Genetic diversity in Chilean strawberry (*Fragaria chiloensis*): differential response to *Botrytis cinerea* infection

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Abstract

Botrytis cinerea Pers. is a necrotrophic and polyphageous pathogen economically important on soft fruits and crops producing severe agricultural losses. The Chilean strawberry, *Fragaria chiloensis* (L.) P. Mill. is one of the wild parents of commercial strawberry and has the capacity to tolerate the infection from the pathogenic fungus. A study was performed to make a genetic analysis of wild and cultivated individuals collected from three different regions of Central South Chile and compare the *Botrytis* infection performance. The dominant ISSR markers were used to generate the genetic fingerprinting and the native samples were contrasted against the commercial *Fragaria × ananassa* Duch. The samples from Contulmo presented the lowest diversity within population and the highest diversity between populations, but at the same time showed the highest tolerance to *Botrytis* infection. Infected leaves and fruits from *F. × ananassa* showed 3.6% of the area covered by the fungus, three days after inoculation, while the plants from Contulmo showed 3.3% of the area covered by the pathogen, five days after inoculation. The plants from Contulmo were classified as highly tolerant (HT), tolerant (T), moderately tolerant (MT), and susceptible (S) where 50% of plants belonged to the group of tolerant. The results, highly significant ($p \leq 0.01$), indicate that the samples of *F. chiloensis* from Contulmo are more genetically homogeneous and can be classified as tolerant to the infection by *Botrytis cinerea*.

Additional key words: grey mould, native strawberry, resistance.

Resumen

Diversidad genética en frutilla chilena (*Fragaria chiloensis*): respuesta diferencial a la infección con *Botrytis cinerea*

Botrytis cinerea Pers. es un patógeno necrótrofo y polífago de importancia económica en cultivos y frutales, ocasionando importantes pérdidas en agricultura. La frutilla chilena, *Fragaria chiloensis* (L.) P. Mill., antecesor silvestre de la frutilla comercial, tiene la capacidad de tolerar la infección de una serie de hongos patogénicos. En este estudio se efectuó un análisis genético de plantas de frutilla chilena silvestres y cultivadas, recolectadas en tres diferentes regiones de la zona centro sur de Chile, en cuanto a su comportamiento frente a la infección de *Botrytis*. Los marcadores dominantes ISSR fueron utilizados para generar el patrón genética y las muestras de frutilla chilena fueron comparadas con las de *Fragaria × ananassa* Duch. Las muestras de Contulmo mostraron la más baja diversidad dentro de la población y la mayor entre poblaciones. Al mismo tiempo presentaron la mayor tolerancia a la infección con *Botrytis*. Las hojas y frutos de *F. × ananassa,* mostraron, tres días después de la inoculación, un 3,6% de su área cubierta por el patógeno. Por el contrario en las plantas recolectadas en Contulmo la infección fue más lenta, observándose un 3,3% del área cubierta por el patógeno, cinco días después de la inoculación. Las plantas de Contulmo fueron clasificadas como altamente tolerantes, tolerantes, moderadamente tolerantes y susceptibles; un 50% de los individuos se ubicó en el grupo de tolerantes. Los resultados, altamente significativos ($p \leq 0,01$), indican que las muestras de *F. chiloensis* colectadas en Contulmo son genéticamente más homogéneas y pueden ser clasificadas como tolerantes a la infección con *Botrytis cinerea*.

Palabras clave adicionales: fresa autóctona, pudrición gris, resistencia.

Introduction

The Chilean strawberry, *Fragaria chiloensis* (L.) P. Mill, is the mother of the octoploid commercial strawberry (*Fragaria × ananassa* Duch.). It grows naturally in the Andes and the Coastal mountains (Hepp and Vera, 1990), and has been domesticated over the last 1000 years by the Chilean mapuches (Hancock *et al*., 1999). *F. chiloensis* is characterized for having an intense aroma, and a remarkable pest and disease resistance spectrum, along with other characteristics that makes it an attractive species for plant research, such as, manipulation tolerance and long post-harvest life (Potter and Dale, 1994; Hancock *et al*., 1999; Lavin and Maureira, 2000). Indeed, research on this species has been focused on its development as a new exotic berry, as well as for genetic improvement of *F. × ananassa* (Hancock *et al*., 2001; Retamales *et al*., 2005).

