Research Article

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Natural hybridization between wheat (*Triticum aestivum* L.) and its wild relatives *Aegilops geniculata* Roth and *Aegilops triuncialis* L.

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Abstract

BACKGROUND: Cultivated bread wheat (*Triticum aestivum* L.) spontaneously hybridizes with wild/weedy related *Aegilops* populations, but little is known about the actual rates at which this hybridization occurs under field conditions. It is very important to provide reliable empirical data on this phenomenon in order to assess the potential crop–wild introgression, especially in the context of conducting risk assessments for the commercialization of genetically modified (GM) wheat, as gene flow from wheat to *Aegilops* species could transfer into the wild species genes coding for traits such as resistance to herbicides, insects, diseases or environmental stresses.

RESULTS: The spontaneous hybridization rates between wheat and *A. geniculata* and *A. triuncialis*, which are very abundant in the Mediterranean area, have been estimated for the first time in the northern part of the Meseta Central, the great central plateau which includes the largest area of wheat cultivation in Spain. Hybridization rates averaged 0.12% and 0.008% for *A. geniculata* and *A. triuncialis*, respectively. Hybrids were found in 26% of *A. geniculata* and 5% of *A. triuncialis* populations, at rates that can be ≤3.6% for *A. geniculata* and 0.24% for *A. triuncialis*.

CONCLUSION: The detection of *Aegilops* spp.–wheat hybrids in *Aegilops* populations indicates that gene flow can occur, although wheat is considered a crop with a low-to-medium risk for transgene escape. These data on field hybridization rates are essential for GM wheat risk assessment purposes.

Supporting information may be found in the online version of this article.

Keywords: *Aegilops* spp.–wheat hybrids; spontaneous hybridization; genetically modified wheat; gene transfer; risk assessment

1 INTRODUCTION

Wheat and related species of the *Aegilops* genus occupy the same habitat in many areas in the world. In Europe, 12 *Aegilops* species are present, including *A. geniculata* Roth. and *A. triuncialis* L. which are the most widespread species of the genus, and both frequent in the Mediterranean area.[1] They are commonly found growing in large stands at roadsides and field edges in close proximity to wheat fields. Both species can overlap their flowering times with wheat in south European fields. Natural hybrids with wheat were mentioned in historical collections[1] and have been detected under field conditions for both species since the 19th Century, mainly in France and Spain.[2–4] *Aegilops cylindrica* Host. and *Aegilops ventricosa* Tausch. hybrids also have been documented in the USA.[5,6] The rate at which two species hybridize depends on the species, their taxonomic proximity and genotype, as well as the cropping circumstances and environmental conditions of the area at the time of hybridization. Interspecific hybridization between wheat and *Aegilops* spp. has been used to transfer traits from *Aegilops* into wheat in wheat breeding.[7,8] There are quite abundant data on *Aegilops*–wheat hybridization rates. However, these rates often are overestimated by the use of techniques that favor the success of crosses. From the review by Zaharieva and Monneveux,[6] we can summarize that wheat × *A. geniculata* hybrids are obtained in a percentage ratio of between 1.2% and 45.1%, whereas the reciprocal cross *A. geniculata* × wheat range between 26.9% and 61.3%. For wheat × *A. triuncialis* hybridization, this potential range is 14.7–32.4% and for *A. triuncialis* × wheat 2.5–81.2%. However, quantitative evaluation of the frequency of interspecific hybridization under natural conditions is less frequent probably as a consequence of its complexity and there are few quantitative data on spontaneous *A. geniculata* and *A. triuncialis* hybridization with wheat. A rate of 0.2% to 6% hybridization was quantified for various *Aegilops* species and wheat

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cultivars (Boguslavski 1978, in Zaharieva and Monneveux[6]). In Spain, the proportion of *A. geniculata* hybrids in one population in Meseta Central was estimated at 0.19%,[3] slightly lower than the 0.24% and 0.39% obtained in two different years in field trials in the same area.[9]

