

Evidence of natural hybridization between *Aegilops geniculata* and wheat under field conditions in Central Spain

Iñigo LOUREIRO, María Concepción ESCORIAL, José María GARCÍA-BAUDÍN and María Cristina CHUECA*

Dpto. Protección Vegetal. INIA. Ctra. La Coruña Km. 7.5, 28040 Madrid, Spain

This study deals with hybrids between *Aegilops geniculata* and bread wheat, *Triticum aestivum* L., detected in two *Ae. geniculata* populations in the “Meseta Central”, Spain’s central plateau where wheat is a major crop. Morphological traits and pentaploid chromosome numbers were used to identify hybrids in 2004 and 2005. The frequency of hybridization under natural conditions was calculated for one of these populations. Six hybrids were detected from a sample of 3158 seeds from this population. The hybrids were mainly sterile but seed set can be found. This study extends the range of documented hybridization to include central Spain. The potential risks associated with natural hybridization in the context of transgenic wheat cultivation are discussed.

Keywords: Biosafety / crop-wild hybrids / hybridization rate / pollen flow / *Triticum aestivum*

INTRODUCTION

Transgenic wheat varieties are being developed and field-tested, and in the next few years certified cultivars of transgenic wheat shall probably be commercially available. In order to prevent the diffusion of a character that could provide adaptative advantages, thus making weed and wild species more invasive (Darmency, 1994), it is important to understand the potential for gene flow and transgene introgression from cultivated wheat into other varieties, species and wild relatives. Any future market launch and use of genetically modified wheat must be undertaken with extreme care, since a number of closely related species, primarily of the genus *Aegilops*, share their habitat with wheat, and some natural hybrids between *Aegilops* spp. and wheat have been documented in field borders (van Slageren, 1994).

As a typical colonizer, *Ae. geniculata* can be found in massive stands, especially at regularly disturbed places such as roadsides and field edges. *Ae. geniculata*, together with *Aegilops triuncialis* (L.), is the most widespread species of the genus. Figure 1 shows the distribution of *Ae. geniculata* in Europe. Relatively few natural hybrids have been found until now in Europe, and the great majority of them are from historic collections. Requier found the first *Ae. geniculata* × *T. aestivum* hybrids two centuries ago

(1825–1827) near Avignon in southern France. The hybrid was described and named *Aegilotriticum triticoides* (Req. ex Bertol.). *A. triticoides* probably occurs under favorable conditions in all regions where both parents grow in sympatry, thus limited by the distribution of *Ae. geniculata*. There is no record of *Ae. geniculata*-wheat hybrids in the USA, although *Ae. geniculata* has a significant presence in some states. However the hybridization with other *Aegilops* species as *Aegilops cylindrica* (Host.) has been documented under field conditions in the USA (Hegde and Waines, 2004).

In Spain, hybridization between wheat and *Ae. geniculata* is limited to only two hybrids mentioned in the review carried out by van Slageren in 1994. One is located in northern Spain (in Pamplona, Vavilov 55918–55920, WIR 1663–1665) and the other at a distance of 900 km to the south, (in Cádiz, Bourgeau s.n. (P)). Both are herbarium specimens. Hybridization between *Ae. geniculata* and wheat can occur, but there is no information available on the frequency of its occurrence. This paper confirms the occurrence of hybrids between these species in Spain. The frequency at which one *Ae. geniculata* population hybridizes with wheat in a population where both species occur in the Meseta Central is estimated.