One of the most important postharvest problems in commercially cultivated strawberry is gray mold which it is caused by the fungus *Botrytis cinerea* Pers. This pathogen is widely distributed, affecting more than 200 species mostly of economic importance (Deighton *et al.*, 2001). The fungus has the capability to develop infection at temperatures from 0°C to 28°C, even though, the growth rate of the fungus is slower at lower temperatures. This characteristic allows the pathogen to produce important fruit losses during post-harvest, transport and storage.

In Chile, gray mold constitutes one of the main pathological problems in several fruit species such as, grapes, apples, pears, raspberries, blueberries, and vegetables like tomato (Apablaza, 2000; Latorre, 2004). This fungus probably causes the highest fruit losses in strawberry production, and it has been estimated that in organic commercial crop the production can be reduced by 55% (Daugaard, 1999).

Botrytis has the ability to colonize different parts of the plant and in addition, its fast life cycle in the field (conidia germination, colonization and spore formation) makes it difficult its control. The disease pressure can be reduce either by some cultural practices such as removing the crop stubbles which can hold mycelium and sclerotia of the pathogen that can be disseminated by soil movement (Auger, 1983) or by using resistant genotypes. However, there is no much information available about specific genes that can confer plant tolerance or resistance to the pathogen. Also, no much research has been developed studying the genetic, biochemical or morphological events related with the higher resistance presented by this species.

Molecular markers have been useful to report the genetic diversity of populations and species (Bornet and Branchard, 2001; Arnau *et al*., 2002; Korbin *et al*., 2002). Several strategies can be used to generate the genetic fingerprinting like amplified fragment length polymorphisms (AFLP) or conventional micro satellites (Bornet and Branchard, 2001; Fossati *et al*., 2001; Korbin *et al*., 2002; Hadonou *et al*., 2004). A general approach has been the use of inter simple sequence repeat (ISSR) primers, which are generally complementary dinucleotide simple sequence repeat motifs that are abundant in genomic DNA, containing one to three base «anchor» to ensure the primer anneals to either the 5' or 3' end of the ISSR (Charters *et al*., 1996; Lanham and Brennan, 1998). This marker has been successfully used in several species (Herrera *et al*., 2002; Herrera *et al*., 2005; Carrasco *et al*., 2007).

According to this, the objective of this study was to determine the genetic diversity of three groups of *F. chiloensis,* compared to *F. × ananassa* and to see the response of both species to the infection with *B. cinerea*.

Material and methods

Plant material

Individual plants of *F. chiloensis* spp c*hiloensis* were collected in Contulmo, Bio-Bio Región Chile (38°04'8.6"S, 73°14'2.96"W), Chillán (36°54'34.21"S, 71°28'41.46"W) and Vilches (35°36'31.87"S, 71°03'38.81"W). The samples in the last two locations were obtained from wild populations growing in the woods. On the other hand, the plants from Contulmo were taken from commercial fields, which represented macro propagated material done by farmers. Individual plants of *F. × ananassa* cv. 'Chandler' were also obtained from Contulmo, Bio-Bio Region, Chile, and used as control in the different PCR reactions performed.

Pathogen isolates

Two of the isolates of *B. cinerea* used in this study corresponded to the collection of the Plant Pathology Laboratory of Universidad de Talca, and were obtained from infected fruit tissue of *Vitis vinifera* and *Malus pumila*. The other two were provided by the IBVB, Uni-

versidad de Talca, and isolated from *F. × ananassa* and *F. chiloensis* infected fruits. Finally a fifth isolate, also obtained from *Vitis vinifera* was kindly provided by the Instituto de Investigaciones Agropecuarias (INIA), La Platina, Santiago, Chile.

Culture media

The different isolates of *B. cinerea* were grown in 2% potato dextrose agar (Hébert *et al.,* 2002). Spores from each isolate were taken, resuspended altogether in sterile water and placed on a Petri plate containing the same culture media. This mixture (stock plate) was used as inoculum to ensure the plant infection. This was cultivated in the same media at 25°C in a growth chamber until the whole Petri plate area was covered.

Experiment design

The experiment was conducted in a completely random design. Each treatment has 10 replicates of one plant. Daily measurements were performed until 100% of the fruit was infected by the fungus. The evaluations were made determining the weight of each fruit (100%) and the weight of the infected area.

