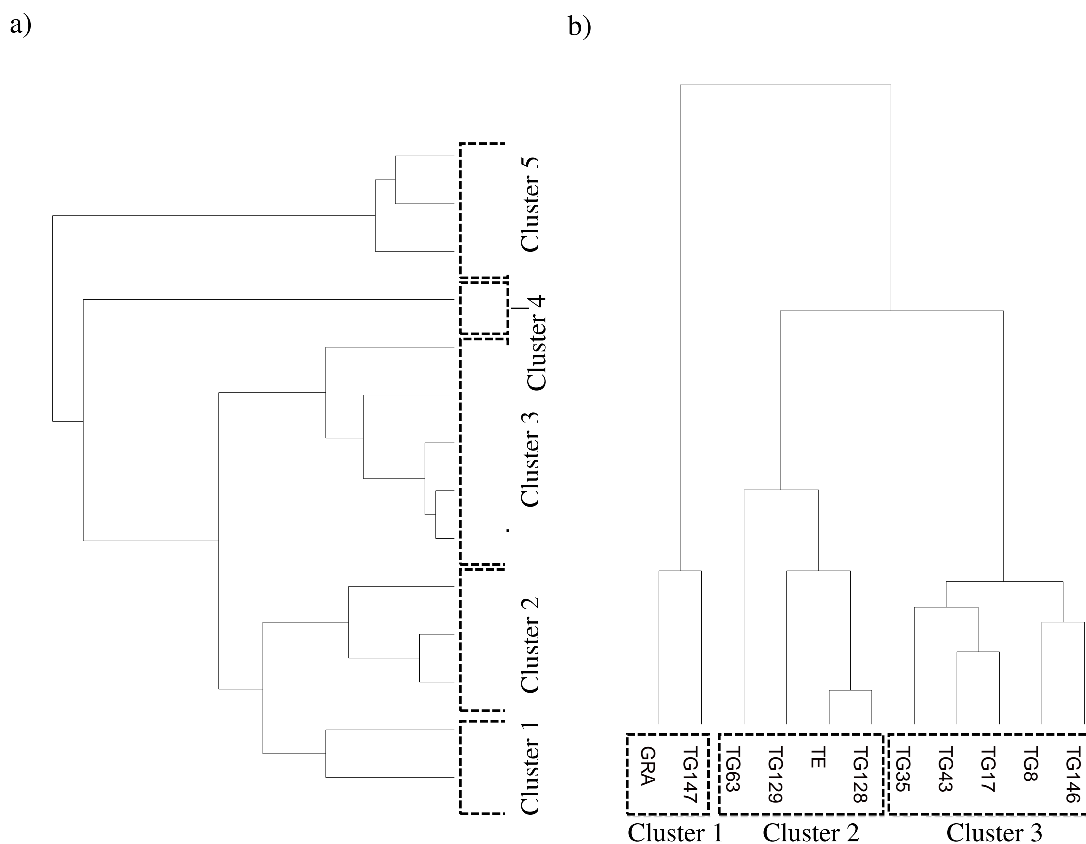
Figure 4.2.1. Correlation circles of sensory descriptors for a) 2017 and c) 2018 vintages projection of wines on the PCA spaces for b) 2017 and d) 2018 vintages.



Cluster analyses yielded five and three main clusters of wines in 2017 and 2018, respectively (Figure 4.2.2). For 2017, the five clusters significantly ($p < 0.05$) differed in reduction (present in TG129), alcohol (lowest in cluster 1 and especially high in TG129), roasted aromas (especially high in cluster 5), sourness (clusters 1 and 4 had higher values than clusters 2 and 5, and cluster 3 had intermediate values), and body perception (highest values in cluster 5). The presence of roasted notes, though not expected in young red wines, has been suggested (Langlois et al. 2010) as olfactory cue that determine wine aging potential, together with other parameters like saturated colour, high astringency and low acidity. Wines from TG35 and TG128 in clusters 3 and 5 would qualify for the production of aged wines since they were described as aromatic with roasted notes and moderate-high astringency in mouth.

Reduction aroma is mainly related to the presence of hydrogen sulfide and methanethiol, both acting as important suppressors of fruity and floral notes. It has been related to ethanol concentration, the amount of assimilable nitrogen, the redox state of the must, and wine and yeast strain (Franco-Luesma, & Ferreira 2016). Reductive notes could be also related to the use of SO_2, since a high dose could lead to discolouration of anthocyanins or the formation of reduction-related flavors (Rubio-Bretón et al. 2018). Any of these factors could be responsible for the high reduction notes presented by TG129 and Graciano (Supplementary 4.2.5) probably leading to the difference in quality scores in both years of the study.

Figure. 4.2.2. Dendrograms derived from Hierarchical Cluster Analysis (HCA) calculated with all the PCs obtained from the PCA (performed with significant sensory attributes for a) 2017 and b) 2018 vintages.



For 2018, three clusters could be identified. Cluster 1 presented the highest alcoholic and leather-like aroma. The presence of the latter has been associated to low-quality wines by experts (Sáenz-Navajas et al. 2013) and could have made GRA and TG147 to be classified in the "low/very low" quality category (Table 4.2.2). Cluster 2 presented the highest dried and red fruit aromas, associated to high quality (Sáenz-Navajas et al. 2013) and cluster 3 was mainly characterized by their fresh grass aroma.

Table 4.2.5 shows how all the attributes present an important vintage effect (vintage or vintage * wine), except for cooked vegetables (highest intensity for TG146) and sweetness (highest for TG35) which show a similar effect on wines regardless the vintage (Supplementary material 4.2.6).

Table 4.2.5. Two-way ANOVA calculated with wine and year as fixed factors and their interaction for descriptors with significant effect in at least one vintage.

	wine			year			wine*year		
	F	**p**	**Sig**	**F**	**p**	**Sig**	**F**	**p**	**Sig**
Red fruit	3.146	0.001	**	0.245	0.621	ns	1.954	0.036	*
Dried fruit	1.600	0.102	ns	1.385	0.240	ns	2.074	0.024	*
Roasted	2.097	0.023	*	4.540	0.033	*	2.333	0.010	*
Fresh grass	1.730	0.070	ns	2.176	0.141	ns	2.527	0.005	**
Cooked vegetables	2.124	0.021	*	2.592	0.108	ns	2.592	0.108	ns
Alcoholic	2.338	0.010	*	6.879	0.009	**	3.960	< 0.0001	***
Reduction	5.074	< 0.0001	***	2.856	0.091	ns	5.612	< 0.0001	***
Leather	1.659	0.086	ns	4.603	0.032	*	1.316	0.217	ns
Sweetness	2.080	0.024	*	2.732	0.099	ns	0.869	0.562	ns
Sourness	2.432	0.007	**	20.093	< 0.0001	***	0.787	0.641	ns
Body	1.680	0.081	ns	12.528	0.0001	***	1.240	0.261	ns
Astringency	3.379	0.0001	***	19.312	< 0.0001	***	1.721	0.072	ns

*p < 0.05; **p < 0.01; ***p < 0.001 by Student–Newman–Keuls test.

Cooked vegetables is considered an off-flavor present in oxidized wines that could affect self-life (Escudero et al. 2000). Oxidation can also lead to aroma deterioration, loss of citric and fresh aromas (Bueno et al. 2016), visible in TG146, where the lowest notes of red fruit were detected. Wines with low phenolic content are more susceptible to oxidation (Gambuti et al. 2017) which could be the case in TG147 and TG146. Low polyphenolic content could be related to the high productivity of these hybrids and not to the cultivars themselves. In addition, high yield is commonly related to the development of vegetal aromas (Etaio et al. 2008, García-Muñoz et al. 2014) an association which was also found for TG146 and TG147. Thus, all these factors could have been responsible for their classification in the lowest quality category.

Conclusions

In this research, two early ripening, low-medium yield genotypes, TG8 and TG63, characterized by high anthocyanin content, high acidity, deep colour, balanced aromas and in mouth properties, were consistently perceived as higher quality than Graciano and Tempranillo, in two very different vintages. Moreover, late ripening selections such as TG129, that present high polyphenolic content, high acidity and red fruit notes could be a good option for future climate conditions. Wines from TG35 or TG128 provided distinct sensory characteristics (roasted notes) valuable for the necessary diversification of the wine market.

This is the first physicochemical and sensorial evaluation of young red wines elaborated with Graciano x Tempranillo intraspecific hybrid grapes. Despite an important effect of vintage on sensory properties of wines, selected genotypes were able to produce quality wines with great sensory variability, confirming our hypothesis that intraspecific hybridization is a useful tool to improve traditional varieties for the adaptation to climate change while increasing wine quality. Besides, new consumer demands provide an opportunity to adopt these selections overcoming the limitations imposed by the traditional viticulture world.

Supplementary material

Supplementary material 4.2.1. Two-way ANOVAs (panelists as random factor and wines as fix factors) calculated on the 28 sensory attributes of wines elaborated in 2017 and 2018 vintages (F, F-ratios; p, p-values; Sig, significance: *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant).

	VINTAGE 2017			VINTAGE 2018		
	F	**p**	**Sig**	**F**	**p**	**Sig**
RED FRUIT	2.327	0.005	**	2.261	0.014	*
BLACK FRUIT	1.663	0.066	ns	0.681	0.742	ns
WHITE FRUIT	0.890	0.564	ns	1.091	0.370	ns
TROPICAL FRUIT	1.166	0.302	ns	0.769	0.658	ns
DRIED FRUIT	1.058	0.394	ns	1.820	0.055	*
CITRIC	0.660	0.802	ns	0.662	0.759	ns
FLORAL	1.616	0.077	ns	1.346	0.204	ns
SPICY	1.377	0.167	ns	1.087	0.371	ns
LIQUORICE	1.594	0.083	ns	0.880	0.552	ns
ROASTED	3.042	0.000	***	1.460	0.153	ns
VANILLA	1.386	0.052	ns	1.153	0.322	ns
VEGETAL	1.362	0.174	ns	1.221	0.276	ns
FRESH GRASS	0.100	0.900	ns	2.263	0.014	*
DRIED GRASS	0.991	0.458	ns	0.950	0.488	ns
COOKED VEGETABLES	2.406	0.004	**	1.510	0.134	ns
BALSAMIC	0.974	0.476	ns	0.867	0.565	ns
REDUCTION	8.246	< 0.0001	***	4.552	< 0.0001	***
ALCOHOL	2.260	0.007	**	4.995	< 0.0001	***
OXIDATION	0.950	0.500	ns	0.982	0.459	ns
SMOKED	0.957	0.493	ns	1.288	0.235	ns
MUSHROOM/EARTHY	1.328	0.193	ns	0.999	0.445	ns
LEATHER	1.353	0.179	ns	2.291	0.013	*
ASTRINGENCY	2.920	0.000	***	2.558	0.005	**
SOURNESS	2.327	0.005	**	1.272	0.245	ns
ALCOHOLIC	0.989	0.461	ns	1.349	0.203	ns
BODY	3.218	0.000	***	0.622	0.795	ns
BITTERNESS	0.100	0.900	ns	1.199	0.291	ns
SWEETNESS	0.100	0.900	ns	3.761	< 0.0001	***

Supplementary material 4.2.2. Detailed information of panel training.

A pre-established list of terms (Supplementary material 4.2.1) was obtained from the literature and included typical attributes usually employed to describe wines produced from Tempranillo and Graciano cultivars. Reference standards representative of all terms were developed and prepared at Laboratorio de Análisis del Aroma y Enología, Universidad de Zaragoza. For in-mouth terms, solutions containing different concentrations of table sugar (0-7 g /L) for sweetness, tartaric acid (0–3 g /L) for acidity, quinine sulphate (0–40 mg /L) for bitterness, potassium aluminum sulphate (0–5 g /L) for astringency, absolute alcohol (0-15% v/v) for alcoholic feeling and carboxymethylcellulose (0-1.5 g /L) for viscosity/body stimuli were prepared. During a typical training session, panelists were presented with references illustrating the different aroma, taste and chemesthetic terms, and 2-4 commercial wines were firstly individually described, and then ratings were discussed until Consensus was achieved.

During final descriptive evaluation of studied wines, participants were provided with a list of 28 terms. This list included the following 22 aroma terms: red fruit (strawberry, cherry, raspberry), white fruit (apple, pear), black fruit (blackberry, blackcurrant), dried fruit (raisin, prune), tropical fruit (banana), citrus (orange, lemon), floral (violet), vanilla, licorice, spicy (black pepper, nutmeg, clove), menthol/balsamic, dry herbs (hay), fresh vegetables (green pepper), fresh grass, roasted (coffee, toasts, toffee), smoky, reduction (cauliflower, rotten eggs), oxidation (acetaldehyde), undergrowth (moldy, mushroom), animal (leather), cooked vegetables (olive, backed potato) and alcohol (ethanol, spirit-like).

