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Short communication. Collection and characterisation of a population of *Triticum boeoticum* Boiss., a wild wheat species not previously found in the Mediterranean western region

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Abstract

A population of wild wheat was collected in July 2010 in an abandoned area near Madrid, Spain. This zone possesses a high botanical biodiversity together to a particular type of soil termed "green clays". A sample of wheat was collected, multiplied and characterised for several agro-morphological traits and glutenin subunits. The 2n chromosome number revealed that it was a diploid wheat species, and characterisation data indicated that the accession was *Triticum boeoticum* Boiss. This species probably arrived as a weed of the einkorn crop grown in the zone until at least the first half of s. XIX. The specific edaphic and climatic characteristics of the habitat and the fact that there were no references so far about this species in the Mediterranean western region make this acquisition very valuable for wheat improvement. The new accession is conserved at the National Plant Genetic Resources Centre and a herbarium sheet has been deposited in the Royal Botanic Garden in Madrid.

Additional key words: cereal; germplasm; glutenins; Spain.

Resumen

Comunicación corta. Recolección y caracterización de una población de *Triticum boeoticum* Boiss., una especie de trigo silvestre no encontrada previamente en la región mediterránea occidental

En julio del 2010 se recolectó una población de trigo silvestre en una zona abandonada cerca de Madrid, España. Esta zona posee una biodiversidad botánica elevada y un tipo de suelo muy peculiar denominado "arcillas verdes". Se recogió una muestra de trigo y se multiplicó y caracterizó para varios caracteres agro-morfológicos y subunidades de gluteninas. El número cromosómico 2n de las semillas demostró que es una especie diploide de trigo y los datos de caracterización indicaron que es *Triticum boeoticum* Boiss. Esta especie llegó probablemente como mala hierba del cultivo de escaña que se producía en la zona hasta al menos la primera mitad del s. XIX. Las características edáficas y climáticas del lugar y el hecho de que no haya referencias hasta ahora de esta especie en la zona oeste de la región Mediterránea aumentan el valor de esta adquisición para la mejora del trigo. La nueva accesión se conserva en el Centro Nacional de Recursos Fitogenéticos y se ha depositado una hoja de herbario en el Real Jardín Botánico de Madrid.

Palabras clave adicionales: cereal; España; germoplasma; gluteninas.

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This work has 1 Supplementary Figure that does not appear in the printed article but that accompany the paper online.

Abbreviations used: CRF-INIA (Plant Genetic Resources Centre - Spanish National Institute for Agricultural and Food Research and Technology); HMW (high-molecular-weight).

Diploid wheats comprise three species: two wild, *Triticum urartu* Tum. and *Triticum boeoticum* Boiss., and the cultivated *Triticum monococcum* L. The three species are closely related to the A genome of polyploidy wheats (Kihara, 1924). The wild species are useful genetic resources as donors of genes for valuable traits for wheat improvement, such as disease resistance, stress tolerance and gluten quality (Gale & Miller, 1987; Appels & Lagudah, 1990; Rogers *et al.*, 1997). Most of the wild einkorn was first spread through the northern part of the Fertile Crescent (Mac Key, 2005) but their distribution area was narrower than that of the cultivated einkorn. So, some populations of diploid wild wheats have been found in the countries of the eastern Mediterranean as Greece, Bulgaria, Lebanon, Syria and Turkey (Kimber & Feldman, 1987) but there is none reference for the western countries as Italy, France, Spain and Portugal. In contrast, T. *monococcum* was cultivated in all Mediterranean countries in the past. On the other hand, the diploid wild wheats presented a wide range of natural habitats, from the sea level in Macedonia to 2000 m in Iran and Iraq, in soils hardly suitable for cultivation, and on virgin and fallow land (Damania, 1998; Mac Key, 2005). High intra-specific variation with different agromorphological types has been also described (Dorofeev, 1968; Filatenko & Hammer, 1997). The objective of this work was to collect, identify and characterise a wild wheat recently found in Spain and not previously described.