DNA extraction from the fungus and infected leaves

The DNA from the *Botrytis* isolates was extracted according to the methodology described by Lee and Taylor (1990) with some modifications. From infected leaves the DNA was extracted following a modified CTAB method described by Herrera *et al*. (2002). The DNA concentration was determined measuring the absorbance at 260 nm in a spectrophotometer.

Genetic fingerprinting

For the genetic analysis of *F. chiloensis* (Contulmo, Chillán and Vilches groups) and *F. × ananassa*, the DNA was amplified using a set of primers ISSR 100/8 (888, 811, 825, 836, 834, 855). The primers were obtained from the Biotechnology Laboratory, University of British Columbia, Canada.

The *B. cinerea* isolates were identified using specific primers (Rigotti *et al*., 2002). The fungus characterization was complemented by the detection of the transposable elements *Boty* and *Flipper*. The primers described by Ma and Michailides (2005) were used for *Boty*, and we designed *Flipper* transposable element. Additionally ISSR (inter simple sequence repeat) molecular markers were used to determine the predominant isolate in the mixture of the fifth isolates used as inoculum. All PCR products were separated by electrophoresis in 2.0% agarose gels run in TAE 1X. PCR products were visualized by UV fluorescence after staining with ethidium bromide.

Data analysis

The DNA profiles were recorded as presence (1) and absence (0) of amplification products and the results assembled in a data matrix table. This data was used to obtain all the genetic parameters to evaluate the samples similarity/dissimilarity. To obtain the dendrogram, the un-weighted pair-group algorithm based on arithmetic averages (UPGMA) (Nested cluster, SAHN, NTSYS-pc softward, 2.11N version) was used. The genetic differences (PhiPT and pairwise PhiPT) among populations were estimated using an analysis of molecular variance (AMOVA) (Excoffier *et al.,* 1992), (GenAlEx6 full pack and Arlequin program, version 2.0). PhiPT was calculated as the proportion of the variance among populations, relative to the total variance. PhiPT represents the correlation between individuals within a population, relative to the total and it is analogous to fixation index (*Fst*) for measuring population differentiation when the data are haploid or binary (Maguire *et al*., 2002).

Response of *Fragaria* to infection with *Botrytis cinerea*

The plants collected from three locations and *F. × ananassa*, were infected by placing a piece of agar of 0.5 cm diameter, containing *Botrytis* mycelium on the leaf surface. The inoculum was located in the middle of the leaf, covered by a bandage and after that, 250 µL of sterile water were added. Finally, leaves were placed inside a plastic bag to provide adequate conditions for the pathogen infection and maintained at room temperature.

Three leaves per plant randomly chosen were marked and inoculated with the pathogen and observed every day until 100% of the leaf area was necrotic. The

plants were classified according to their response to the fungus infection using a severity scale proposed by Moraga (2003) and a field monitoring table developed by Polack and Mitidieri (2005). The area of infection was determined using lineal scale.

Fruit inoculation

The fruit infection was performed following the same procedure described for leaf infection with few modifications. Mature fruits (or stage 4 according to Figueroa *et al.,* 2008) were used. To eliminate any possible inoculum coming from the field, the fruits were previously disinfected with 0.5% hypochlorite solution. Then, sepals were cut. The fruit was placed in the center of a plastic container and the progress of the infection was recorded daily. Inoculated fruits were incubated at $24 \pm 2°C$. The area of infection was determined using lineal scale.
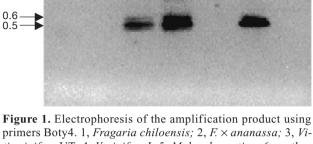
Results

Genetic diversity analysis from fungus isolate

The genetic diversity of both the fungus and plants was performed in order to establish the homogeneity before making the plant infection. In terms of the fungi, DNA was extracted from all *B. cinerea* isolates and a 750 bp was observed in all samples after PCR amplification with the marker described by Rigotti *et al.* (2002). In the case of *Boty* transposable elements a fragment of 510 bp was amplified for the samples collected from *V. vinifera* INIA, *V. vinifera* UTalca and the mix stock (Fig. 1). Nevertheless, this fragment was not detected for the fungus isolates from *F.* × *ananassa, F. chiloensis* and *Malus domestica*. On the other hand, all samples showed the amplified fragment with the expected size of 800 bp which is associated to *Flipper* transposable elements. This indicates that the predominant fungus in the mixture is genetically closer to the one containing *Boty* transposable element and obtained from grapevine.