Supplementary material 4.2.3. Mean values (n=12) and SD represented as error bars for the phenology periods between April 1st and flowering date (green), flowering date and start veraison (yellow) (F-SV), start veraison and end veraison (blue)(SV-EV) and end veraison and ripening (EV-R)(pink) of each sample. Period length from April 1st till ripening with SD (n=12) is indicated for each sample.

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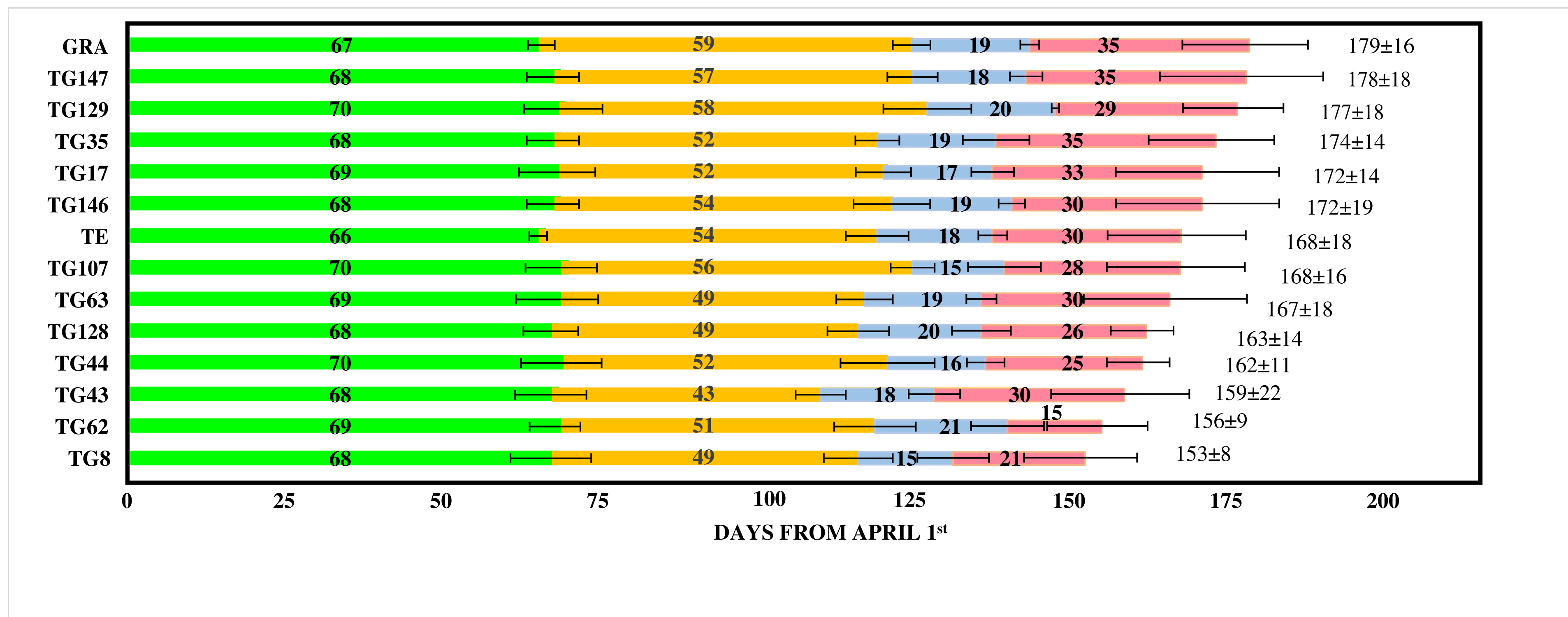
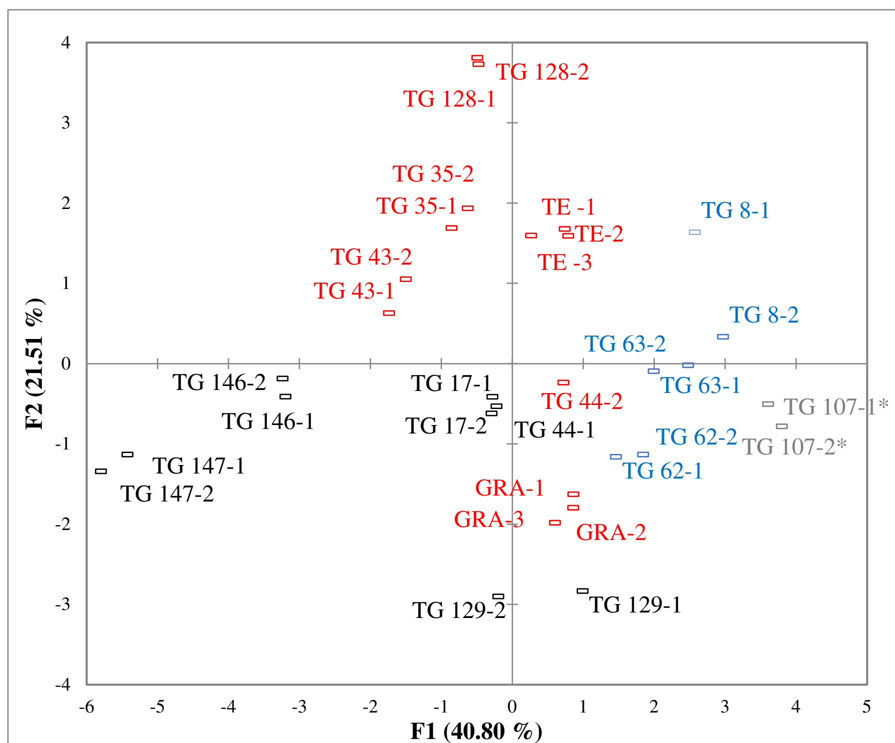
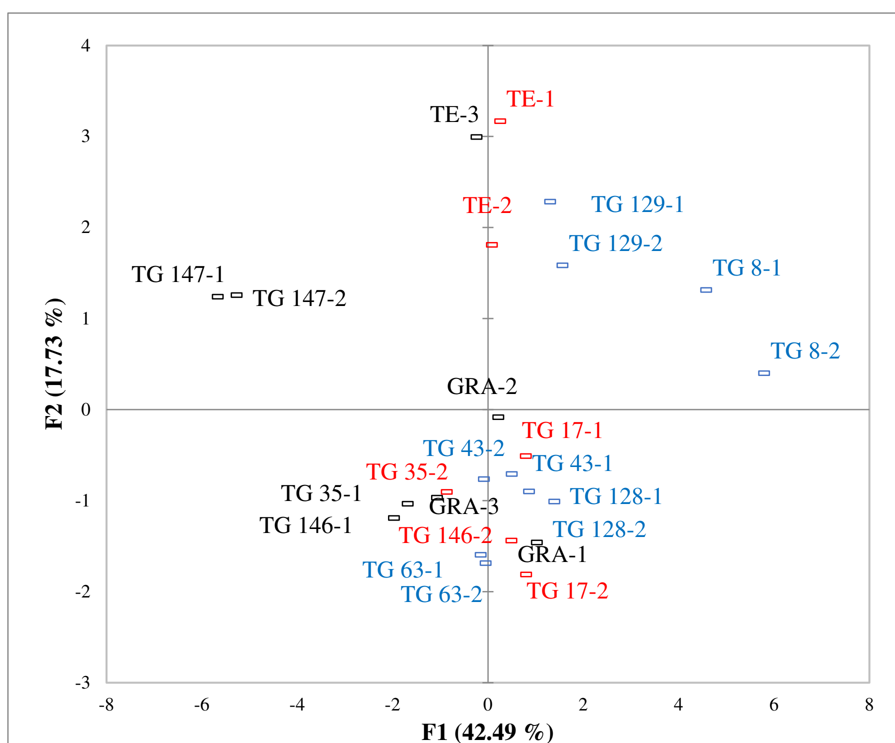


Supplementary material 4.2.4. PCA with chemical data of each wine as active variables for year 2017 a) and 2018 b). Average quality (in red); high/very high quality (in blue); low/very low quality (in black) based on CA-HCA calculated on quality categorization task; *not assessed for quality (in grey).



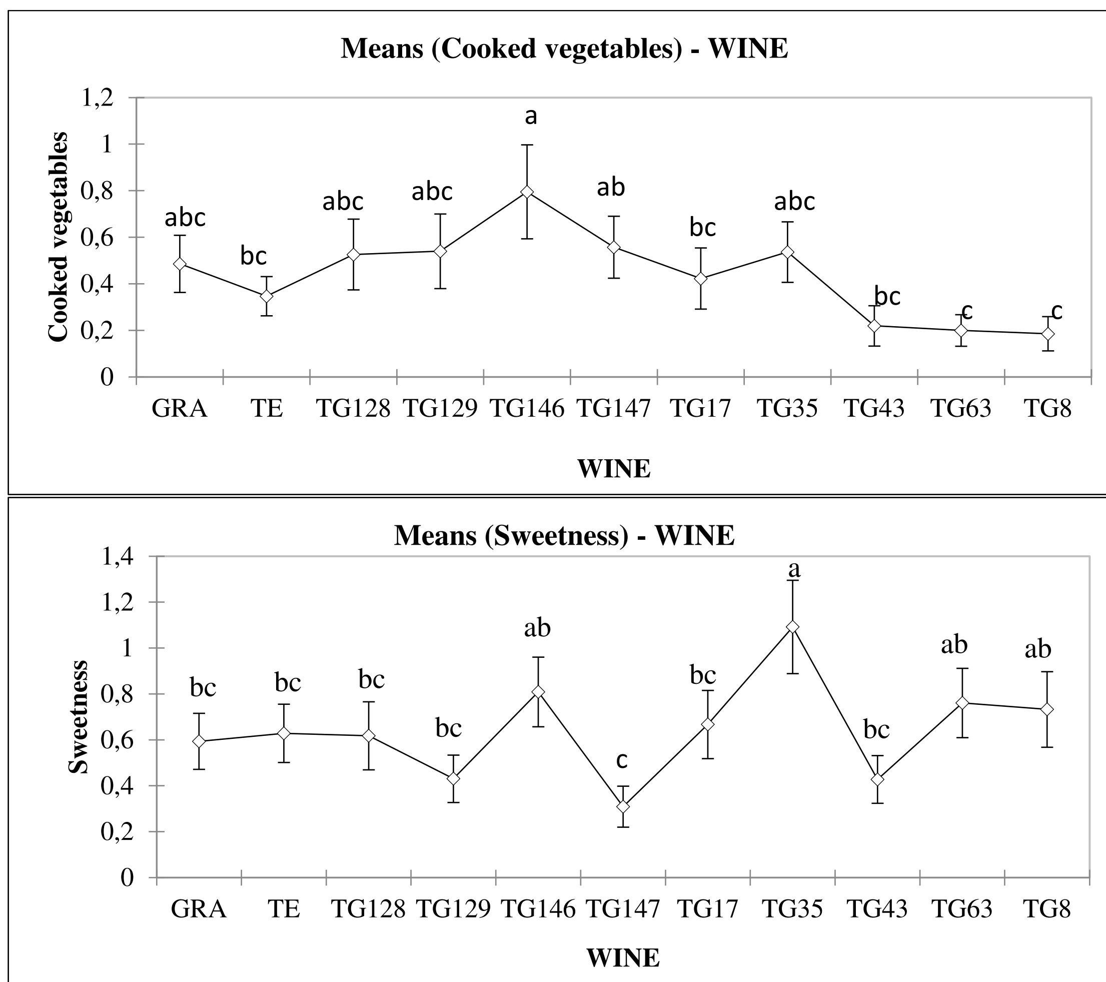


Supplementary material 4.2.5. Mean ± SEM scores (SD/ (panelist number)$^{0.5}$) of significantly differing attributes among all the wines in 2017 (above) and 2018 (below) vintages. Means followed by different letters in the same column differ by LSD test ($p < 0.05$).