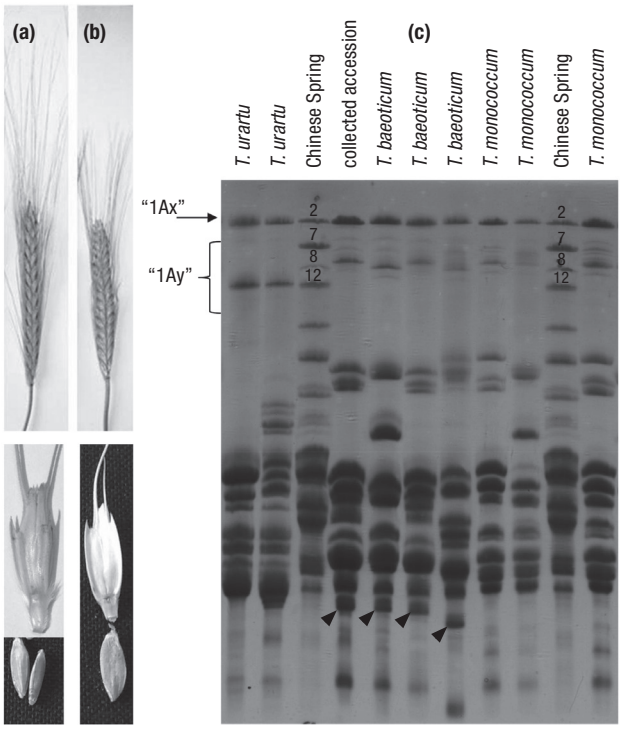

Figure 1. Spikes, spikelet and grains of (a) the collected accession, (b) a Spanish cultivated einkorn, (c) Glutenin electrophoresis of accessions of *T. urartu*, 'Chinese Spring' bread wheat control, the collected wheat accession, *T. boeoticum* and *T. monococcum.* The regions of "1Ax" and "1Ay" subunits in diploid wheats are indicated. Arrowheads show faster subunits present in some *T. boeoticum* accessions. The subunits of the bread wheat have been numbered according to Payne & Lawrence (1983).

In July 2010, a population of wheat with brittle rachis has been located in an abandoned area in the locality of Coslada (Madrid), Spain. The geographic parameters were 40º 26'14'' N of latitude, 3º 33' 55'' W of longitude and 609 m of altitude. The collecting area occupies 10 ha of difficult access, isolated by factory premises and the train rails. Suppl. Figs. 1 b and c (pdf) shows two photographs of the zone at the present time. The topographic map of the region of 1875 reported that the zone was cultivated, and the aerial image of the zone in 1946 shows the crop plots (Suppl. Fig. 1a [pdf]). In the 70's the crops were abandoned and only some grazing continued till 2005. The wheat population was located in an area of about 1,400 m^2 (Suppl. Fig. 1b [pdf]). The plants had brittle rachis, so most of the spikelets were collected from the soil although some spikelets remained in the plant (Suppl. Fig. 1d,e,f [pdf]). The zone has a Mediterranean Continental climate typical of the centre of Spain, with cold in winter and hot and drought in summer. Climatic parameters from a weather station 2.5 km far from the collecting zone were 765.90 mm for ETP, 404.40 mm for annual rainfall and 14ºC for annual temperature (38-year mean taken since 1961). The soil (Suppl. Fig. 1e [pdf]) termed as "Green Clays" is uncommon in Spain (Martínez Labarga, 2010). It is composed almost exclusively by phyllosilicates and small quantities of quartz and feldspar. The greatest part of the phyllosilicates is magnesium smectite (saponite) of high purity (García Romero et al., 1990; García Romero, 2004). These soils strongly retain the nutrients and water, so they are fertile but the available water for plants is low. The wheat was in a sloping depression where the humidity was higher. The pH of the soil is alkaline which agrees with wild einkorns have affinity for basic soil types like marls, clays and limestones (Zohary & Hopf, 2000). The collecting site possesses a high biodiversity and more than 150 species have been catalogued

by Martínez Labarga (2010). Other wild species of the *Triticeae* tribe, as *Aegilops lorentii* Hochst, *Aegilops geniculata* Roth and *Aegilops triuncialis* L., were collected and maintained in the CRF-INIA. Dorofeev (1968) reported that wild einkorns were often observed growing together with plants belonging to *Ae. biuncialis* Vis. (synonym: *Ae. lorentii* Hochst.) and *Ae. triuncialis* L. It was also found one of the greatest populations in the world of *Cynara tournefortii* Boiss. & Reut., and the best Iberian populations of *Malvella sherardiana* (L.) Jaub. & Spach and *Linaria caesia* (Pers.) F. Dietr. (Martínez Labarga, 2010). *Cynara tournefortii* and Malvella sherardiana are catalogued as CR (critically endangered) and VU (vulnerable), respectively in the IUCN Red List of threatened plants (Moreno, 2008). Other dicotyledonous species not located before or infrequent in the Madrid province were discovered, like *Teucrium spinosum* L. and *Convolvulus humilis* Jacq., *Klasea flavescens* (L.) Holub subsp. *flavescens, Scolymus maculatus* L. or *Ziziphora hispanica* L. Traditional agricultural and livestock uses and, in particular, the proximity of the enclave to several cattle tracks has surely contributed positively in the richness in species (Martínez Labarga, 2010).