Genetic diversity within plants

Four out of eight ISSR primers were selected and used for PCR amplification, 49 bands were obtained

Figure 1. Electrophoresis of the amplification product using primers Boty4. 1, *Fragaria chiloensis;* 2, *F.* × *ananassa;* 3, *Vitis vinifera* UT; 4, *V. vinifera* I; 5, *Malus domestica;* 6, mother stock plate; C-, negative control.

for all the populations ranging from 500 bp to 1,200 bp with 100% repeatability, being 30 polymorphic fragments. The UPGMA analysis showed a genetic similarity among the groups studied, considering fragments at the same weight as located at the same locus. The dendrogram obtained based on the fingerprinting generated shows a clear separation among the groups studied where clearly three groups can be identified (Fig. 2). The five samples from Contulmo presented a narrow genetic variation, which varies between 90 to 100%, indicating that the samples are genetically close (indicated with a black circle, group 1). Three samples from Vilches also are grouped in this main branch but in a different small group with a similarity of 80%. For the Vilches and Chillán group the genetic variation ranges between 68 to 85% (group 2). Even though, this cluster analysis shows that the samples are not as homogeneous as the one obtained from Contulmo.

F. × *ananassa* showed a higher genetic diversity to the other *F. chiloensis* samples (50%), nevertheless, the similarity among individual plants for the commercial strawberry varied between 60 and 90% indicating that the group of *F.* × *ananassa* is less related to the others (group 3). This result is confirmed with statistical analysis (AMOVA), with a variance of 36% within groups and 64% among them. This indicates that there is a higher genetic variation between the *F. chiloensis* groups from Contulmo, Chillán, Vilches and the control *F.* × *ananassa,* compared to the one observed within each group. At the same time, a high genetic variability between groups was found considering PhiPT values and also showed in the dendrogram (Fig. 2). The Nei
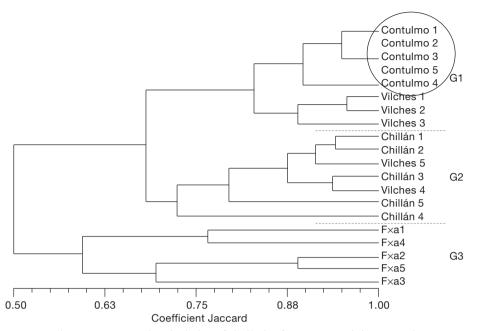
Figure 2. Dendrogram representing the index of similarity for *Fragaria chiloensis* and *F. × ananassa* plants based on the polymorphism generated by primers (825-834-836-855).

genetic identity and distance (Table 1) showed that samples from Contulmo and Vilches have higher similarity and identity, with values of 0.05 and 0.951 respectively. This value was corroborated by PhiPT where samples from these two places had a value of 0.220. Most importantly, *F. × ananassa* showed the lowest value (ranging from 0.352 to 0.23) when it was compared to the other groups, being the population with the most different values.

Plant and fruit response to the infection with *Botrytis cinerea*

Different inoculation strategies were used in order to generate the infection. The best results were obtained by patching leaf and fruits with the inoculate (data not shown). The pathogen was able to infect the plants and an increase in the infected area over the days was observed. The progress of infection was measured during summer 2006 (February). The first groups of plants showing necrotic lesions (2-3.6 % of the leaf area covered) were those from Chillán, Vilches, and the commercial *F. × ananassa* (Fig. 3). Ten days after inoculation, 100% of the inoculated leaf area was necrotic. The samples from Contulmo showed the first symptoms with necrotic lesions covering 3.3% of the leaf area only five days after inoculation. Thirteen days later the Contulmo group showed 100% of the inoculated leaf necrotic.