2017	Cooked vegetables	Reduction	Alcohol	Red fruit	Roasted	Astringency	Sourness	Body
GRA	0.33 ± 0.17cde	0.83 ± 0.27abc	0.35 ± 0.14e	0.4 ± 0.19de	0.29 ± 0.14efg	0.75 ± 0.23de	2.60 ± 0.34a	0.23 ± 0.11cde
TE	0.42 ± 0.17b-e	1.06 ± 0.35ab	1.27 ± 0.34ab	0.38 ± 0.23de	0.31 ± 0.18efg	1.96 ± 0.36a	1.94 ± 0.35a-d	0.23 ± 0.13cde
TG8	0.09 ± 0.07e	0.28 ± 0.14de	0.78 ± 0.31b-e	0.66 ± 0.23b-e	0.66 ± 0.22c-f	1.00 ± 0.27bcd	1.78 ± 0.32b-e	0.38 ± 0.15cd
TG17	0.38 ± 0.18b-e	1.16 ± 0.35a	0.59 ± 0.22cde	0.88 ± 0.24bcd	0.44 ± 0.18d-g	1.13 ± 0.27bcd	1.41 ± 0.32c-f	0.44 ± 0.19cd
TG35	0.16 ± 0.11cde	0.09 ± 0.07e	1.19 ± 0.31abc	0.44 ± 0.20cde	0.56 ± 0.21c-f	1.41 ± 0.34abc	1.84 ± 0.34bcd	0.19 ± 0.1de
TG43	0.13 ± 0.10de	0.44 ± 0.21cde	0.31 ± 0.15e	1.56 ± 0.35a	0.50 ± 0.22c-g	0.63 ± 0.17de	2.06 ± 0.26abc	0.00 ± 0.00e
TG44	0.50 ± 0.19a-d	0.69 ± 0.27a-d	1.31 ± 0.31ab	0.56 ± 0.23cde	0.00 ± 0.00g	0.38 ± 0.18e	0.94 ± 0.25f	0.19 ± 0.10de
TG62	0.53 ± 0.19abc	0.56 ± 0.22b-e	0.91 ± 0.23b-e	0.97 ± 0.32bc	0.38 ± 0.18efg	0.75 ± 0.25de	1.81 ± 0.3bcd	0.34 ± 0.17cd
TG63	0.16 ± 0.09cde	0.38 ± 0.17cde	0.97 ± 0.27bcd	1.22 ± 0.28ab	1.19 ± 0.32ab	1.59 ± 0.31ab	1.63 ± 0.29b-e	0.41 ± 0.18cd
TG107	0.25 ± 0.13cde	0.28 ± 0.16de	0.81 ± 0.25b-e	0.69 ± 0.27b-e	0.78 ± 0.25b-e	1.13 ± 0.29bcd	2.13 ± 0.31ab	0.13 ± 0.06de
TG128	0.72 ± 0.28ab	0.41 ± 0.24cde	0.56 ± 0.23de	0.75 ± 0.27b-e	0.91 ± 0.28a-d	1.13 ± 0.32bcd	1.59 ± 0.26b-f	1.03 ± 0.27a
TG129	0.31 ± 0.15cde	0.63 ± 0.27a-e	1.78 ± 0.44a	0.22 ± 0.12e	0.22 ± 0.15fg	0.84 ± 0.28cde	2.13 ± 0.42ab	0.25 ± 0.12cde
TG146	0.81 ± 0.31a	1.03 ± 0.35ab	0.75 ± 0.28b-e	0.84 ± 0.32bcd	1.00 ± 0.28abc	0.78 ± 0.22de	1.13 ± 0.28ef	0.53 ± 0.21bc
TG147	0.28 ± 0.14cde	0.63 ± 0.21a-e	0.78 ± 0.31b-e	0.56 ± 0.21cde	1.34 ± 0.37a	0.59 ± 0.19de	1.28 ± 0.32def	0.84 ± 0.24ab

2018	Fresh grass	Reduction	Alcohol	Dried fruit	Red fruit	Leather	Astringency	Sweetness
GRA	0.08 ± 0.06d	1.72 ± 0.40a	1.11 ± 0.33ab	0.22 ± 0.11c	0.75 ± 0.21bcd	0.58 ± 0.19a	0.58 ± 0.16b-e	0.50 ± 0.16b
TE	0.44 ± 0.15a-d	0.33 ± 0.15c	0.44 ± 0.2cde	0.64 ± 0.22abc	0.81 ± 0.22a-d	0.11 ± 0.11bc	0.97 ± 0.20ab	0.78 ± 0.21b
TG8	0.47 ± 0.19a-d	0.25 ± 0.14c	0.17 ± 0.08de	0.36 ± 0.17c	0.42 ± 0.18cd	0.14 ± 0.07bc	1.11 ± 0.25a	0.78 ± 0.21b
TG17	0.81 ± 0.20a	0.72 ± 0.22bc	0.19 ± 0.14cde	0.39 ± 0.17bc	0.39 ± 0.11d	0.14 ± 0.08bc	0.69 ± 0.18abc	0.53 ± 0.18b
TG35	0.28 ± 0.19bcd	0.33 ± 0.18c	1.17 ± 0.31ab	0.22 ± 0.17c	0.28 ± 0.16d	0.00 ±.0.00c	0.22 ± 0.13de	1.50 ± 0.29a
TG43	0.34 ± 0.13bcd	0.54 ± 0.16bc	0.66 ± 0.18bcd	0.49 ± 0.16abc	0.51 ± 0.17bcd	0.03 ± 0.03c	0.74 ± 0.21abc	0.54 ± 0.17b
TG63	0.22 ± 0.10cd	0.16 ± 0.11c	0.73 ± 0.20bc	0.27 ± 0.13c	1.35 ± 0.24a	0.19 ± 0.14bc	0.51 ± 0.19b-e	0.46 ± 0.13b
TG128	0.67 ± 0.20ab	0.19 ± 0.10c	0.42 ± 0.15cde	0.89 ± 0.26ab	0.94 ± 0.26abc	0.39 ± 0.13ab	0.78 ± 0.2abc	0.61 ± 0.20b
TG129	0.19 ± 0.10cd	0.61 ± 0.21bc	0.36 ± 0.17cde	0.97 ± 0.25a	0.61 ± 0.23bcd	0.28 ± 0.12abc	0.42 ± 0.15cde	0.36 ± 0.11b
TG146	0.56 ± 0.22abc	1.00 ± 0.42b	0.00 ± 0.00e	0.22 ± 0.17c	1.00 ± 0.35ab	0.28 ± 0.14abc	0.17 ± 0.12e	1.56 ± 0.37a
TG147	0.19 ± 0.10cd	0.53 ± 0.21bc	1.53 ± 0.35a	0.56 ± 0.22abc	0.56 ± 0.18bcd	0.53 ± 0.17a	0.25 ± 0.09de	0.31 ± 0.12b

Supplementary material 4.2.6. Average ratings (for panelists scores and both years) and SEM of the attributes sweetness and cooked vegetables.



Error bars were calculated as SD / (panelist number)$^{0.5}$. Different letters represent statistical differences by LSD test ($p < 0.05$)

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4.3. Variability in berry traits, must and wine composition in Pinot Noir clones in the region of Marlborough in New Zealand

Abstract

New Zealand is considered one of the New World best regions for the production of high-quality Pinot Noir (PN) grapes and wines. In an increasingly competitive market, the offer of a wide range of wines better adapted to consumers can be a hallmark for these new regions. Know the potential variability on quality parameters of Pinot Noir clones and how berry size and environmental conditions could influence on them was the objective of this research. First of all, a preliminary study using 8 different clones to select 3 (PN_115, PN_ Abel and PN_UCD5) in base on berry size. Samples were collected from 3 different vineyards, located in two different subregions, and with two different rootstocks. Morphological traits of berries and seeds, and chemical parameters of berry extracts, musts and wines were measured. Significant differences were found among clones in berry morphology (PN_115 presented the lowest berry size) or aminoacid accumulation in musts, being the concentration of these compounds higher in PN_UCD5 independently of the sub region studied. The influence of berry size on studied variables was weak, smaller berries presented higher total acidity than bigger berries. In the warm and humid subregion, with poor fertile soil conditions (Wairau valley), berry weight was smaller, amino acid accumulation lower, instead phenolic composition and colour index in must were higher. Rootstock effect was also observed, the RSK 101-14 produced higher seed weight, pH, and phenolic and nitrogen composition in musts.

Introduction

Wine grapes are one of the most valuable perennial crops in the world (FAO 2018). Wine quality relies on high quality grapes (Nimii et al. 2018), which are influenced by several factors as viticultural management, selecting suitable varieties/clones; and winemakers supervising the correct fermentation of grapes. Grape quality has traditionally been associated to berry size, with small berries leading, in theory, to high quality wines. However, the relationship between berry size and berry composition has been matter of debate, between supporters (Rolle et al. 2015, Wong et al. 2016) and detractors (Roby et al. 2004, Walker et al. 2005).

Grape berry weight shows high genetic diversity within the *Vitis* genus, ranging from < 0.5 to > 10 g (Houel et al. 2013) and varies among clones of a given cultivar (Dai et al. 2011). Grape berry composition is a highly complex trait under the control of complex interactions among genotype, environment and cultural practices, also showing a high genetic diversity. As a result, different clones have the capacity to produce wines with different chemical composition. Thus, distinct color, and aromatic profile was found between Albariño clones (Zamuz et al. 2007) or phenolic content in Monastrell (Gómez-Plaza et al. 1999, 2000), Cabernet Sauvignon (Burin et al. 2011), Pinot Noir (Schueuermann et al. 2018), Cabernet Franc, Cabernet Sauvignon and Merlot (Forveille et al. 1996).

Grape berry and must composition variability may be the result of berry-to-berry differences within a bunch; bunch-to-bunch differences on a shoot; shoot-to-shoot differences on a vine; or vine-to-vine differences in a vineyard (Trought et al. 2017). Many studies have found internal variability among clusters from the same vine for different physical and chemical

parameters (Tarter et al. 2005, 2008, Trought et al. 2017). Bunch position on the shoot and shoot position on the cane influence phenology, with shoots from distal buds and inflorescences developing earlier, and advancing fruit ripeness. In lower cane node positions a greater number of shoots with looser bunches were found whilst at higher leaf node positions bunch size was smaller and berry size greater (Martin & Vasconcelos unpublished data). Even berries from the tip and shoulder of the same cluster exhibited different aroma profiles (Noguerol-Pato et al. 2012). These results appear very important for quality modelling because both bunch and berry size are strongly and significantly related to the leaf node positions count of the basal bunch.

The influence of environmental and climatic factors as temperature and pluviometry in grape composition has been widely observed in many wine regions (Resco et al. 2016). These factors linked to others such as the variety, rootstock and viticultural management determine the chemical composition of the berry, influencing variables such as the accumulation of nitrogen or phenolic compounds (Gutiérrez-Gamboa et al. 2017, Vidal et al. 2017).

In this context, in the present research it was assessed the influence of berry size, environment conditions and rootstock on berry, must and wine composition in Pinot Noir clones. First, a pilot study was made to select clones in base on berry size. This study also provided information about Pinot Noir genetics influence berry morphology and must composition. Two assays were made in different subregions to prove the following hypotheses:

- **Study 1.** PN clones differing in berry size will present differences in grape, must and wine composition.

- **Study 2.** Different environmental conditions will affect berry morphology, must and wine characteristics of different PN clones.

Material and Methods

Climatic data and subregion characteristics

Marlborough wine region is formed by three sub-regions: Awatere Valley located at south - east (30 % of vineyard area), Wairau Valley (45% of plantings) and the Southern Valleys zone (25 %) (Figure 4.3.1). In order to maximize differences, the first two sub-regions were chosen for this work. Awatere valley is characterised by free - draining soils fertile soils formed from loam and alluvial gravel and vines grow deep root systems. Climate is greatly influenced by the ocean, characterised by intense sunlight cooled by ocean winds, (Table 4.3.1) and often with a degree of elevation, which promotes a delay in harvests in comparison to Wairau Valley (https://www.nzwine.com).

Wairau Valley is a wide river valley that follows the Wairau River, and is separated in its upper part from the city of Nelson by Richmond Mountains, and the Wither Hills in the south protect the valley from harsh weather from the south - east. The effect of mountains and hills create a Foehn effect, with the west being subjected to wet weather, whilst Blenheim city enjoys the warmth and sunshine. Soils are not very fertile, located along the river terraces, being usually shallow, formed by clay, silt and stones which aids fast-draining. As a result, it has a warmer, more sheltered climate than in the Awatere Valley (https://www.nzwine.com). Full details of the

regional weather conditions are available on the Marlborough Research Centre web page (http://www.mrc. org.nz/category/weather-data/).