Wheat is one of the main food grains consumed in the world. Genetically modified (GM) wheat cultivars have been successfully developed and field-tested. However, the GM wheat commercialization has been difficult because of concerns about consumer acceptance. This fact led to the withdrawal of a GM glyphosate-resistant wheat cultivar that was close to its commercialization. In recent years, modern biotechnology approaches, including RNA interference (RNAi) and CRISPR/Cas9 gene editing, have emerged as promising tools to produce GM wheat with improvements of traits such as drought tolerance, resistance to pests and diseases, and yield.[10–14] Other traits such as the reduction in the accumulation of asparagine, the precursor for the formation of the carcinogen acrylamide during cooking and processing[15] or the reduction of the gluten content in the grain to produce a wheat for patients with celiac disease have been developed and are being field-tested.[16,17] In 2020, Argentina's Ministry of Agriculture approved the first GM wheat cultivar for growth and consumption, making it the first country to adopt it. This GM wheat, drought-tolerant and resistant to glufosinate herbicide, was sown on >50 000 ha in the 2021–22 growing season.[18]

The potential use of GM plants in agriculture requires an ecological risk-assessment.[19] With the introduction of GM wheat, there are specific hazards which have to be assessed in regard to the persistence and invasiveness of the wild relatives in case of introgression of the traits involved in the GM crop. These events are related mainly to changes in the fitness of the wild relatives and can have important evolutionary consequences such as increased weediness, reduced flora and fauna abundance, or local extinction risk of these wild relatives. *Aegilops geniculata* and *A. triuncialis* generally are not considered weedy in the agronomic sense. However, *A. triuncialis* is a troublesome weed in California and both species were characterized as high risk in a weed risk assessment conducted by USDA owing to their potential for establishment and spread favored by a wide range of traits associated with weedy and invasive species.[20] Furthermore, these colonizing species have the capacity to develop large stands, up to many hectares in area, which can favor the abundance of hybrids. If these hybrids acquire a herbicide resistance gene from wheat, they could increase their invasiveness under selection pressure of the herbicide. Any wheat cultivar requires tolerance to some herbicide to be grown under an extensive production system. The fact that hybrids between wheat and these *Aegilops* species can be produced, and that the hybrids can be partially fertile after backcrossing to any of the parents or by spontaneous amphiploidy,[21,22] indicates that wheat genes can be introgressed to the wild *A. geniculata* and *A. triuncialis* populations, as has been recently shown using wheat diagnostic alleles.[4,23,24] The transference of herbicide resistance genes from wheat to other *Aegilops* species, *A. cylindrica*, a noxious weed in wheat-producing areas of the western United States, has been detected under field conditions, which could compromise its control with the herbicide to which the wheat is resistant.[25,26]

Information on the amount of outcrossing rate between wheat and *Aegilops* species under natural conditions is very scarce, but this information is relevant in order to avoid crop–wild transgene flow, as hybridization is the first step in introgression. So, data on the spontaneous hybridization under field conditions will be of great value for risk assessment purposes. The aim of this work is to empirically calculate the spontaneous hybridization rate between wheat and its wild relatives *A. geniculata* and *A. triuncialis* at sites of sympatry under semiarid conditions in Spain.

2 MATERIALS AND METHODS

2.1 Field survey

A total of 54 *A. geniculata* and 55 *A. triuncialis* wild accessions were collected in several field surveys conducted yearly for 5 years across the 'Meseta Central' (Central Plateau), the main bread wheat (*T. aestivum*) cropping area of Spain (Fig. 1). In this area, the average yearly rainfall is ≈400–600 mm, with unequally distributed rainfall, mostly in autumn and spring, monthly mean temperatures are from 4 to 20 °C, with extreme temperature events of −15 and 40 °C also recorded, and with more than 100 days of frost-risk every year, so winter and facultative wheat types are commonly grown.[27] The surveys were conducted by driving throughout the area and stopping when *Aegilops* species were detected growing in close proximity to bread wheat fields (<1 m). The surveys were conducted across the eight provinces of Castilla y León (Ávila, AV; Burgos, BU; Segovia, SG; León, LE; Palencia, PA; Salamanca, SA; Valladolid, VA and Zamora, ZA), located in the northern part of the Meseta, and in the provinces of Madrid (MA) and Guadalajara (GU, Castilla-La Mancha), located in the central part. Each *Aegilops* accession was georeferenced and a random representative sample of mature spikes of the parental population was harvested. To ensure that the samples were representative of the field population, spikes were collected from different plants at short intervals (2–3 m). Seeds from all of these spikes were manually threshed in the laboratory, mixed by accession, and counted and stored for several months before being planted.