The same infection pattern was observed when fruits were infected (data not shown). A lesion affecting 2.3%

Table 1. Nei's genetic distance between Contulmo, Chillan, Vilches and *F. × ananassa* (F × a) populations using GenAlEx6 full pack program

	Pair Nei genetic distance				Pair Nei genetic identity			
	Contulmo	**Chillán**	**Vilches**	**F × a**	**Contulmo**	**Chillán**	**Vilches**	**F × a**
Contulmo	0.000	—	—	—	1.000	—	—	—
Chillán	*0.195	0.000	—	—	*0.823	1.000	—	—
Vilches	*0.050	*0.171	0.000	—	*0.951	*0.843	*1.000	-
F × a	*0.352	*0.272	*0.345	0.000	*0.703	*0.762	*0.708	1.000

Analysis was carried out using pairwise population matrix of Nei genetic identity. The probability values based on 100 permutations are shown above diagonal. *$P \leq 0.01$.

Fragaria chiloensis: **Contulmo**

| Day 1 | Day 3 | Day 5 | Day 7 | Day 9 | Day 11 | Day 14 |

Fragaria chiloensis: **Chillán**

| Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 |

Fragaria chiloensis: **Vilches**

| Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 10 |

Fragaria × ananassa **cv Chandler**

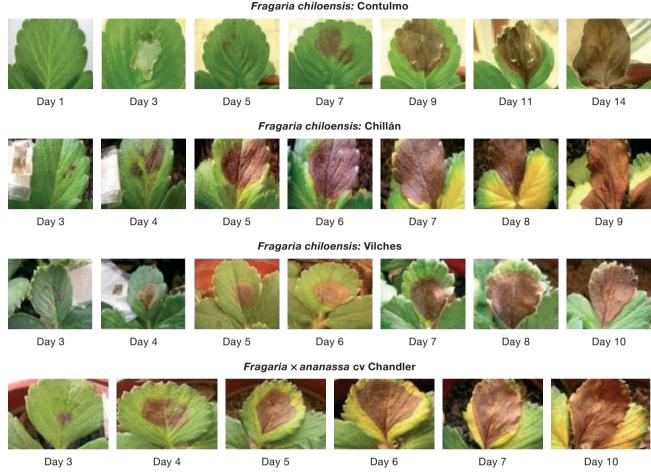| Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 10 |

Figure 3. *Botrytis cinerea* infection progress in *Fragaria chiloensis* (Vilches, Chillán and Contulmo) and *F. × ananassa* leaves.

of the fruit weight was observed one day after inoculation in the fruits from *F. × ananassa*. The fruits from Contulmo showed small lesions that represented only 1.05% of the fruit weight, only three days after inoculation. Fruits presented 100% of their tissue covered by *B. cinerea,* six days after inoculation in the case of *F. × ananassa*, and nine days after inoculation in those collected from *F. chiloensis* (Contulmo) (Fig. 4).

Contulmo plants response to *Botrytis cinerea* infection

Plants from Contulmo were infected with *B. cinerea* during winter 2006. Evaluations were made daily until all plants presented 100% of their leaf area infected. As it is reported in Table 2, statistical differences ($p \leq 0.01$) were found for the percentage of infected leaf area by the pathogen. Some plants (17, 19 and 22) can be classified as highly tolerant with an average of infected leaf area of 25%. On the other hand, two plants (numbered 8 and 10) presented 82% and 95% of necrotic leaf area, being classified as highly sensitive to *B. cinerea*. In these plants the pathogen developed faster, with the presence of necrotic lesions four days post-inoculation, earlier than in the other plants.

No plants of this group were classified as highly resistant or resistant. However, an important number were intermediate in terms of infection level. The fungus covered 26 to 76% of leaf area 13 days post-inoculation. According to these results, these individuals were classified as tolerant to moderately tolerant.

Discussion

Identification of *Botrytis cinerea*

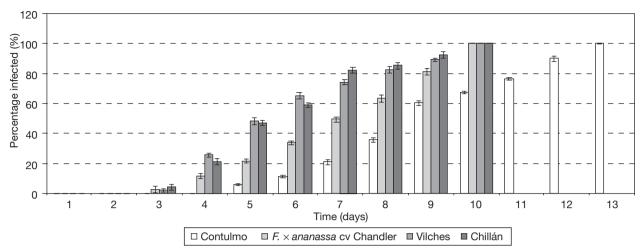Several studies have been carried out in order to detect genetic differences among isolates of pathogenic

Figure 4. Percentage of *Botrytis cinerea* infected leaf area in *Fragaria chiloensis* (Contulmo, Chillán and Vilches) and *F. × anannasa* plants.