Figure 4.3.1. Subregions of Marlborough region. A. Southern Valleys, B: Wairau Valley and C: Awatere Valley. Source: https://www.nzwine.com/

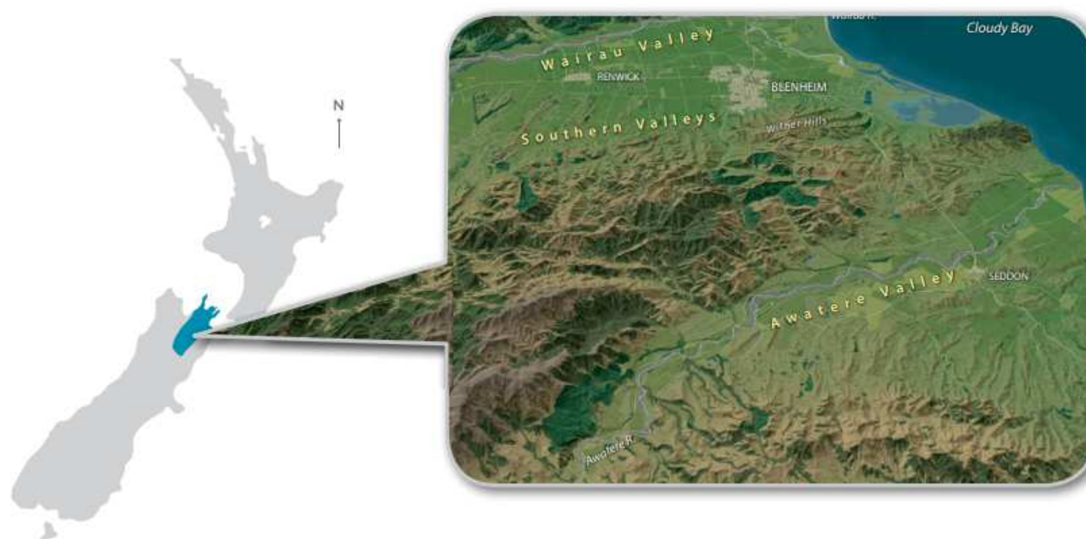


Table 4.3.1. Climatic data of Awatere and Wairau valley during September 2017 to April 2018.

	Period Sept-April	Awatere	Wairau
Rain (mm)	Total rain	675	487.8
Humidity	RH	63.9	71.8
	Max mean	26.9	28.2
T^a	Min mean	3.8	5.4
	Max	30.5 (Jan. Feb)	32.5 (Jan)
	Min	-0.7 (April)	0.3 (April)
Radiation	Mean day	401.6	548.7
(MJ/m2)	Max	474.0 (Sept)	790.1 (Dec)
	Min	167.1 (March)	320.0 (April)

Plant material

The three studies were conducted in commercial vineyards were vines grown using a Double Guyot, bilateral 12 - node canes training system. Pest and disease management followed Sustainable Winegrowing New Zealand guidelines (http://www.nzwine.com). On April 2018, twenty-five cluster samples of each clone were harvested separately from both the north (exposed) and south (shaded) sides of the same vine in cane node position 2, inflorescence leaf node position 4. Careful cluster selection was undertaken to ensure the clusters were picked from the correct position on the vine. Not field replicates were collected in the Pilot Study, three was the number of replicates in Study 1 and Study 2, where seventy-five clusters per clone were picked.

A Pilot study was conducted with eight different PN clones with the aim to select three with the greatest differences in berry morphology (Table 4.3.2). Three of them were assessed for Studies 1 and 2 for Studies 2 in two different regions and grafted in two different rootstocks. Vines of 25-year-old grafted onto 3309 rootstock were used for Pilot study and Study 2, being the spacing between vines of 1.5 × 1.25 m and 2.2 × 1.44 m, respectively. Study 1 were conducted in Wairau subregion grown on 101 - 14 rootstock, with a spacing of 1.5 × 1.22 m.

Table 4.3.2. Summary of the main features of the studies conducted.

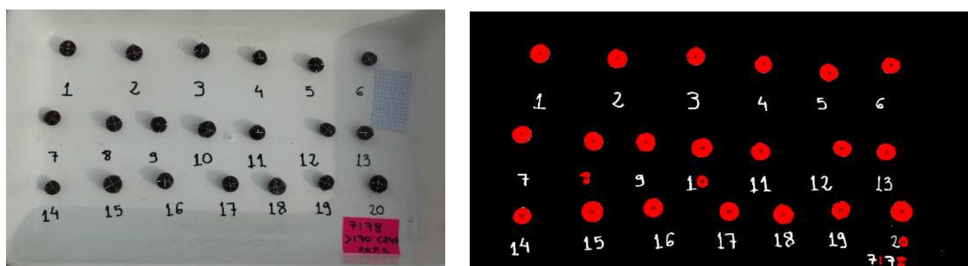
Study	Clones	Rootstock	Subregion	Plot
Pilot Study	PN_UCD5, PN_115, PN_Abel, PN_777, PN_Am, PN_Mariafeld, PN_667, PN_UCD6	3309	Wairau Valley	Clayvin vineyard (Giesen)
Study 1	PN_UCD5, PN_115, PN_Abel	110-14	Wairau Valley	Bankhouse vineyard (Indevin)
Study 2	PN_UCD5, PN_115	3309	Awatere Valley	Ballochdale vineyard

Berry and seed parameters

Berry morphology and berry extracts

Berry data were collected on April 2018 at ripening stage when random grapes picked from the top, medium and bottom of the clusters reached technological maturity (21° Brix). At harvest date, 200 whole berries from each clone were sampled from representative clusters and mean berry weight (g) was calculated. Berries were after frozen at - 20ºC to assess berry morphology and for subsequent extraction and analysis. In 40 berries per plant, length and diameter (mm) were measured in ImageJ software after photographs were taken (Figure 4.3.2), and shape coefficient was calculated as the ratio between length and diameter (Houel et al. 2013). Measures were analyzed in replicate.

Figure 4.3.2. Picture of the berries before and after being analyzed.



With the remaining 160 berries, 2 extracts were made for each clone, following the protocol guide showed in AWRI Grape Portal (http://www. https://www.awri.com.au/), and consists in the extraction of colour and phenolics using a solution of 1.0M Hydrochloric Acid and Acidified 50% v/v ethanol. Total anthocyanin content (mg/L), monomeric anthocyanin content

(mg M3G/L), total phenolics (AU), total polyphenolic and colour indexes (AU) were measured by spectrophotometric assays.

Seed analysis

Mean seed number per berry (SN) and mean seed fresh weight (SW, mg) were obtained in duplicate from a sample of 20 berries per clone randomly selected. Seed mass was also measured by digital image with ImageJ software (Figure 4.3.3). The number of seeds per g of marc was also measured in a representative sample of 20 g. Measures were analyzed in replicate.

Figure 4.3.3. Picture of the seeds before and after being analyzed.

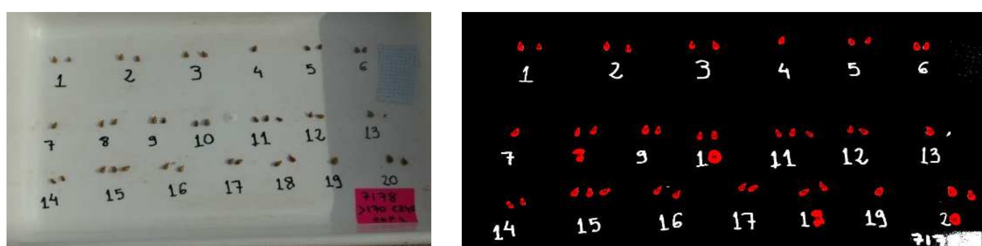


Image analysis techniques were confirmed as a useful tool for the measurement of phenotypic characteristics of berry and seeds as it was previously confirmed in other works (Wycislo et al. 2008, Rodriguez-Pulido et al. 2012). In the present study, image analysis was confirmed to be a lower time-consuming tool in the measurement of berry morphology, since berry area, volume and mass obtained correlated to berry weight and berry length or diameter.

Analysis of phenolics in grapes and wines

Direct optical density analysis was used to estimate the concentration of phenolic compound using the Somers Colour essay (Somers & Evans 1977). With this method Total anthocyanins (mg / L), colour density (AU), Hue, and Total phenolics (AU) were estimated from absorbance lectures. Total phenolics were estimated by the magnitude of absorbance at 280 nm. Absorbance at 420 nm gives an estimate of the concentration of yellow/brown pigments (mainly tannins) but also some oxidative phenolic breakdown products under natural wine pH / SO_2 conditions. Absorbance at 520 nm gives an estimate of the concentration of all red coloured pigment present under natural wine pH / SO_2 conditions (Somers & Evans 1977). Besides, total phenolics in musts were also evaluated by Folin - Ciocalteu method and expressed mg GAE / L.

Monomeric anthocyanins (mg M3G / L) were quantified by the pH difference method (Lee et al. 2005), which is a rapid and simple spectrophotometric method based on the chromophore anthocyanin structural change between pH 1.0 (colored) and 4.5 (colorless). Monomeric anthocyanin pigments reversibly change colour with a change in pH. The coloured oxonium form exists at pH 1.0, and the colourless hemiketal form predominates at pH 4.5. The difference in the absorbance of the pigments at 520 nm is proportional to the pigment concentration. Concentration is expressed on a malvidin-3-glucoside basis.

Nitrogen compounds and organic acid determination

Amino acid profiles were quantified on an Agilent 1200 series HPLC using a gradient elution programme of phosphate/borate buffer (10 mM each, pH 8.2) and organic solvent (MeOH: MeCN: H_2O, 45:45:10) on a Phenomenix Kinetix C18 column (5 um, 240_4.6 mm) as is described in Martin et al. (2016). Primary amino acids were derivatised online with o-phthalaldehyde and 3-mercaptopropionic acid and detected by fluorescence (340 nm excitation, 450 nm emission). Samples were treated with iodoacetic acid to aid in the reduction of cysteine. Secondary amino acids derivatised online with 9-fluorenylmethyl chloroformate and detected by fluorescence (260 nm excitation, 315 nm emission). A standard mix of 17 amino acids was purchased from Agilent. All standards and samples contained the internal standards sarcosine (100 mg / L) and a-aminobutyric acid (100 mg / L). All samples were diluted fourfold in water and filtered through a 0.45-um syringe filter before injection. All samples were run in duplicate and quantified on a four-point standard curve (R2 > 0.98) (Henderson & Brooks 2010). Ammonium was analyzed to assess the YAN requirements of the ferments, being quantified by enzymatic assay (Vintessentials Laboratories, Victoria, Australia).

Tartaric and malic acids were quantified on a Shimadzu Prominence, high performance liquid chromatography (HPLC) (Shimadzu Corporation, Kyoto, Japan) system using isocratic elution with a phosphate buffer (140 mM, pH 2.4) on an Allure Organic Acids Restek column (5 um, 240 _ 4.6 mm) as is described in Martin et al. (2016). All samples were diluted ten-fold in a solution containing thiourea as an internal standard and filtered through a 0.45-um syringe filter before injection (Shi et al. 2011). All samples were run in duplicate and quantified on a five-point standard curve. The correlation coefficient (R^2) of actual vs predicted concentration was > 0.98.

Must analysis

Must samples were subjected to a range of primary metabolite analyses. Soluble solids concentrations (° Brix) were determined with an Atago refractometer PAL-1 (Atago Co. Ltd, Japan). Titratable acidity (TA) and pH were determined on a Mettler Toledo T70 autotitrator (Mettler Toledo, Columbus, OH, USA) using an equivalence point titration. Aqueous sodium hydroxide (0.1 M) was used as titrant and TA was expressed as tartaric acid equivalents. TSS (measured as _Brix) was determined using a Mettler Toledo RM40 refractometer (Iland et al. 2004). Samples were centrifuged or filtered before analysis and analyses were carried out in duplicate. Spectrophotometric assays were run on a Molecular Devices Spectramax 384 Plus, UV transparent 96 well microplate.

Winemaking

At harvest (on April 2018) fruit from three field replicates of Studies 1 and 2 were combined, to give three fermentation replicates. Clusters were processed using the standard Plant and Food Research (PFR) winemaking protocol. Samples were chilled overnight at 10 ºC and then crushed in a manual crusher (Marchisio Cervino 400 / 600 kg / H). A standard sulphur dioxide (SO_2) quantity (40 ppm) was added as potassium metabisulphite at crushing. Musts were cold soaked for 3 days at 6 °C and then warmed to 18 °C and inoculated with RC212 yeast (Lallemand, Denmark) (rate 250 mg/·L). Grapes of each clone were fermented in triplicate at 25 °C and di-ammonium phosphate (DAP) was added where yeast available nitrogen (YAN, mg N / L) concentrations were below 250 ppm. Ferments were plunged three times a day. Fermentation soluble solids concentrations (measured as °Brix) were monitored daily using a portable density meter (Anton-Paar DMA 35, Austria) and when residual sugar was less than 2 g / L as determined

by Clinitest® (Bayer, USA), ferments were given three days of post-fermentation maceration before pressing. Ferments were pressed in a compressed air operated 6-kg sample press (Stainless Steel Systems, Blenheim, New Zealand) under a cover of carbon dioxide (CO_2). A pressing regime of two minutes at 1 Bar followed by another two minutes at 2 Bar was applied. Wine was settled for one week and then racked off yeast lees. An addition of 50 mg / L SO_2 (as potassium metabisulphite) was made.

Wine samples were analyzed for pH, total phenolics and titratable acidity as for juice analysis one month after bottling. Reducing sugars (g / L) were quantified by an enzymatic assay kit (Megazyme International, Ireland). Alcohol (%) was measured using an Anton Paar wine alcolyzer (Anton-Paar, Austria). All measurements were taken in duplicate from each of the three fermentation replicates and variation was < 0.02 % v / v.