The chromosome number of the collected seeds of wheat showed that it was a diploid species (2n = 14). The ears collected had brittle and hairy rachis, and two awns and two grains (one of them smaller and darker) per spikelet (Suppl. Fig. 1f [pdf] and Fig. 1a). Since cultivated einkorn usually has one awn and one grain per spikelet (Fig. 1b), and weaker rachis hairiness and brittleness (Filatenko *et al.*, 2002) these data point to the wild wheats *T. urartu* or *T. boeoticum*. In some cases both wild species are very similar morphologically, but they can be distinguished with some molecular markers as glutenins and short-sequence DNA repeats (SSRs) (Waines & Payne, 1987; Hammer *et al.*, 2000). So, High Molecular Weight (HMW) glutenin subunits were analysed to distinguish between *T. boeoticum* and *T. urartu.* Fig. 1c shows the glutenin pattern of the collected accession, together with the patterns of other accessions of *T. urartu* and *T. boeoticum* conserved at the CRF-INIA. Three Spanish accessions of *T. monococcum* were also included. Comparisons with *T. urartu* indicated that the collected accession had less prominent "1Ay" subunits, typical of *T. boeoticum*, and different from the major one common in *T. urartu* (Waines & Payne, 1987; Hu *et al.*, 2012). Also, subunit "y" in our sample was clearly slower than subunit 12 of 'Chinese Spring', whereas the mobility was similar in *T. urartu*. Subunit "1Ax" of the collected accession was slightly faster than subunit 2 of 'Chinese Spring'. According to Waines & Payne (1987) we can conclude that the protein genotype of this new accession did not corresponded to *T. urartu* but to *T. boeoticum*. The range of mobilities of the subunits of the Spanish cultivated einkorns and the other *T. boeoticum* accessions were also in agreement with the ranges obtained by Waines & Payne (1987) and Hu *et al.* (2012). It can be observed that all the *T. boeoticum* analysed, ours included, had a prominent faster band (►) not present in the cultivated einkorn.

Table 1. Agro-morphological data of the collected accession

Character	Value
Grow habit	Prostrate
Stem hairiness	In line
Top nude stem hairiness	High
Leaf hairiness	Very hairy
Spike length (cm)	12.5
No. spikelets per spike	34.8
Days to heading	183
Days to maturity	225
Plant height (cm)	116.6
Lemma awn barbs	Rough
Lemma awn colour	White
Awn length (cm)	11.9
Spike density	Dense
Glume hairiness	Hairless dull
Glume colour	White
Seed colour	Brown

A sample of the collected seeds was sown for multiplication and agro-morphological characterisation during 2011 in the experimental fields of the CRF-INIA in Alcalá de Henares, 20 km far from the collecting site. Table 1 shows the agro-morphological data of the accession. The variables were measured according to IBPGR (1985) and Ruiz *et al.* (2007). Some agro-morphological traits such as very high hairy leaf, and long and less dense spike were clearly different from the Spanish accessions of cultivated einkorn (Fig. 1b), which had hairless leaf, and shorter and very dense spikes (Ruiz *et al.*, 2007). Agro-morphological data also indicated that the sample was different from other *T. boeoticum* accessions maintained at the CRF-INIA. Two main eco-geographic races of *T. boeoticum* have been recognized. One is a relatively small one-seeded type typical of the cooler Balkans and western Anatolia usually referred to as *T. boeoticum* subsp. *aegilopoides*. The other race is a larger two-seeded race

present in the warmer dry summer areas of southern Turkey, Iraq and Iran commonly referred as *T. boeoticum* subsp. *thaoudar* (Reut. ex Hausskn.) Grossh. The first grain of this race is smaller and darker (Mac Key, 2005). In Anatolia all intergradations and intermediates between these two races occur often in mixed stands (Zohary, 1969). Our data indicate that the accession collected was *T. boeoticum* subsp. *tahoudar* as proposed Dorofeev et al. (1979) or *T. monococcum* subsp. *aegilopoides* var. *thaoudar* according to Mac Key (2005). A herbarium sheet of the specimen has been deposited in the Royal Botanic Garden in Madrid (No. MA-840865).

Wild wheats carry interesting traits for adaptation to climatic change in the Mediterranean region (Mac Key, 2005). The specific edaphic and climatic characteristics of the habitat make the new accession of *T. boeoticum* found in Spain a valuable germplasm for wheat improvement. Moreover, this accession is an important genetic resource since there were no references so far about this species in the Mediterranean western region. The current distribution area of wild wheats may be larger than 10,000 years ago because these species were spread as admixture with seeds of cultivated species. Some of them like *Hordeum spontaneum, T. boeoticum, T. urartu* and *Ae. tauschii* developed weedy races well adapted to growing in cereal field. We have found a reference about that einkorn was grown in the locality in the past (Madoz, 1845). So, it is possible that *T. monococcum* was cultivated in the collecting zone and the wild einkorn found in the present work was a weed of the crop similar to that found in other countries.

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