* Corresponding author: chueca@inia.es

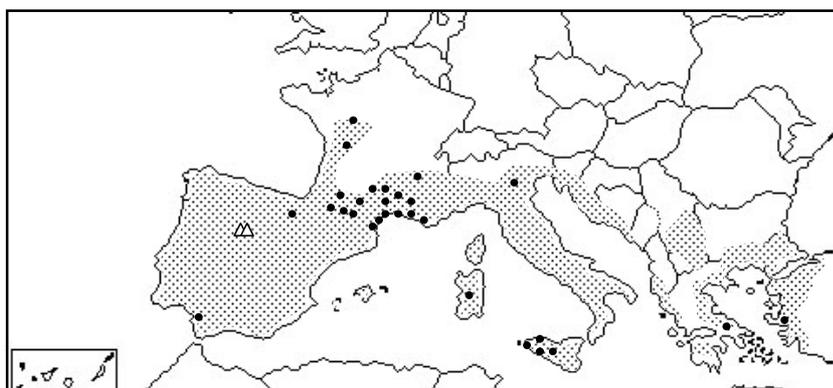


Figure 1. Distribution of *Ae. geniculata* in Europe (shaded area). ● Indicates areas where hybrids were found (from van Slageren, 1994). △ Indicates new hybrids detected in the Zamora area, Spain.

RESULTS AND DISCUSSION

In spring of 2003 a survey of weeds in cereals was carried out in the Meseta Central, which comprises 67% of the area cultivated with bread wheat in Spain (MAPA, 2006). In the course of this survey, by chance, we detected in the Zamora region several natural *Ae. geniculata* × wheat hybrids (Fig. 1). These hybrids occurred in two populations of *Ae. geniculata* (Figs. 2A, 2B and 2C) located at <1 m from the wheat fields. These populations were designated Population 324 and Population 325. We found four hybrids in Population 324 and two in Population 325. This is the first reference of hybridization between wheat and wild relatives in the Meseta Central. Hybrids were identified in the field by their intermediate spike morphology (Fig. 2D), similar to that of the hybrids previously obtained by hand crossing. The hybridity of the plants was confirmed by their chromosome number ($2n = 35$ chromosomes).

In order to study the extent of natural hybridization, we returned to collect *Ae. geniculata* spikes from both populations in the same area in spring 2004. However, one of the wheat field edges had been cleaned of plants, and Population 325 had been eliminated. Population 324 spread extensively along the wheat field (Fig. 2A).

This area comprises many hectares of cereals, primarily wheat and barley. The dryness of the soil and the great density of plants made it impractical to count the number of *Aegilops* maternal plants. Besides, these plants can have up to several hundred tillers, and when spikes are mature, disarticulation takes place at the base of the lowest fertile spikelet (whole-spike type) (Zaharieva and Monneveux, 2006), making it difficult to know precisely the number of the plants the broken-off spikelets came from.

A total of 3200 seeds from Population 324 were collected and grown in the greenhouse in autumn 2004.

Six hybrid individuals were identified from 3158 germinated seedlings, so the spontaneous hybridization rate was of 0.19%. Assuming that hybrid and nonhybrid seeds were equally likely to germinate, this value represents the proportion of *Aegilops* flowers that produce viable seeds after pollination by a wheat pollen grain. In the wild, these seeds can germinate or remain viable in the soil for more than one year, and the resulting hybrid plants may be established or not. This natural hybridization rate was similar to the 0.24% and 0.39% obtained in the assays carried out in 2003 and 2004 under simulated field conditions (Loureiro et al., 2006). Our semiarid field conditions, with frequent high temperatures and low relative humidity during the flowering periods, negatively affect to the viability and dispersal of the wheat pollen (Waines and Hegde, 2003; Loureiro, 2005). Therefore, rates of crop-wild hybridization may be higher under environmental conditions that are more favorable to hybridization.

Hybridization is also possible between *Ae. geniculata* and durum wheat (*Triticum turgidum* L. var. *durum*) under field conditions (David et al., 2004). Although mainly sterile, these hybrids can produce fertile spontaneous amphiploids (estimated at 10^{-6} in sympatric populations) from unreduced gametes, which may be the main avenue for transferring genes from cultivated durum wheat to *Ae. geniculata*.