At each collection site, if the presence of hybrids was detected growing within the *Aegilops* populations along the edges of a wheat fields, which were identified by their intermediate spike morphology similar to that obtained by hand-crossing under glasshouse conditions in the laboratory (Supporting Information Fig. S1),[3,21,28] the number of hybrids was recorded.

2.2 Hybridization screening

The seeds of the *Aegilops* spp. accessions harvested in summer were sown as soon as possible in the following autumns and screened for hybridization in a glasshouse under controlled conditions (25/16 ± 2 °C, day/night temperature) and without additional illumination at the INIA experimental station (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, 40° 27′ N, 3° 44′ W) in Madrid, Spain.

Seeds from each sample were sown at 1–2 cm depth in 15-L plastic trays (36 × 26 × 16 cm, 111 seeds per tray) containing manure, sand and soil (1:1:1 by volume) and watered as required. Germination and seedling emergence were recorded. In spring *Aegilops* × wheat hybrids were identified by their spike appearance, similar to those obtained by hand-crossing under glasshouse conditions in our laboratory.[21,29] The number of *Aegilops* seedlings that germinated in each population and the number of *Aegilops*–wheat hybrids was recorded.

2.3 Hybridity confirmation

The different ploidy levels of bread wheat *T. aestivum* (2*n* = 6x = 42, AABBDD) and *A. geniculata* (2*n* = 4x = 28, MMUU)