Statistical analysis

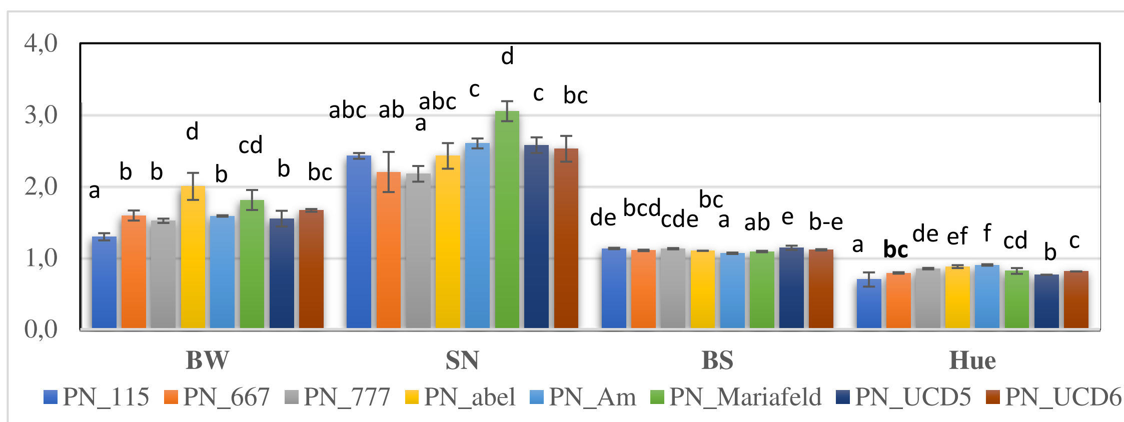
Normality distribution was checked by the Kolmogorov-Smirnov test. Data that significantly deviated from normality were analyzed by non-parametric Kruskal-Wallis test. ANOVA analysis performed with LSD test were carried out to detect differences between clones, plots and rootstocks. A MANOVA test was conducted to detect interactions between clone, rootstock and plots factors on the traits analyzed. Analysis were conducted with SPSS v.25. Principal Component Analyses (PCA) among the samples of each study were calculated using PAST software.

Results

Pilot study

Samples from eight PN clones were collected from the same position in the vine to study differences in berry, seed and must parameters. Clones presented statistical differences in berry morphology, seed traits and phenolic compounds of berry extracts as well as musts parameters (Supplementary material 4.3.1.). Figure 4.3.4 shows the main statistical differences of the parameters analysed. PN_Abel presented the highest berry weight (2.0 ± 0.19) and PN_115 the lowest (1.29 ± 0.05), both were selected to perform the two following studies along with PN_UCD5 because it showed intermediate values. PN_Mariafeld presented the highest number of seeds (3.05 ± 0.14), whilst PN_777 the lowest (2.18 ± 0.11). Regarding berry extracts, PN_115 presented the lowest value in Hue (0.7 ± 0.03) and PN_Am the highest (0.9 ± 0.06).

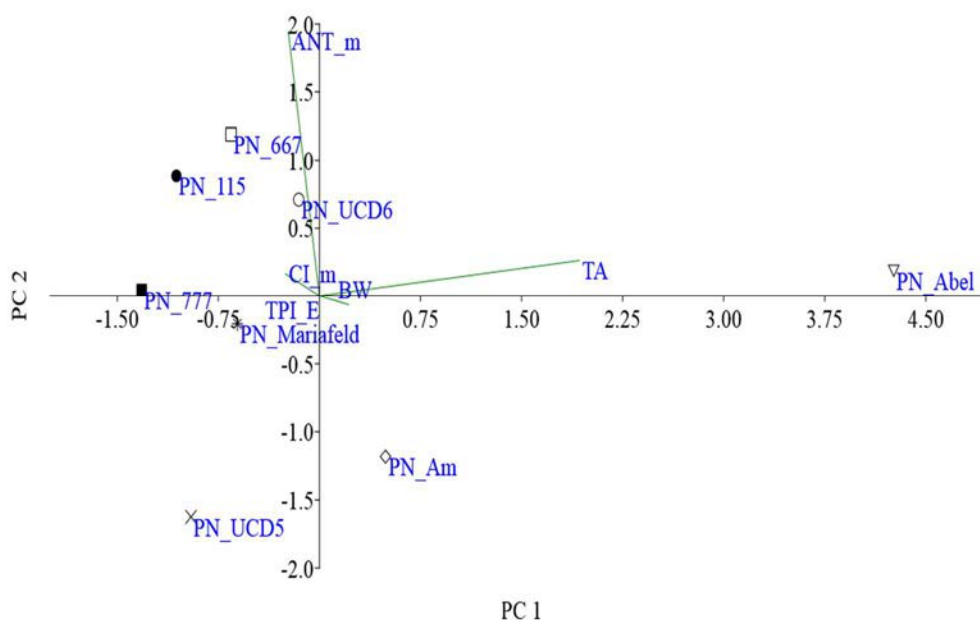
Figure 4.3.4. Differences between PN clones studied for berry weight (BW), seed number (SN), berry shape (BS) and Hue.



In musts, the widest ranges among clones were obtained for total acidity (11.3 g / L in PN_UCD6 and 5.9 g / L in PN_Abel), content of ammonium (149.4 mg / L in PN_777 and 78.1 mg / L in PN_UCD5), colour intensity (2.8 AU in PN_777 and 1.3 AU in PN_Mariafeld) and tonality (4.4 in PN_Am and 2.3 in PN_777). Major nitrogen compounds were arginine, alanine and proline while glycine, tyrosine, methionine, phenylalanine and lysine with the lowest concentration (Supplementary material 4.3.1.).

With the aim to identify the features that best described each clone, a PCA was performed including the traits analysed (Figure 4.3.5). Clones resulted clearly separated in the dimensional plot. Total variance explained by the first two PCs was 95.1 %.; PC1 explained 73.5 % with anthocyanin (ANT) and colour index (CI) discriminating among clones in the biplot. Thus, PN_115 and PN_667 were located in the negative side of first dimension mainly influenced by ANT, whilst PN_Abel and PN_Am were located in the opposite side. Berry weight (BW) and musts total acidity (TA) discriminated samples in PC2, where PN_Am and PN_UCD5 where located in the negative side.

Figure 4.3.5. PCA plot considering clones and the traits analyzed.



Relationship between berry size and chemical composition of grape, must and wine

Study 1. Wairau Valley

The aim of the study was to assess the influence of berry weight on berry, must and wine composition of three PN clones previously selected. PN_Abel, presented the highest berry weight and higher number of seeds with lower seed weight compared to PN_UCD5 and PN_115. In berry extracts, differences in colour index and total phenolics were found, being higher in PN_Abel compared with PN_115. PN_UCD5 showed significant differences in berry diameter, weight, area and volume relative to PN_Abel and PN_115 (Table 4.3.3.).

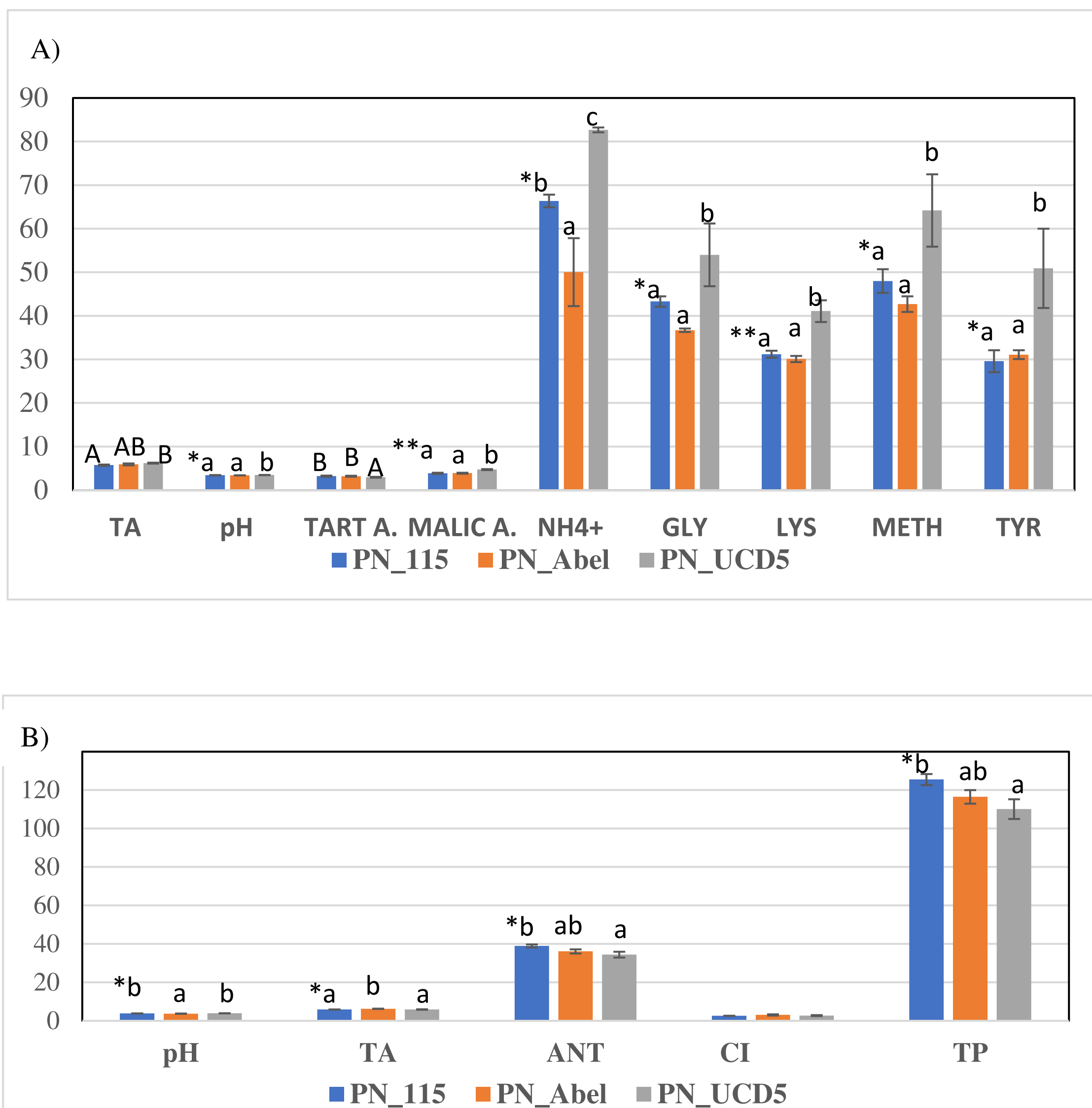
Table 4.3.3. Summary of berry, seed and berry extracts parameters in PN_115, PN_Abel and PN_UCD5.

		PN_115	PN_Abel	PN_UCD5
BERRIES	**Berry length (mm)**	**15.0 ± 0.3a****	**16.4 ± 0.1b**	**15.3 ±0.2a**
	Berry diameter (mm)	**13.4 ± 0.2a****	**15.1 ± 0.04c**	**14.0 ±0.2b**
	Berry shape	**1.1 ± 0.02b ***	**1.09 ± 0.01a**	**1.10 ± 0.01a**
	Berry weight (g)	**1.26 ± 0.05a****	**1.83 ± 0.1c**	**1.50 ± 0.04b**
	Berry area	**157.6 ± 4.6a****	**195.3 ± 0.7c**	**168.4 ± 4.6b**
	Berry volumen	**1409 ±55a****	**1980 ± 6c**	**1580 ± 62b**
	Berry mass	**11030 ± 477a***	**13857 ± 679b**	**12674 ±524b**
SEEDS	**Seed nº/g marc**	5.7 ± 0.3	5.9 ± 0.8	4.9 ± 0.3
	Seed weight (mg)	**53.8 ± 3ab***	**49.6 ± 0.3a**	**57.0 ± 1.1b**
	Seed number	**1.5 ± 0.1a****	**2.5 ± 0.1b**	**1.5 ± 0.3a**
	Seed mass	**1467 ± 239**	**1538 ± 101**	**1648 ±43**
BERRY EXTRACTS	**º Brix**	17.8 ± 0.3	18.6 ± 1.0	17.9 ± 0.4
	Colour Index	**1.0 ± 0.1a***	**1.8 ± 0.5b**	**1.2 ± 0.2a**
	Monomeric Anthocyanins	**47.0 ± 4.7a***	**52.7 ± 1.8ab**	**58.0 ±2.6b**
	Total Anthocyanins (mg/L)	60.4 ± 3.9	64.6 ± 2.7	69.0 ± 4.4
	Colour Density (AU)	1.6 ± 0.2	1.8 ± 0.0	1.8 ± 0.0
	Hue	0.9 ± 0.1	0.9 ± 0.0	0.9 ± 0.0
	Total Phenolics (AU)	**7.5 ± 1.4a***	**9.6 ± 0.9b**	**8.7 ±0.4ab**

** reflects statistical differences at 0.01 level and * at 0.05. Differences are highlighted in bold.

In musts, PN_Abel had the lowest values in ammonium. PN_UCD5 was significantly distinct from the other clones, with higher pH and malic acid content, and highest content of glycine, lysine, methionine and tyrosine amino acids (Figure 4.3.6 A). Wines derived from the smallest berry size clone (PN_115) presented higher pH and lower total acidity than the larger berry size clone PN_Abel (Figure 4.3.6 B). Thus, variables related to colour and phenolic compounds only presented differences associated to berry size in berry extracts.