Our data confirmed the presence of *Ae. geniculata*-wheat hybrids in nature, thus overcoming the first step in gene flow. The second step would be the ability of the hybrids to survive and reproduce in nature for the first generation. In general, most of the *Ae. geniculata* × *T. aestivum* hybrids are almost completely sterile (David et al., 2004; Loureiro, 2005; Zaharieva and Monneveux, 2006), as occurred with the hybrids between *Ae. cylindrica* and wheat (Zemetra et al., 1998). However, F_2 seeds can be produced occasionally in *Ae. geniculata* ×

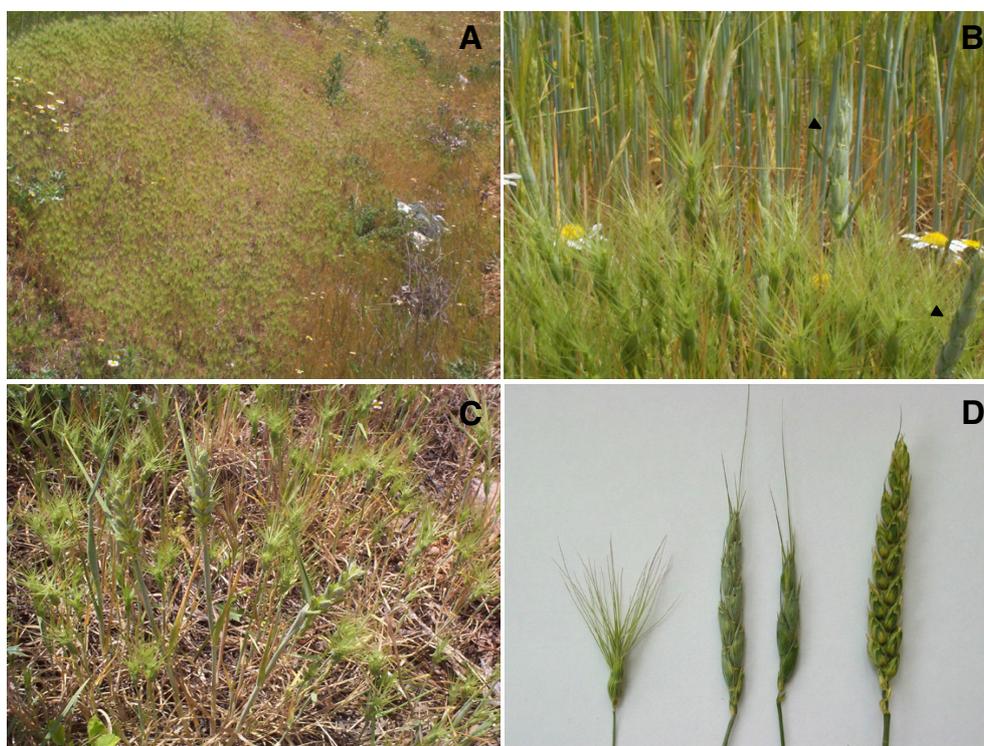


Figure 2. **A:** An extensive stand of *Ae. geniculata* (Population 324) on a roadside near Zamora, Spain; **B** and **C:** natural hybrid plants between *Ae. geniculata* and *T. aestivum* on the edge of wheat fields; and **D:** two spikes of a natural hybrid between *Ae. geniculata* (left) and *T. aestivum* (right).

wheat hybrids under greenhouse conditions (e.g., three from 25 hybrid plants, I. Loureiro, unpublished data).

Backcrosses of F₁ hybrids with the wheat parent are known only from botanical or private gardens so far; BC₁ plants called *Aegilotriticum speltaeforme* (Jord.) have not been described under natural conditions (Jacot et al., 2004). However, Loureiro et al. (2006) obtained BC₁ seeds from hybrid plants at rates of 8% (seeds/spikelets) under simulated field conditions; these rates can reach up to 22% in some hybrid plants with other wheat genotypes as parents (I. Loureiro, unpublished data). It is not clear if further selfing or backcrossing generations could be produced and/or distinguished from the wild relatives or the wheat species under field conditions.