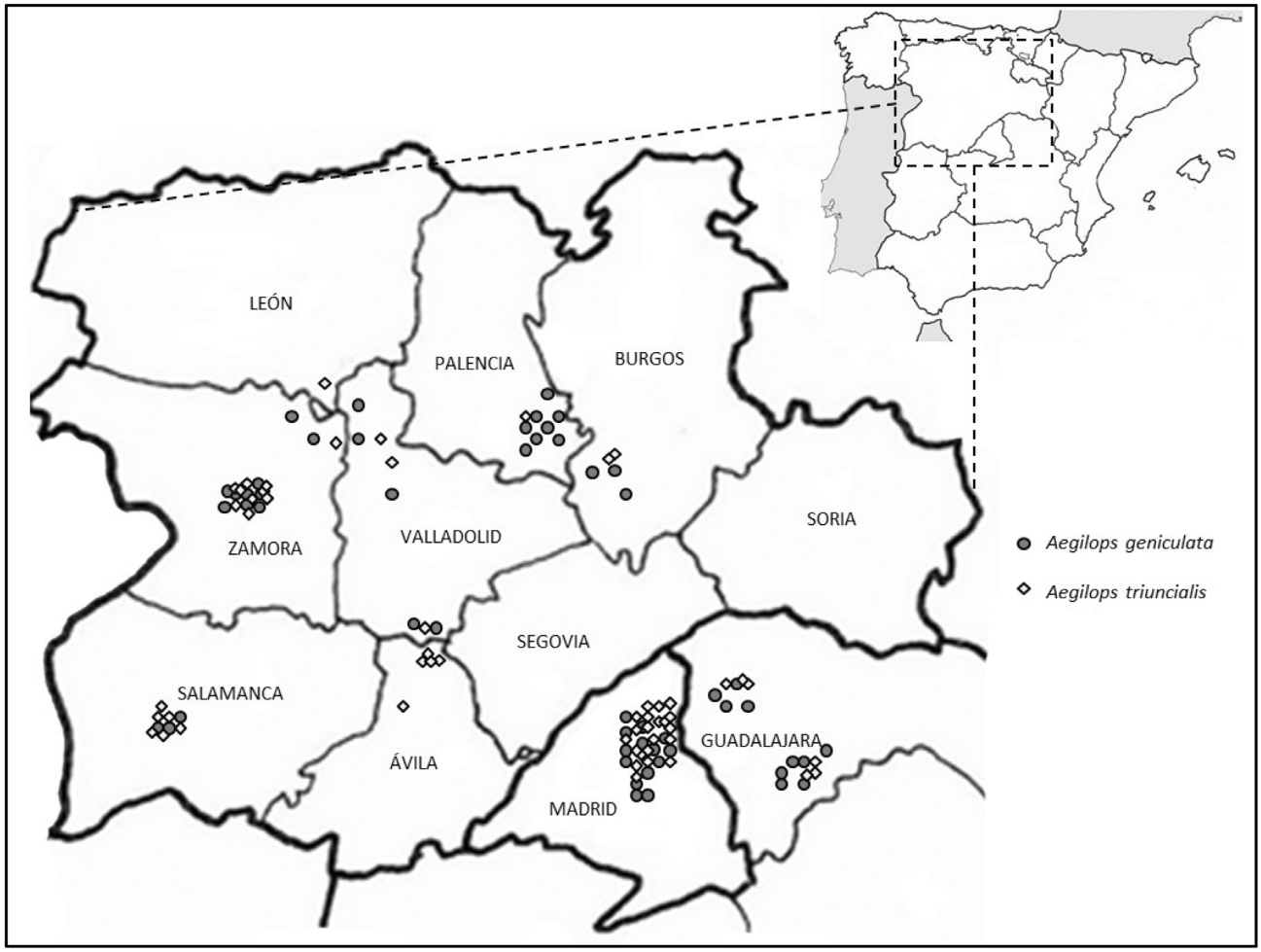

Figure 1. Geographical origin of the *Aegilops* accessions collected in field surveys conducted across the 'Meseta Central' (Central Plateau), Spain. The location of each population is indicated by a point. The accession number, the year of collection and the georeferenced *A. geniculata* and *A. triuncialis* accessions dataset are in Supporting Information Tables S1 and S2, respectively.

and *A. triuncialis* (2*n* = 4x = 28, UUCC) enabled us to confirm the putative hybrids on the basis of their chromosome number in root meristems. *Aegilops*–wheat F_1 hybrids would be pentaploids (2*n* = 5x = 35 chromosomes), ABDMU or ABDCU depending on the *Aegilops* species involved in the hybridization. Chromosome counts were conducted on roots regenerated in pots in the glasshouse using young shoots from the hybrids. Root meristems for mitotic chromosome number counts were collected from each plant and were treated in α-bromonaphthalene at 4° C during 16 h, 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 hydrolyzed during 10–12 min. at 60 °C HCl 1 N, then stained in Schiff reactive for 60 min. and squashed in 1% Belling's aceto-carmine before light microscopy observation.

2.4 Data analysis

The hybridization rate between wheat and the different *Aegilops* populations was estimated as the ratio of the number of hybrid plants to the total sample of germinated *Aegilops* seedlings. Chi-square tests were used to determine significant differences between observed and estimated overall hybridization rates in the *Aegilops* populations, whereas binomial distribution was used to determine the probabilities of finding at least one *Aegilops*–wheat hybrid in each *Aegilops* progeny. The absence of hybrids in many *Aegilops* spp. populations resulted in non-normality of the data, do nonparametric Kruskal–Wallis *H*-tests ($P = 0.05$) were carried out to determine significant differences among years for the hybridization rates measured in the different *Aegilops* populations. Statistical analyses were performed using CENTURION XVI.II statistical software (Statgraphics Technologies, Inc., The Plains, VA, USA).

3 RESULTS

3.1 *A. geniculata* × wheat natural hybridization

Aegilops geniculata × wheat hybrids were observed under field conditions in 13 of the 54 (24.1%) of the populations identified in the survey. The hybrids were growing among wild *A. geniculata* stands near to the wheat field edges [Fig. 2(A)]. The hybrid phenotype, with greater spike length and greater height of these hybrid plants exceeding that of *Aegilops*, allowed us to identify those F_1 hybrids under field conditions [Fig. 2(B)]. A total of 65 830 seeds were harvested from the 54 *A. geniculata* field accessions. These seeds were grown in the glasshouse and a total of 92 *A. geniculata* × wheat putative hybrids were detected

Figure 2. (A) A mixed stand of *A. geniculata* and *A. triuncialis* plants growing in close proximity to a bread wheat (*T. aestivum*) field. (B) Spikes of a natural hybrid between *A. geniculata* and wheat, some of them fallen on the ground after disarticulation; (C) A natural hybrid between *A. triuncialis* and wheat. (D) Hybrid detection in the glasshouse after sowing the *Aegilops* accessions. (E and F) Spike morphology of the *A. geniculata*– and *A. triuncialis*–wheat hybrids detected in the glasshouse, respectively.