Figure 4.3.6. Differences in must (A)and wine parameters (B) between PN_115, PN_UCD5, PN_Abel.



Abbreviations: TA Total acidity (g / L), TART A. Tartaric acid (g / L), MALIC A. Malic Acid (g / L), GLY Glycine (mg / L), LYS Lysine (mg / L), METH Methionine (mg / L), TYR Tyrosine (mg / L), ANT Total Anthocyanin content (mg / L), CI Colour intensity and TP Total phenolics (mg GAE / L). ** reflects statistical differences at 0.01 level, * at 0.05.

Study 2. Awatere Valley

This study focused on the influence of berry weight on berry and wine composition of PN_ UCD5 and PN_115 clones collected in Awatere Valley sub-region, with distinct climatic and edaphic features compared to the first two studies. Clones presented statistical differences in seed and berry traits (Table 4.3.4), and in several berry extracts and musts traits. PN_ UCD5 showed higher berry length, weight and area than PN_115; and presented higher seed number and weight. Total phenolic content in berry extracts was higher in PN_115 and in musts only pH was significantly lower.

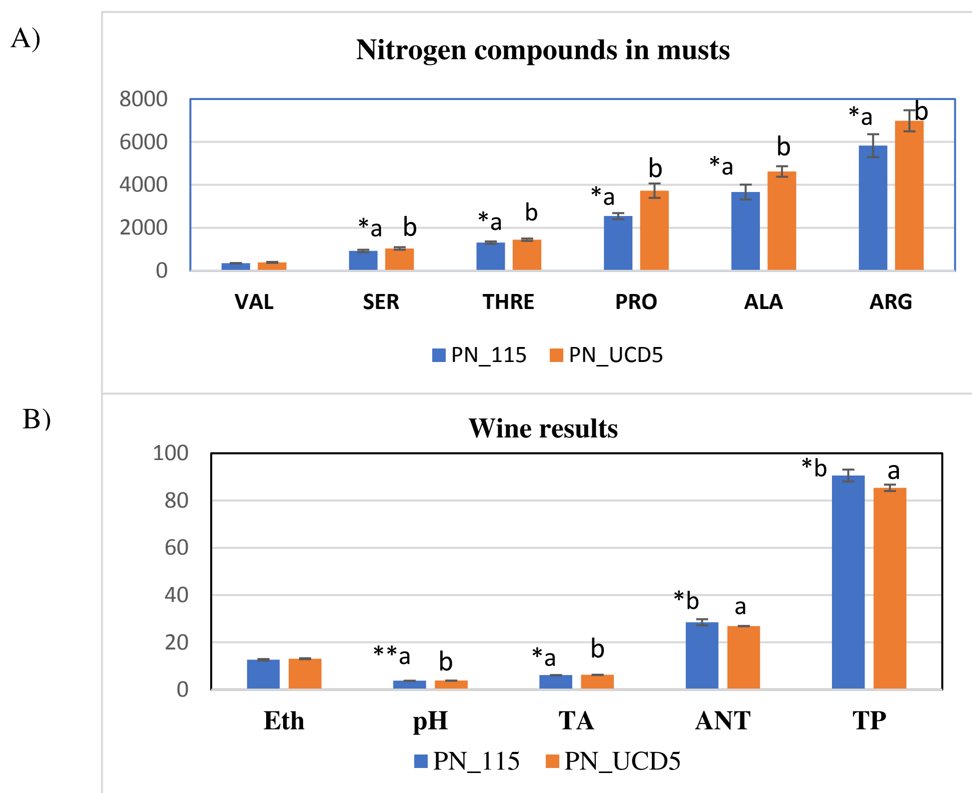
Table 4.3.4. Summary of seed, berry and must parameters in PN_115 and PN_UCD5.

		PN_115	PN_UCD5
BERRIES	**Berry length (mm)**	**16.4 ± 0.6 ***	**17.2 ± 0.5**
	Berry diameter (mm)	**14.7 ± 0.5***	**15.8 ± 0.6**
	Berry shape	1.1 ± 0.0	1.1 ± 0.0
	Berry weight g)	**1.7 ± 0.0****	**2.2 ± 0.1**
	Berry area	**190.5 ± 13.2***	**213.2 ± 13.2**
SEEDS	**Nº seeds/g marc**	5.0 ± 0.6	5.0 ± 0.7
	Seed nº/ berry	**1.7 ± 0.1****	**2.3 ± 0.2**
	Seed weight (mg)	**35.5 ± 7.4***	**41.1 ± 1.4**
BERRY EXTRACTS	**Monomeric Anthocyanins (mg/L)**	44.3 ± 4.1	43.3 ± 1.4
	Total Anthocyanins (mg/L)	58.9 ± 2.6	56.4 ± 1.7
	Total phenolics (AU)	**9.2 ± 0.6***	**7.7 ± 0.5**
MUSTS	**ºBrix**	21.9 ± 0.3	22.4 ± 0.6
	Total Acidity (g/L)	7.5 ± 0.3	7.4 ± 0.1
	pH	**3.35 ± 0.01****	**3.41 ± 0.01**
	$NH4^+$ (mg N / L)	93.4 ± 6.3	104.8 ± 10.3
	YAN (mg N / L)	383.0 ± 23.8	434.8 ± 30.0
	Glucose + Fructose (g / L)	224.5 ± 2.9	229.1 ± 8.8
	Colour intensity (AU)	**1.0 ± 0.2***	**0.7 ± 0.1**
	Tonality (AU)	4.1 ± 0.5	4.7 ± 0.7

** reflects statistical differences at 0.01 level, and * at 0.05. Differences are highlighted in bold.

Main differences among clones were found in amino acids content (Figure 4.3.7 A), having PN_UCD5 higher accumulation of arginine, alanine, serine, threonine and proline, as reported in Wairau Valley subregion. Results for other amino acids are presented in Supplementary material 4.3.2. In wines, PN_115 presented lower pH and TA, and higher total phenolics and anthocyanin content than PN_UCD5 (Figure 4.3.7 B). Therefore, in this study differences between clones were consistent in berry extracts, musts and wines.

Figure 4.3.7. Differences in nitrogen compounds (A) and wine parameters (B) in Awatere sub-region in PN_115 and PN_UCD5.



Abbreviations: VAL Valine (mg / L), SER Serine (mg / L), THRE Threonine (mg / L), PRO Proline (mg / L), ALA Alanine (mg / L), ARG Arginine (mg / L), Eth % Ethanol, TA Total acidity (g / L), ANT Total anthocyanins (mg / L). *show statistical differences at 0.05 level.

Influence of environment in berry morphology, must and wine characteristics

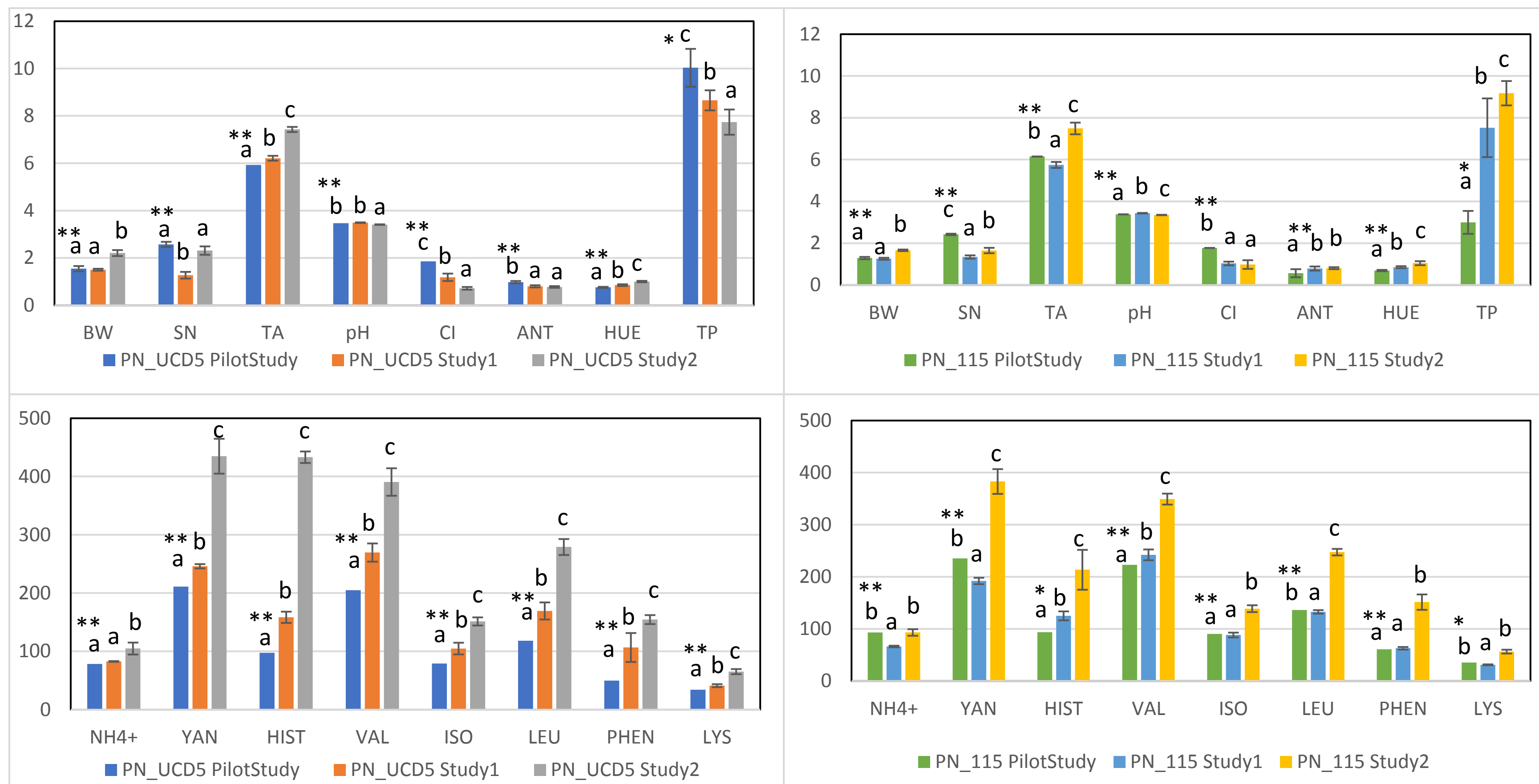
A MANOVA was performed to evaluate the influence of sub region and rootstock in clones PN_115 y PN_UCD5, and to assess the interactions between these factors (Table 4.3.5). Sub region was significant for most of the parameters, whilst rootstock had more influence in must variables. Few interactions were found among these factors, anthocyanidins and total phenolics of berry extracts, pH, YAN and anthocyanidins in musts, and amino acids as leucine showed interactions between clone and rootstock and with sub region factor.

Table 4.3.5. MANOVA results considering clone (PN_115 and PN_UCD5), subregion (plot) and rootstock (RSK) factors.