Table 2. Contulmo plants classification in response to *Botrytis* infection, according to the percentage of infected leaf area after 13 days post inoculation

Infection response classification	Scale (in infection percentage)	Plant number	Covering percentage with *B. cinerea*[1]
Highly resistant (HR)	0	—	—
Resistant (R)	1-10	—	—
Highly tolerant (HT)	11-25	17	24.9[a]
		19	25.3[a]
		22	25.3[a]
Tolerant (T)	26-50	18	26.79[b]
		21	28.2[c]
		13	34.7[d]
		24	37.6[e]
		16	40.60[f]
		11	41.93[g]
		1	42.3[gh]
		6	42.8[hi]
		4	43.4[ij]
		14	44.03[j]
		23	48.6[k]
		15	49.9[l]
Moderately tolerant (MT)	51-75	7	50.9[m]
		9	51.1[m]
		20	53.33[n]
		12	53.7[n]
		2	57.7[o]
		3	60.8[p]
		5	75.1[q]
Susceptible (S)	76-100	8	82[r]
		10	94.6[s]

[1] Different letters indicate significant difference according to Duncan (*p* = 0.05).

fungus species, early diagnosis of a particular disease in a specific crop, determine the predominant pathogen strains and establish levels of variability (Giraud *et al.*, 1997, 1999; Martínez *et al.*, 2003). In this research, isolates of *B. cinerea* were identified using PCR, where all isolates presented the 0.75 kb band specific for Botrytis (Rigotti *et al.*, 2002). Complementarily, specific primers were used to detect the presence of the transposons *Boty* and *Flypper*. In the isolates from *F. × ananassa*, *F. chiloensis* and *M. domestica*, the transposable element *Boty* was not detected, however, the transposon *Flipper* was present. Muñoz *et al.* (2002) indicated that there were no isolates containing the transposable element *Flipper*, nevertheless, isolates of fungus with this transposon was found in strawberry (Albertini *et al.*, 2002), as was reported years later by Milicevic *et al.* (2006). On the other hand, the fungus with the transposable element *Flipper* found in *M. domestica* has not been reported in strawberry, so this is the first time reporting this transposon in *Botrytis* populations.

In the isolates from *V. vinifera* INIA, *V. vinifera* Universidad de Talca and the stock plate, it was possible to determine the presence of both transposable elements, which have been described previously in different fruit species (Giraud *et al.*, 1997, 1999; Muñoz *et al.*, 2002; Ma and Michailides, 2005). Both elements were present in the stock plate, which indicates that one of the isolates from *Vitis* was highly competitive being the predominant in the plate, belonging to *V. tramposa*. The analysis with ISSR supports this conclusion, indicating a high similarity of the stock plate (56%) with the isolate from *V. vinifera,* Universidad de Talca.

Analysis of the plants genetic diversity

Molecular markers have been developed for the identification of individual plants allowing the characterization of germoplasms (Bhat *et al.*, 1997). However, it is important to notice that this technique may evaluate the polymorphism when fingerprinting strategy is applied (Bornet and Branchard, 2001; Culley and Wolfe, 2001; Arnau *et al.*, 2002). ISSR molecular markers appear as an excellent tool to study polymorphism in the four *Fragaria* groups included in this experiment. The results agreed with previous work done in the commercial strawberry (Arnau *et al.*, 2002) and Chilean strawberry (Carrasco *et al.*, 2007). It was possible to discriminate between wild groups (G2) collected in the Andes Foothills (Chillan and Vilches) and the commercial group *F. × ananassa* Chandler (G3). In the wild plants a higher degree of diversity could be observed, which it can be explained because this plants come from natural areas where a higher genetic variability is expected. On the other hand, we observed a high degree of similarity in the Contulmo group, which can be explained by the fact that plants were vegetative propagated. Finally, for *F. × ananassa* (G3) the similarity degree was between 60 and 100%, similar to the values found by Arnau *et al.* (2002), indicating that the origin of the plant material is genetically wider, and in the case of Chilean strawberry, our results differ from those obtained by Carrasco *et al.* (2007). *F. chiloensis* ssp. *chiloensis f. chiloensis* and *F. chiloensis* ssp. *chiloensis f. patagonica* were compared showing a genetic diversity of 89.6% at species level. In our study (*F. chiloensis* ssp. *chiloensis f. patagonica*) this value was 67.7% which is still in the range expected. The differences with the work developed by Carrasco *et al.* (2007), can be explained by the number of plants they studied (22 of *chiloensis* form*,* and 194 of *patagonica* form) which represent the natural area of Chilean strawberry distribution. In this study the number of plants and the locations were reduced. The percentage of polymorphism also could be affected by the fact that the Contulmo plants were clones due to the propagation system employed in the field.