(Table 1). The hybrids were easily identified in the glasshouse by their height and intermediate spike morphology [Figs 2(D), (E) and S1]. All of them were confirmed as hybrids in the laboratory by chromosome counting. These hybrids were found in the glasshouse in 14 accessions. Average hybridization between *A. geniculata* and wheat was 0.12 ± 0.50% (range 0–3.64%) (Table 1). The *A. geniculata* seed sample evaluated in this study, allowed us to estimate with 95% certainty that the frequency of finding at least one hybrid in the *A. geniculata* progeny was below 0.17%. There were no differences among sampling years in the spontaneous hybridization rates measured in the *A. geniculata* populations (Kruskal–Wallis test: $H = 4.44$, $P = 0.3491$).

The hybridization rates for those accessions in which hybrids were detected is shown in Fig. 3. In four of the accessions (30.9%; accessions 324, 402, 436 and 438), hybrids had been observed *in situ* under field conditions, which would allow backcrossing. The observed hybridization rates of accessions 332 (0.03%) and 434 (3.64%), which had the lower and the higher hybridization, were significantly different from the overall hybridization ($\chi^2 = 391.58$, df = 13; $P < 0.05$). In relation to the geographical origin, hybrids were found in six of the seven regions where *A. geniculata* accessions were collected, with the province of Salamanca being the only region where no hybrids were detected.

3.2 *A. triuncialis* × wheat natural hybridization

In the field surveys, *A. triuncialis* × wheat hybrids were observed in only two of the 55 *A. triuncialis* populations collected growing in sympatry with wheat [Fig. 2(A)]. A total of 70 637 seeds were harvested and sown in the glasshouse for hybrid identification. Only four *A. triuncialis* × wheat hybrids were detected in the

Table 1. *Aegilops geniculata* × wheat (*Triticum aestivum*) hybridization. Spontaneous hybridization rates between bread wheat and its wild relative *A. geniculata* measured under field conditions

Location	No.*	Accessions with hybrids in field (%)	Seeds analysed per accession (mean ± SD)	Total seeds (*N*°)	Accessions with hybrids in glasshouse (%)	Hybrids (*N*°)	Hybridization (%, mean ± SD)	Range (%)
Burgos	4	0	1056 ± 465	4225	50.0	6	0.18 ± 0.27	0–0.56
Guadalajara	12	25.0	1211 ± 546	14 534	25.0	67	0.39 ± 1.04	0–3.64
Madrid	11	63.6	1816 ± 1032	19 977	18.2	3	0.02 ± 0.04	0–0.13
Palencia	8	0	604 ± 318	4834	25.0	3	0.04 ± 0.07	0–0.20
Salamanca	3	0	1163 ± 619	3489	0	0	0	---
Valladolid	5	40.0	467 ± 343	2336	20.0	1	0.03 ± 0.06	0–0.13
Zamora	11	9.1	1494 ± 1048	16 435	36.4	12	0.04 ± 0.07	0–0.19
Total	54	24.1	1219 ± 849	65 830	25.9	92	0.12 ± 0.50	0–3.64

*Number of *A. geniculata* field accessions collected.