	Factor	Clone		Plot		RSK		Clone x Plot		Clone x RSK	
	Trait	F	Sig	F	Sig	F	Sig	F	Sig	F	Sig
BERRIES / SEEDS	Berry weight	73.8	0.00	88.5	0.00	ns	ns	7.0	0.03	ns	ns
	Seed number	8.7	0.02	30.1	0.00	148.8	0.00	ns	ns	ns	ns
	Seed weight	8.9	0.02	ns	ns	6.6	0.03	ns	ns	ns	ns
	Anthocyanins	6.0	0.02	ns	ns	ns	ns	32.0	0.00	26.0	0.00
	Total phenolic index	7.1	0.01	5.5	0.03	ns	ns	ns	ns	ns	ns
	Colour Intensity	5.6	0.03	4.5	0.04	ns	ns	ns	ns	ns	ns
	Hue	ns	ns	73.9	0.00	13.4	0.00	ns	ns	ns	ns
	Total phenolics	ns	ns	ns	ns	ns	ns	23.0	0.00	11.1	0.01
MUSTS	º Brix	ns	ns	131.4	0.00	25.2	0.00	9.7	0.01	ns	ns
	Total acidity	ns	ns	167.8	0.00	ns	ns	ns	ns	ns	ns
	pH	96.7	0.00	33.0	0.00	27.6	0.00	5.8	0.04	11.1	0.01
	NH_4	9.0	0.01	17.4	0.00	21.2	0.00	16.8	0.00	ns	ns
	YAN	15.4	0.00	282.0	0.00	ns	ns	9.1	0.01	40.7	0.00
	Anthocyanins	6.9	0.03	10.3	0.01	70.0	0.00	11.4	0.01	18.9	0.00
	Total phenolic index	ns	ns	79.0	0.00	46.3	0.00	ns	ns	ns	ns
	Colour intensity	ns	ns	141.3	0.00	75.6	0.00	ns	ns	ns	ns
	Hue	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Total phenolics	6.3	0.03	7.2	0.02	82.0	0.00	10.4	0.01	17.6	0.00
	Serine	7.1	0.03	125.3	0.00	ns	ns	ns	ns	ns	ns
	Histadine	ns	ns	9.0	0.02	ns	ns	ns	ns	ns	ns
	Glycine	ns	ns	45.7	0.00	ns	ns	ns	ns	ns	ns
	Threonine	17.4	0.00	223.1	0.00	ns	ns	ns	ns	ns	ns
	Arginine	14.6	0.01	154.5	0.00	ns	ns	ns	ns	ns	ns
	Alanine	23.8	0.00	198.0	0.00	8.8	0.02	5.7	0.05	ns	ns
	Tyrosine	24.5	0.00	174.4	0.00	ns	ns	6.0	0.04	ns	ns
	Valine	5.7	0.05	156.0	0.00	8.7	0.02	5.7	0.05	ns	ns
	Methionine	ns	ns	15.2	0.01	ns	ns	ns	ns	ns	ns
	Phenylalanine	ns	ns	83.2	0.00	ns	ns	ns	ns	ns	ns
	Isoleucine	5.7	0.05	118.1	0.00	ns	ns	ns	ns	5.8	0.05
	Leucine	14.6	0.01	291.6	0.00	6.3	0.04	9.7	0.02	8.6	0.02
	Lysine	13.9	0.01	92.1	0.00	ns	ns	ns	ns	ns	ns
	Proline	6.7	0.04	88.8	0.00	ns	ns	29.0	0.00	ns	ns

In Figure 4.3.8 differences between plots and rootstocks in both clones were showed; it was noteworthy the high amino acid contents obtained in musts from Awatere sub region berries. Other parameters as total acidity, berry weight and Hue resulted also higher in Awatere sub region independently of the clone, whilst total phenolics presented a different behaviour depending on the clone.

Figure 4.3.8. Summary of the main differences in PN_115 and PN_UCD5 in the different studies.



Abbreviations: BW Berry weight (g), SN seed number, TA Total acidity (g / L),
CI colour index, ANT anthocyanins (mg / L), TP Total phenolics (UA), HIS Histadine (mg / L), VAL Valine (mg / L) ISO Isoleucine (mg / L), LEU Leucine (mg / L) PHEN Phenilalanine (mg / L) LYS Lysine (mg / L).
** reflects statistical differences at 0.01 level, * at 0.05.

Discussion

Significant differences between PN clones were detected in berry morphology, seed parameters, must and wine composition. Our results support other studies performed in different cultivars (Burin et al. 2011, Schueuermann et al. 2018) which show that clones from the same grape variety can differ in must and wine composition, relevant for industrial use. Clonal selection with a focus on berry size is presumably a useful tool in grapevine genetic improvement to produce wines with distinct colour, aromatic profile and phenolic content. Remarkably, PN_UCD5 must amino acid content was almost as twice as that of the other two clones studied. Must nitrogen components play a key role on wine quality by affecting yeast growth during alcoholic fermentation (Gutierrez-Gamboa et al. 2018), and the link with volatile compounds produced in wine (Valdés et al. 2019). Gutierrez- Gamboa et al. (2018) found that Carignan Noir can be considered as a proline accumulator cultivar, in parallel, Pinot Noir could be regarded as an arginine accumulator variety. Schueuermann et al. (2018) reported arginine and proline as the two more abundant compounds in musts of PN_ 115 and PN_777.

In the present work, smaller berries bore lower number of seeds as previously reported (Walker et al. 2005, Gil et al. 2015), although no relationship between berry and seed size was obtained. Extracts from the smaller berry clones had lower anthocyanidins and phenolic contents and a low colour index. In wines, lower acidity was associated with clones with smaller berries. Barbagallo et al. (2011), and Friedel et al. (2016), also reported wines from small berries having less total acidity, however, Poni et al. (2009), Barbagallo et al. (2011), reported that smaller berries can retain more acidity in terms of pH. Anthocyanidin and phenolic compounds in wines were not related to berry size as no differences between PN_115 and PN_Abel were found. However, PN_UCD5 presented less content than PN_115. That points out that phenolic content in red grapes is largely dependent not only on cultivar and species (Dai et al. 2011), but also on clones.

Environmental factors such as climate, soil characteristics and viticultural practices, as the vine rootstock, caused variability for most of studied traits. Berry shape or total acidity variables were not altered neither by the plot nor by the rootstock. Comparing the two sub regions, berry weight was smaller in Wairau Valley, most likely provoked by water deficit (Roby et al. 2004) and/or high temperatures (Holt et al. 2008), as Wairau is warm and humid with poor fertile soils. Anthocyanidins, phenolic compounds and colour index in musts were higher in this region, as well as wine phenolic content. The moderate stress caused by higher temperatures and low soil fertility could have increased the accumulation of these compounds, since they are involved in the stress response (Koundouras et al. 2006, Schreiner et al., 2013). In studies with Pinot Noir variety, warm temperatures during early berry development increased phenolic concentrations (Nicholas et al., 2011, Blank et al. 2019). Clones from Awatere Valley presented higher accumulation of nitrogen compounds, in agreement with Gutierrez-Gamboa et al. (2018), who found the highest nitrogen compound concentration in grapes in the coolest plot leading to a faster alcoholic fermentation. Therefore, presumably low night temperatures before harvest could lead to a higher synthesis of amino acids.

Rootstock 3309 positively stimulated seed weight, pH, anthocyanidins and phenolic compounds in must compared to 101-14, but not influenced berry parameters. Also nitrogen compounds were higher; as previously reported (Gutierrez-Gamboa et al. 2018) rootstock seem to have some influence in the accumulation of these compounds. A high amount of amino acids can lead to an improvement in wine aroma, due to the fact that certain amino acids are precursor of volatile compounds.

Conclusions

In this work, variability in morphologic and chemical composition of berry, must and wine was found among Pinot Noir clones. Chemical composition of must and wine was mainly determined by the clone. According to the results obtained, clonal selection may result in differences in Pinot Noir wine quality better adapted to different environments. The influence of berry size on studied variables was weak, grapes with lower berry size presented in berry extracts lower content of anthocyanidins or total phenolic compounds, parameters related to quality. In wines could be only related to acidity parameters. On the other hand, berry weight was smaller at the plot with warmer and humid conditions and lower soil fertility, where anthocyanidins, phenolic compounds and colour index resulted increased. Remarkably, the cooler environment presented an increase of nitrogenous compounds. Berry size was not influenced by rootstock, however variability in chemical composition of berries, musts and wine were related to vine rootstock. Results are useful in order to design different wine styles at different plots based on different clones. An evaluation of the aromatic compounds derived from the amino acids detected in musts should be further studied.

Supplementary material

Supplementary material 4.3.1. Summary of the parameters studied in the eight PN clones in Pilot study.

		PN_115	PN_667	PN777	PN_Abel	PN_Am	PN_Maria	PN_UCD5	PN_UCD6
Berry traits	**Berry weight (g)**	1.29 ± 0.05a	1.59 ± 0.07b	1.52 ± 0.03b	2.0 ± 0.19d	1.58 ± 0.01b	1.81 ± 0.14cd	1.55 ± 0.11b	1.66 ± 0.02bc
	Berry length (mm)	15.1 ± 0.75	16.19 ± 0.91	16.01 ± 0.72	16.85 ± 0.18	16.0 ± 0.12	15.2 ± 0.32	16.51 ± 0.05	16.78 ± 0.01
	Berry diameter (mm)	13.36 ± 0.47a	14.69 ± 0.74b	14.2 ± 0.72ab	15.36 ± 0.15b	15.1 ± 0.0b	16.53 ± 0.51c	14.52 ± 0.3b	15.12 ± 0.11b
	Berry shape	1.13 ± 0.01de	1.10 ± 0.01bcd	1.10 ± 0.01cde	1.1 ± 0.0bc	1.06 ± 0.01a	1.09 ± 0.01ab	1.14 ± 0.03e	1.11 ± 0.01b-e
	Berry mass	12005 ± 528	14025 ± 815	12837 ± 235	14034 ± 67	13301 ± 1095	13853 ± 413	13163 ± 344	13177 ± 2039
Seed traits	**Seed number**	2.4 ± 0.0abc	2.2 ± 0.28ab	2.18 ± 0.11a	2.4 ± 0.18abc	2.60 ± 0.07c	3.05 ± 0.14d	2.58 ± 0.11c	2.53 ± 0.18bc
	Seed weight (mg)	37.0 ± 0.9a	45.05 ± 0.73c	42.9 ± 0.54bc	40.0 ± 1.7ab	38.29 ± 1.11a	54.33 ± 0.30d	37.95 ± 1.50a	42.51 ± 2.33bc
	Seed nº/marc	8.31 ± 0.52d	6.4 ± 0.2abc	6.85 ± 0.13bc	5.71 ± 0.92a	6.88 ± 0.24bc	7.14 ± 0.09cd	5.94 ± 0.03ab	5.9 ± 0.35ab
	Seed mass	1370 ± 45	1630 ± 51	1300 ± 16	1468 ± 132	1353 ± 12	1345 ± 51	1387 ± 14	1555 ± 294
Berry extracts	**Ant mono. (mg / L)**	46.0 ± 10.0	56.15 ± 0.87	58.58 ± 1.87	46.24 ± 1.24	49.44 ± 5.34	54.91 ± 6.63	51.71 ± 1.55	54.73 ± 3.16
	Total anthocy (mg/L)	60.9 ± 8.7ab	65.51 ± 0.2b	72.22 ± 3.06c	57.83 ± 0.5a	62.77 ± 6.17ab	69.82 ± 5.85bc	72.1 ± 16.7ab	68.47 ± 3.9bc
	Colour intensity (UA)	0.34 ± 0.1	0.43 ± 0.01	0.43 ± 0.01	0.32 ± 0.02	0.36 ± 0.01	0.39 ± 0.04	0.37 ± 0.0	0.42 ± 0.0
	Hue	0.7 ± 0.03a	0.79 ± 0.04bc	0.85 ± 0.02de	0.88 ± 0.03ef	0.90 ± 0.06f	0.82 ± 0.03cd	0.76 ± 0.03b	0.81 ± 0.01c
	Total phenolics (UA)	5.56 ± 0.01a	7.75 ± 1.03b	9.55 ± 0.13c	7.91 ± 0.13b	10.21 ± 0.49c	9.64 ± 1.97bc	10.03 ± 0.8bc	8.35 ± 0.27b
Musts	**ºBrix**	19.9	21	19.5	18.7	20.5	19.8	19.7	19.9
	Total acidity (g / L)	6.15	6.54	5.89	5.93	7.06	7.37	6.46	11.3
	pH	3.38	3.34	3.3	3.47	3.34	3.29	3.37	3.1
	NH4+ (mg N / L)	93.18	110.24	149.45	78.35	99.73	88.22	78.11	101.02
	Glucose +Fructose (g/L)	199.42	211.94	192.12	184.93	210.93	198.00	199.56	198.51
	Total antochyanins (UA)	10.83	11.11	9.93	8.32	10.50	8.62	9.66	9.47
	Color intensity (UA)	1.77	1.66	2.82	1.86	2.27	1.28	2.23	1.55
	Hue	4.07	4.31	2.35	3.26	4.42	2.55	4.34	3.59

Continue

		PN_115	PN_667	PN777	PN_Abel	PN_Am	PN_Maria	PN_UCD5	PN_UCD6
Aminoacid compounds	**Serine (mg/L)**	591.7	743.7	1147	565.6	882	833.4	985.1	1184
	Histidine (mg/L)	93.6	118.5	221.2	97.37	157.5	179.1	201.1	162.7
	Glycine (mg/L)	49.55	80.23	121.5	47.34	79.09	71.74	62.08	78.74
	Threonine (mg/L)	816.8	1038	1460	793.6	1200	922.4	1186	1450
	Arginine (mg/L)	2447	2938	6212	2306	3551	3690	4311	4148
	Alanine (mg/L)	1403	2140	2833	1437	2681	2022	2383	2728
	Tyrosine (mg/L)	31.53	44.05	56.93	29.89	49.75	62.6	44.41	55.21
	Valine (mg/L)	222.9	273.5	356	204.9	321.9	208.3	267.8	331.6
	Methionine (mg/L)	48.66	58.35	71.93	41.85	65.89	66.88	47.99	51.95
	Phenylalanine (mg/L)	60.93	71.33	78.6	49.66	79	63.33	79.69	85.44
	Isoleucine (mg/L)	90.43	100.9	126.5	79.11	115.7	83.1	106.8	128.3
	Leucine (mg/L)	136.3	169.6	226.9	118.2	197.2	174.6	191.2	232.6
	Lysine (mg/L)	35.46	42.46	64.31	33.95	46.97	53.91	42.76	50.25
	Proline (mg/L)	1879	2466	2248	1281	2277	1810	2303	2389

** reflects statistical differences at 0.01 level, * at 0.05.

Supplementary material 4.3.2. Summary of nitrogen compounds results in must in Study 2.