The fact that hybrids between wheat and *Ae. geniculata* can be produced, that they can be partially fertile, and that pairing between *Ae. geniculata* and wheat chromosomes is possible (Fernandez-Calvin and Orellana, 1992; Cifuentes et al., 2006), indicates that a wheat gene could be transferred to the wild. Our data and the data from available literature show that introgression from wheat to *Ae. geniculata* could occur in areas where

the two species grow together and hybridize. Wang et al. (2001) have proved this introgression in greenhouse experiments for *Ae. cylindrica*, another weed growing in U.S wheat fields. Hybridization rates were related to the genotype of each parent and to their interaction with environmental conditions.

Ae. geniculata is generally not weedy in the agronomic sense, although it grows profusely in ruderal habitats adjacent to wheat fields. In certain cases, for instance in the states of California and Oregon in the USA, *Ae. geniculata* spreads locally and has been described as 'a weed of known economic importance' (USDA, 2005). In the above circumstances, the transfer of transgenes that confer traits such as herbicide resistance could produce plants that can no longer be controlled easily and may be more problematic than the wild type; this type of problem must be studied further.

Gene flow dynamics need to be considered in planning future field experiments with transgenic wheat. Agricultural reality shows that the degree of autogamy is high in wheat and that, generally, gene flow can be managed, provided that some precautionary measures are taken, such

as keeping enough spatial isolation from *Ae. geniculata*. More research in this field is needed, since the information available about the potential for wheat crops to cross-pollinate with wild relatives of allied genera is scarce and must be studied case by case and region by region (Zaharieva and Monneveux, 2006).

MATERIALS AND METHODS

Plant material

Aegilops geniculata (Roth) (= *Ae. ovata* (L.)) is an annual allotetraploid species ($2n = 4x = 28$, MMUU) that can cross-pollinate with bread wheat (*Triticum aestivum* (L.), $2n = 6x = 42$, AABBDD) and create pentaploid ($2n = 5x = 35$, ABDMU) hybrids. The genome symbols follow Waines and Barnhart (1992) for *Aegilops* and Kimber and Feldman (1987) for *Triticum*.

In 2004 a representative random sample of around 1000 spikes was harvested at maturity at short intervals along (6–7 meters) *Ae. geniculata* Population 324 without eliminating the parental population. This population, located in the local village of Manganeses de la Lampreana (41° 45' North; 5° 42' West), in the Zamora region (Spain), occupies an area of approximately 15 m² and *Aegilops* plants were almost in direct contact with wheat. Seeds from all of these spikes were manually threshed, mixed together and seeds counted and stored for several months before being planted.

In October seeds were sown at a depth of 2–2.5 cm in 3 L plastic pots (16 cm diameter, 5 seeds per pot) containing compost: sheep manure, sand and soil in a 1:1:1 (v/v/v) mixture. The pots were placed in a cold greenhouse.

In late May 2005, *Ae. geniculata* × wheat hybrids were identified by their spike appearance. Percent hybridization data was estimated as a proportion of hybrids in the total sample of germinated *Aegilops* seedlings (N = 3158). Non-germinated seeds were omitted from the calculation.

Chromosome counts

The different ploidy levels of *T. aestivum* ($2n = 42$) and *Ae. geniculata* ($2n = 28$) enabled us to confirm the putative hybrids on the basis of their chromosome number in root meristems. Chromosome counts were done on roots regenerated in pots in the greenhouse using young shoots from the natural hybrids. Root meristems for mitotic chromosome number counts were collected from each plant and were pre-treated in α -bromonaphthalene at 4 °C

during 16 hours, fixed in a 90% acetic acid solution during 30 min, washed twice with 95° ethanol and stored in 70° ethanol. After a minimum of 10–14 days, root meristems were stained in Schiff reactive for 60 min after a 10–12 min hydrolysis at 60 °C HCl 1N, and squashed in 1% Belling's aceto-carmin prior to light microscopy observation.

ACKNOWLEDGEMENTS

This work has received financial support from the Comisión Interministerial de Ciencia y Tecnología (CICYT) from Spain; project AGL2004-07101-C02-01/AGR.

Received January 2, 2006; accepted December 19, 2006.

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