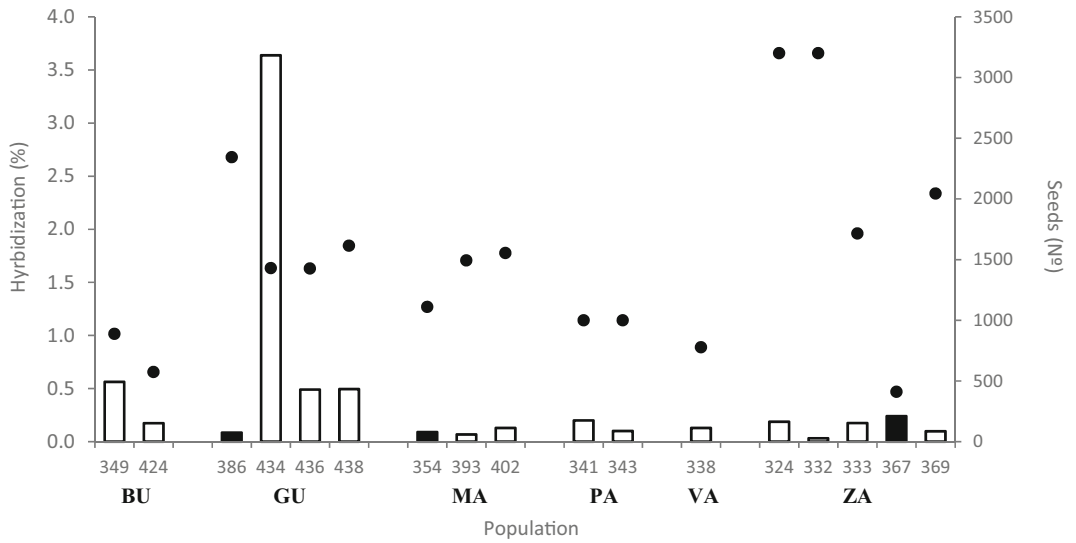

Figure 3. Hybridization rates found in 14 *A. geniculata* (white bars) and three *A. triuncialis* accessions (black bars) growing in close proximity to wheat fields under semi-arid conditions in different regions in Spain (BU, Burgos; GU, Guadalajara; MA, Madrid; PA, Palencia; VA, Valladolid; ZA, Zamora). Dots show the sizes of the seed samples of the *Aegilops* accessions.

Table 2. *Aegilops triuncialis* × wheat (*Triticum aestivum*) hybridization. Spontaneous hybridization rates between bread wheat and its wild relative *A. triuncialis* measured under field conditions

Location	No.*	Accessions with hybrids in field (%)	Seeds analysed per accession (mean ± SD)	Total seeds (*N*°)	Accessions with hybrids in glasshouse (%)	Hybrids (N°)	Hybridization (%, mean ± SD)	Range (%)
Ávila	5	0	2023 ± 1167	10 117	0	0	0	---
Burgos	2	0	695 ± 313	1390	0	0	0	---
Guadalajara	9	0	1142 ± 753	10 274	14.3	2	0.009 ± 0.032	0–0.09
León	1	0	1332	1332	0	0	0	---
Madrid	19	5.3	1567 ± 1066	29 774	5.3	1	0.005 ± 0.021	0–0.09
Palencia	1	0	360	360	0	0	0	---
Salamanca	6	16.7	1268 ± 506	7605	0	0	0	---
Valladolid	3	0	965 ± 451	2895	0	0	0	---
Zamora	9	0	765 ± 218	6889	11.1	1	0.027 ± 0.081	0–0.24
Total	55	3.6	1278 ± 882	70 637	5.4	4	0.008 ± 0.036	0–0.24

*Number of *A. triuncialis* field accessions collected.

glasshouse (Table 2). The detected hybrids, identified by their phenotype [Fig. 2(F)], came from three *A. triuncialis* accessions and were confirmed by chromosome counting. Average hybridization between *A. triuncialis* and wheat was 0.008 ± 0.036% (range 0–0.24%) (Table 2), with no significant differences in the hybridization rates among *A. triuncialis* populations and the overall estimated hybridization ($\chi^2 = 0.17$, df = 2; $P = 0.9196$). This *A. triuncialis* seed sample size, >70 000, provided a 95% confidence that 0.014% was the maximum spontaneous hybridization rate. There were no significant differences among sampling years in the spontaneous hybridization rates measured in the *A. triuncialis* populations ($H = 4.72$, $P = 0.3170$). As in the case of *A. geniculata*, one of the *A. triuncialis* accessions yielding hybrids in the glasshouse (accession 354) also had hybrids under field conditions in the previous year, so backcrossing might also have been possible. In relation to the geographical origin, *A. triuncialis* × wheat hybrids were found in three of the nine regions where *A. triuncialis* accessions were collected.

4 DISCUSSION

In order to preserve the environment and to avoid agricultural problems, the potential use of genetically modified plants in agriculture requires an ecological risk-assessment.[19] There are specific hazards which have to be assessed in regard to the persistence and invasiveness of the wild relatives in case of introgression of the traits involved in the GM crop. Although the contact between wheat crops and potential *Aegilops* wild recipients is extensive in Europe,[1] the amount of spontaneous hybridization between wheat and *Aegilops* species generally is unreported; however, this information is relevant in order to avoid the escape of the transgenes by hybridization, the first step in introgression. In Colorado (United States), Gaines *et al.*[30] assessed gene flow between *A. cylindrica* and wheat to evaluate the potential transfer of herbicide-resistance genes from imidazolinone-resistant (IR) winter wheat cultivars and found that the average hybridization across sites and years when wheat and *A. cylindrica* grew in sympatry was 0.1%, with a maximum of 1.6%.