	PN_115			PN_UCD5		
	Mean ± SD	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**
Serine	923.5 ± 54.2*A	861.3	960.8	1039.8 ± 56.8B	974.5	1077.0
Histadine	213.6 ± 38.3*A	179.1	254.8	433.1 ± 169.8b	277.8	614.4
Glycine	83.8 ± 7.8	74.9	89.0	92.9 ± 8.5	83.4	100.0
Threonine	1312 ± 57.3*a	1249.0	1360.0	1445.3 ± 59.0b	1379.0	1492.0
Arginine	5827 ± 532.7*a	5217.0	6197.0	6985.3 ± 495b	6467.0	7454.0
Alanine	3665 ± 347.8*a	3281.0	3959.0	4621 ± 245.1b	4342.0	4799.0
Tyrosine	83.2 ± 0.9**a	82.2	84.0	105.1 ± 5.8b	101.6	111.8
Cystine	178.2 ± 15.4	168.7	196.0	164.1 ± 13	151.9	177.8
Valine	349.2 ± 10.5*a	338.7	359.6	390.7 ± 23.6b	365.0	411.3
Methionine	97.5 ± 23.1	80.4	123.8	106.7 ± 22.5	87.0	131.2
Phenylalanine	151.5 ± 14.8	136.4	165.9	154.5 ± 7.7	145.7	159.9
Isoleucine	138.9 ± 6.5*A	134.0	146.3	151.4 ± 7.0B	143.5	156.7
Leucine	247.5 ± 6.4**a	241.9	254.4	279 ± 13.6b	266.9	293.8
Lysine	56.6 ± 3.6A	52.7	59.9	65.1 ± 4.4B	60.2	68.6
Proline	2546 ± 141**a	2423.0	2701.0	3728 ± 336.8b	3352.0	4002.0

** reflects statistical differences at 0.01 level, * at 0.05 and capital letters at 0.1.

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CHAPTER 5.
GENERAL CONCLUSIONS

5. General conclusions

1. The phenotypic segregation of 12 traits including berry, flower, and seed-related parameters was assessed in two wine-grape segregant populations with Tempranillo as common parent, consisting of 130 and 151 plants derived from a cross with Grenache and Graciano varieties, respectively. Fourteen traits related to must composition, productivity and phenology stages were also studied in four consecutive years and two different plots in G x T progeny. All the parameters presented transgressive segregation and continuous variation. Year effect resulted significant for all traits except berry weight, flower diameter and seed weight. Plot effect resulted significant for all the traits analyzed. Broad-sense heritability estimatation resulted higher in T x G progeny, being particularly high for flower traits. Significant correlations among traits were observed, and moderate associations between berry length and berry shape, and between berry shape and pistil shape were found in both genetic backgrounds. Eleven white and red genotypes were pre-selected in Grenache x Tempranillo progeny, based on ripening date, cluster weight, yield, acidity and Brix degree by cluster analysis, that will need to be further analyzed.

2. Female plants showed rounder flower shape, larger flower diameter, lower number of seeds, and a delay in flowering and start veraison dates compared with hermaphrodites in both genetic backgrounds. A QTL region in LG2 was detected for flower-morphology, seed, productivity traits, and phenological stages (flowering date, veraison), confirming the influence of flower sex in the genetic determinism of these characters. Effects of sex resulted particularly strong in flower morphology traits as ovary shape in both progenies.

3. Significant QTL regions were detected for berry size and productivity parameters in LG17 in Grenache x Tempranillo progeny. In Tempranillo x Graciano population, regions in LG3 and LG5 resulted associated mainly to berry size and seed traits. In Tempranillo x Graciano progeny, a QTL on LG5 for berry, seed and flower traits covered the region of *FERONIA* locus and a QTL on LG18 for seed traits resulted associated to locus *SDI*. For flower morphology, QTL on LG8, LG11 and LG14 were identified, with QTL on LG11 showing the strongest and most stable effect over the two years. A candidate gene VIT_11s0016g03650 with a function associated to pollen morphology is proposed associated to the highly significant QTL detected in LG11 for flower traits in both progenies.

4. In Grenache x Tempranillo progeny, main QTL were detected on LG17 and LG18 for yield, fertility index, cluster number, and cluster weight. A QTL on LG1 was detected for sugar content, and for total acidity five stable QTL were found on LG4, LG12, LG13, LG14 and LG17. Concerning phenological traits, QTL were detected on LG10 and LG14 for Sprouting, on LG7 and LG10 for flowering, while veraison showed significant associations with genomic regions in LG11 and LG17, being ripening date significantly associated to LG8, LG11 and LG13. A QTL region on

LG17 was found significantly associated to berry size, productivity traits, phenology stages, and on LG7 and LG13 QTL for flower morphology and flowering date suggesting close linkage or pleiotropic effects. In Tempranillo × Graciano progeny, co-localizations of QTL for flower morphology, seed traits and phenology events were detected in LG3 and LG11.

5. The research conducted in Grenache x Tempranillo hybrids confirmed that smaller berries showed a higher extractability of anthocyanins and phenolic compounds than larger berries, presenting deeper colour. In Grenache x Tempranillo hybrids, wines from small berry size genotypes resulted in sensorialanalysis more astringent and sweeter with higher fruity notes than those from larger berries, which were plain and more alcoholic. These wines achieved consistently higher quality scores than those derived from large berry-genotypes. In Pinot Noir variety, a clear relationship between berry size and the accumulation of anthocyanidins, phenolic compounds was not found, probable because plot and environmental conditions also triggered great differences between clones, especially in berry weight, total acidity and nitrogen compounds.

6. The sensory profiles and quality scores of wines derived from twelve Graciano × Tempranillo selections were obtained in two different years. Based on wine expert's perception, two high quality hybrids, TG8 and TG63 were shown to consistently improve Tempranillo and Graciano due to a higher anthocyanin content, colour intensity, acidity, and positive aroma related to red fruit. Another selection, TG129, appears to be an interesting alternative to Tempranillo and Graciano in the context of global warming due to its late-ripening cycle, high polyphenol content and fruity aroma. Other selections with herbal and dried fruit aroma notes appeared as potential cultivars suitable to satisfy distinct consumer demands.

7. The genetic study of hybrids between traditional wine-grape cultivars proved useful to understand the genetic control of key traits that are linked with wine quality and to select new premium genotypes better adapted to future climate scenarios.

Conclusiones

1. La segregación fenotípica de 12 caracteres de baya, flor y semilla, fueron evaluados en dos poblaciones segregantes de uva de vinificación con Tempranillo como parental común, en un total de 130 y 151 plantas derivadas de un cruce con las variedades Garnacha y Graciano, respectivamente. Catorce parámetros relacionados con la composición del mosto, la productividad y la fenología se estudiaron en cuatro años consecutivos y dos ambientes diferentes en la progenie Garnacha x Tempranillo. Todos los parámetros presentaron segregación transgresiva y variación continua. El efecto del año resultó significativo para todos los rasgos, excepto el peso de la baya, el diámetro de la flor y el peso de la semilla, mientras que el efecto parcela resultó significativo para todos los rasgos analizados. Las estimaciones de heredabilidad en sentido amplio resultaron más altas en la progenie Tempranillo x Graciano, especialmente en los parámetros de flor. Se observaron correlaciones significativas entre caracteres, siendo moderadas entre la longitud y la forma de la baya, y entre la forma de la baya y el pistilo en ambos fondos genéticos. Once genotipos de uva tinta y once de uva blanca fueron preseleccionados en la progenie de Garnacha x Tempranillo en función de la fecha de maduración, el peso del racimo, el rendimiento, la acidez y el grado, que deberán ser analizadas en profundidad en el futuro.

2. Las plantas femeninas mostraron una forma de flor más redondeada, mayor diámetro de flor, un menor número de semillas y un retraso en la floración y fecha de inicio de envero en comparación con las hermafroditas en ambas poblaciones. Se detectó una región QTL en GL2 para la morfología de la flor, parámetros de semilla, productividad y estadíos fenológicos (fecha de floración, envero), confirmando la influencia del sexo en la determinación genética de estos caracteres. El efecto del sexo resultó particularmente significativo en los caracteres de morfología de la flor como la forma de ovario en ambas progenies.

3. Se detectaron regiones QTL significativas para el tamaño de la baya y los parámetros de productividad en GL17 en la progenie de Garnacha x Tempranillo. En la población Tempranillo x Graciano, regiones en GL3 y GL5 resultaron asociadas principalmente al tamaño de la baya y caracteres de semilla. En la progenie Tempranillo x Graciano una región QTL en GL5 para parámetros de baya, semilla y flor cubrió la región del locus *FERONIA* y un QTL en GL18 para rasgos de semilla resultó asociado al locus SDI. Para la morfología de las flores, se identificaron QTL en GL8, GL11 y GL14, siendo el localizado en GL11 el más significativo y estable en los dos años y ambas progenies y proponiéndose un gen candidato VIT_11s0016g03650 con una función asociada a la morfología del polen asociado a dicho QTL.

4. Se detectaron QTL significativos en GL17 y GL18 para rendimiento, índice de fertilidad, número de racimos y peso del racimo. Se detectó un QTL en GL1 para el contenido de azúcar, y para la acidez total se encontraron cinco QTL estables y

altamente significativos en GL4, GL12, GL13, GL14 y GL17. En cuanto a los parámetros fenológicos, se detectaron QTL asociaciones significativas para la fecha de envero con regiones genómicas en GL11 y GL17, y en el GL8, GL11 y GL13 para la fecha de maduración. Una región QTL en GL17 resultó significativamente asociada con parámetros como tamaño de la baya, productividad y fenología, y en los GL7 y GL13 para la morfología de la flor y la fecha de floración, lo que sugiere una estrecha vinculación entre estos caracteres o efectos pleiotrópicos. En la progenie Tempranillo × Graciano, se detectaron co-localizaciones de QTL para morfología de la flor, parámetros de semilla y estadíos fenológicos en los GL3 y GL11.

5. La investigación realizada en híbridos de Garnacha x Tempranillo confirmó que las bayas más pequeñas presentan una mayor capacidad de extracción de antocianinas y compuestos fenólicos que las bayas más grandes, presentando los vinos obtenidos un color más intenso. Los vinos de genotipos de bayas pequeñas resultaron sensorialmente más dulces, astringentes con mayores notas frutales más altas que los procedentes de baya más grande, que eran más planos aromáticamente y alcohólicos. Por ello, consiguieron puntuaciones de calidad más altas por parte de los expertos que los vinos derivados de genotipos de bayas grandes en los dos años de estudio. En la variedad Pinot Noir, no hubo una relación consistente entre tamaño de baya y acumulación de antocianos y compuestos fenólicos, quizá debido a que las condiciones ambientales y de la parcela desencadenaron grandes diferencias entre los clones, especialmente en el peso de la baya, la acidez total y los compuestos aminoácidos.

6. Los perfiles sensoriales y las puntuaciones de calidad de los vinos derivados de doce selecciones de Graciano × Tempranillo se realizaron en dos años diferentes. Según la percepción de los expertos, dos híbridos poseen una alta calidad, TG8 y TG63, mejorando consistentemente a Tempranillo y Graciano debido a un mayor contenido en antocianos, intensidad de color, acidez y aroma relacionado con la fruta roja. Otra selección, TG129, parece ser una alternativa interesante a Tempranillo y Graciano en el contexto del calentamiento global debido a su maduración tardía, alto contenido en polifenoles y aroma afrutado. Otras selecciones con perfiles sensoriales diferentes asociados a aromas herbáceos o fruta seca se presentan como adecuados para satisfacer las distintas demandas de los consumidores.

7. El estudio genético de híbridos entre variedades tradicionales de uva de vinificación demostró ser útil para comprender el control genético de los caracteres clave vinculados con la calidad del vino y para seleccionar nuevos genotipos mejorados y mejor adaptados a nuevos escenarios climáticos.