The results obtained with the AMOVA support the similitude analysis, because the dendrogram showed the same high similarity among the Chilean strawberry groups. Also, PhiPT total, pair PhiPT and Nei's genetic distance agreed the statistic differentiation between the different groups. In this sense, samples collected from Contulmo and Vilches showed the higher similarity and *F. × annanasa* 'Chandler' showed the major genetic distance from the rest of the groups. Also, the markers used showed a high efficiency in the analyzed polymorphisms, and represent the difference described between groups. The variance molecular analysis reported by Carrasco *et al.* (2007) showed a percentage of variation between the groups of 14.9%. In our case, 64% of the variation explains the difference between the groups. This value is expected due to the inclusion of a different species which was used as control throughout this study. As conclusion, the plants from the area of Contulmo were suitable as model population for any research study due to its high genetic homogeneity.

Response to *Botrytis cinerea* infection in leaves and fruits

The infection methodology developed in this study was efficient and reproducible, making possible to study the progression of the disease. According to this, the same technique could be used to inoculate other necrotrophic fungus like *B. cinerea* in their hosts.

We could observe important differences in the infection process for both, inoculated leaves and fruits. The fruit infected area in *F. × ananassa*, increased rapidly from the third day after inoculation, ranging from 5.41% (2nd day) to 38.46% (3rd day). A completely different situation was observed in the fruit from the plants collected in Contulmo where the increase in the infected area was slower. Different Chilean strawberry populations have been described as resistant to several diseases (Hancook *et al*., 1999; Daubeny*,* 2003), however there were not previous reports showing this tolerance to *B. cinerea.* Nishizawa *et al.* (2003), made a first approach when they observed that *F. chiloensis* fruits*,* seven days after storage presented only a 7% incidence of gray mold. Even though, our research gives different results, this can be explained by the different methodology used. Nishizawa *et al.* (2003), did not inoculate the fruits, but our results indicate that in the fruits from the Contulmo plants the pathogen grows slower taking more time in developing symptoms (necrotic lesions). According to this, the *F. chiloensis* plants presented a certain tolerance degree to *B. cinerea* infection*.*

The results obtained in our experiments agree with the statement of Daugaard (1999). He indicates that there is no commercial strawberry cultivar resistant to

B. cinerea, but there are genotypes with different levels of susceptibility. However, Chandler *et al.* (2000) mentioned that cv. 'Florida Festival' (*F. × ananassa*) is less susceptible to *B. cinerea* than cv. 'Sweet Charlie'.

The infective ability of a particular *B. cinerea* isolate depends mostly of the host, and the adaptability of the pathogen to a particular environmental condition. The fact that the isolates used in this study where obtained from different commercial crops, it give them a natural infective capacity, which is necessary for the fungus to complete its life cycle. Clearly, the mixture of isolates used in the experiments to infect the plants was able to produce disease and the individuals that resulted tolerant constitute the base for the breeding program of Chilean strawberry. Statistical analysis was made 13 DPI (days post inoculation), but the observations continue until 30 DPI (information not shown), where the leaf area of the highly tolerant plants were not still completely covered by the pathogen. On the contrary, the sensitive plants presented 15 DPI, 100% of their leaf area necrotic due to the action of the pathogen. According to their differential response to *Botrytis* infection, the *F. chiloensis* plants from Contulmo, can be separated in four different groups: Highly tolerant, tolerant, moderately tolerant and sensitive. Most of the plants had necrotic lesions five days after inoculation. The samples from Contulmo were classified as tolerant and moderately tolerant, but just few resulted as highly tolerant or susceptible.

Acknowledgements

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