Our results show that the hybridization between cultivated wheat and wild *A. geniculata* is very likely, with hybrids found in almost a quarter of the studied populations; however, it is less frequent in the case of *A. triuncialis*, with 3.6% of the populations harboring hybrids. We found that on average 0.12% of the *A. geniculata* harvested seeds were hybrids if the *A. geniculata* wild populations were growing in close proximity to wheat fields. This rate of spontaneous gene flow agrees with the results of 0.19% of natural hybrids previously reported for one *A. geniculata* population growing in close proximity to wheat in the same area of the Meseta Central in Spain,[3] and are slightly lower than the 0.24% and 0.39% obtained in two different years in field trials in the same area, but in this case the hybridization was favored by overlapping the flowering periods between species and by diminishing pollen competence introducing only one *A. geniculata* plant into a wheat pollen source.[9] Assuming this 0.12% rate of spontaneous hybridization and an average seed production per plant of 80.2 seeds plant^{-1},[9] in an *A. geniculata* population with ≈100 plants growing near a wheat field, almost 10 (9.6) hybrids would be formed, which could germinate or remain viable in the soil for >1 year. This could be a conservative estimate as the average seed number in *A. geniculata* plants grown under Mediterranean conditions can reach 1400 seeds,[6] although variations in the environmental conditions may cause great differences in plant characteristics such as plant height and number of tillers.

In the case of *A. triuncialis*, with 0.008% spontaneous hybridization rate and 150 seeds plant^{-1} (data from 2 years, with 10 plants year^{-1}, unpublished data), 1.2 hybrids would be formed in the same size population. Such rates could be much higher in hybridization hotspots, where probably the overlap in the flowering periods between the crop and its related species *Aegilops* would be larger, and environmental conditions – so important for successful pollination[9,31] – more favorable. Probably, as evidenced in field studies for wheat and *A. cylindrica* Host,[26] the hybridization rates varied both by year and sites. Such may have been the case for accession 434 of *A. geniculata* for which, under the above conditions, ≤291 hybrids could be expected. These results demonstrate the importance of performing risk-assessment at multiple sites and detecting variability between regions.

There were spontaneous *Aegilops*–wheat hybrids observed at variable rates in some locations while hybrids were not detected in others. There are many factors that contribute to successful hybridization. This study implies field surveys with different wheat cultivars and *Aegilops* spp. accessions, and thus, plant genetic factors may have played an important role in the differences found in hybridization.[32] However, because the survey was conducted in different geographical regions and over several years, the extent of *Aegilops*–wheat hybridization probably was influenced by the environmental conditions during the overlap in their flowering times. Spain is within the ten most climatically diverse countries in the world and the most climatically diverse country in Europe with 13 different Köppen climates.[33] Only in the surveyed regions can we can find four different climates: the hot-summer and warm-summer Mediterranean climates (Csa and Csb, respectively, which predominate in Valladolid, Salamanca, Ávila and Segovia); the oceanic climate (Cfb, predominating in Burgos, León, Palencia and Soria); and the semiarid climate (BSk, present in Zamora, Guadalajara and Madrid). To this fact, we must add the high interannual climatic variability. In the surveyed area wheat flowering occurs in late April and May. As an example of this variability, during the last 10 years May was classified as warm and dry in 6 years, whereas in 2 years it was classified as cold and dry and in another 2 years as cold and wet (based on average monthly temperatures and precipitation in relation to the 1981–2010 climatic reference).[34] These environmental factors affect the anthesis of the plants – the extent of floret opening, stigma size, duration of stigma receptivity, stigma exsertion and anther size and filament length – and the pollen production, viability and transport, attributes that are important in cross-pollination[5] and that, even in the same environmental conditions, can be different among wheat cultivars and *Aegilops* accessions, influencing the formation of hybrids.

These are the first data available for this crop and these two related *Aegilops* species in Europe at this scale. Considering the hybridization rates estimated in this study, between 0% and 3.64%, and that these species are widely distributed in Spain, very often appearing in ruderal habitats,[1,3] it is reasonable to think that hybrids can be formed annually. In one of the few studies carried out at these scales, Wilkinson *et al.*[35] assessed the natural hybridization between rapeseed (*Brassica napus* L.) and *B. rapa* L. at the national scale across the UK, estimating that 32 000 hybrids could be formed annually in *B. rapa* populations, considering hybridization rates between 0.4% and 1.5%,[36] which are in the range found in our study for *A. geniculata*. Although the hybridization rates were not reported, the presence of abundant spontaneous

A. cylindrica–wheat hybrids also have been reported in Bulgarian wheat fields.[37]

Ostrowski *et al*.[38] predicted that the distribution of these *Aegilops* wild relatives will change in Europe by 2050 as a consequence of global warming. The estimated change for the global mean across species was +6.4% for *A. geniculata* and up to +37% for *A. triuncialis*, whereas the potential sympatry level with wheat will increase in countries such as Russia, France and Ukraine, which are currently among the main wheat producers in Europe.[38] These predictions of *Aegilops* species occurring where wheat is frequently grown can increase the area where hybrids between these species can be produced. However, the introgression of genes depends not only on the F_1 hybrid formation in the field, but also on the viability and fertility of the hybrids and their successive generations and on the persistence of introgressed genes. Hybrids between *Aegilops* species and wheat often are considered self-sterile; however, F_2 seeds, commonly formed by spontaneous amphiploid production,[21] can be found at rates of 0.08% (0.05–0.16) and 1.82% (0.17–5.44) seeds per spikelet for *A. geniculata*– and *A. triuncialis*–wheat hybrids, respectively. Seed-set was higher when F_1 hybrids were backcrossed with wheat,[22] with an average of 2.87% (0–9.26) for *A. geniculata* under experimental field conditions,[39] whereas fertility increased from F_1 to the BC_1 and F_2 generations of hybrid progenies.[22] These backcrosses, recombination and spontaneous amphiploidy, can result in wheat–*Aegilops* gene introgression.[1–4,21,22,40] Different studies conducted in the United States on the hybridization between wheat and *A. cylindrica* (CCDD), also in the context of risk assessment of GM herbicide-tolerant wheat, have demonstrated that hybrids and backcross derivatives can be formed under field conditions between both species.[25,26] The selection pressure from the herbicide could give a selective advantage to the hybrids and/or backcrosses that carry the resistance gene from wheat, a situation that may allow gene flow between these two species and the introgression of the wheat gene conferring the selective advantage into the weedy population. Once introgressed in *A. cylindrica*, the odds of an allele being herbicide-resistant under selection pressure were 1.6-fold greater than the odds of the allele being resistant without selection pressure.[41] Despite the fact that *A. geniculata* (MMUU) and *A. triuncialis* (UUCC) not sharing homologous genomes with common wheat (AABBDD), the natural introgression of DNA sequences from wheat into related nonhomologous wild species has been described previously, as was the case of *A. peregrina* (SSUU).[42] Evidence of these wheat DNA introgressions in natural *Aegilops* populations surveyed in the Mediterranean area have been reported for these species using molecular markers, questioning the strength of reproductive barriers. Arrigo *et al*.[4] detected that a mean of 2.8% of the *A. geniculata*, 24% of the *A. triuncialis* and 16% of the *A. neglecta* individuals from naturally occurring populations showed signs of substantial wheat introgression. Parisod *et al*.[23] showed that 57.9% of the *A. triuncialis* Californian populations and 81.8% of the Spanish populations had introgressed individuals, which were more frequently reported in Spain than in California (27.3% *versus* 9.14%). This higher rate observed in Spain probably was a consequence of the repeatedly encountered sympatry between species in the Old World. The analysis of Spanish *A. triuncialis* populations collected in areas where introgression of wheat alleles was documented showed that autogamy can reduce but not avoid the introgression of crop alleles into wild counterparts,[24] and that when spatial and temporal scales are properly considered, even rare introgression signatures can be detected.

5 CONCLUSIONS

This study provides information on the potential for gene introgression from common wheat into wild relative species in case of the release of any GM wheat. We report the rates at which hybrids between wheat and its wild relatives *A. geniculata* and *A. triuncialis* are produced and thus, that the first barrier of gene flow – sexual incompatibility between species – can be overcome naturally in the field. These spontaneous hybridization rates between *A. geniculata* and *A. triuncialis* and wheat under field conditions, are baseline information toward a more quantitative risk assessment posed by the introduction of GM wheat. The presence of wheat sequences detected in *Aegilops* spp. populations indicates that gene flow is possible and that, even if wheat is considered a crop with low–medium risk of transgene scape,[43] introgressed genes can remain in the recipient populations. Crop–wild gene flow can transform wild species into weeds, mainly if they carry herbicide resistance genes, and promote the presence in field borders of more competitive and aggressive genotypes. Hybrid fitness and other factors affecting the likelihood of change should be assessed.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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