Spontaneous wheat-*Aegilops biuncialis*, *Ae. geniculata* and *Ae. triuncialis* amphiploid production, a potential way of gene transference

I. Loureiro, C. Escorial, J. M. García-Baudin and M. C. Chueca*

*Dpto. Protección Vegetal. Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). Ctra. La Coruña Km. 7.5, 28040 Madrid. Spain.*

**Abstract**

Some F$_1$ hybrid plants between three species of the *Aegilops* genus and different hexaploid wheat *Triticum aestivum* cultivars show certain self-fertility, with averages of F$_1$ hybrids bearing F$_2$ seeds of 8.17%, 5.12% and 48.14% for *Aegilops biuncialis*, *Aegilops geniculata* and *Aegilops triuncialis* respectively. In the *Ae. triuncialis*-wheat combination with ‘Astral’ wheat cultivar, the fertility was higher than that found in the other combinations. All the F$_2$ seeds studied were spontaneous amphiploids (2n=10x=70). The present study evidences the possibility of spontaneous formation of amphiploids between these three *Aegilops* species and hexaploid wheat and discusses their relevance for gene transference. Future risk assessment of transgenic wheat cultivars needs to evaluate the importance of amphiploids as a bridge for transgene introgression and for gene escape to the wild.

**Additional key words:** *Aegilops*, hybrid self-fertility, risk assessment, *Triticum aestivum*, wild relatives.

**Introduction**

One of the concerns raised over the introduction of genetically modified crops is the stable transference of the transgenes to the wild relatives causing ecologically significant changes in fitness that could lead to increased weediness or invasiveness (Darmency, 1994), which constitutes an important basis for risk assessment studies. Therefore, over the last decade much attention has been paid to crop to weed and wild hybridization as potential avenues for the escape of transgenes into natural populations.

Prior to the commercialization of genetically modified crops the research on the natural hybridization between crops and related wild species was very limited. Most of the research was done with the purpose of breeding and with the aim of transferring desirable traits between species and crops were always used as female parent in
intergeneric and interspecific crosses. But the picture is quite different and numerous crops are known to have wild relatives that can hybridize with them somewhere in the world. Gene flow between cultivated species and their weedy and wild relatives has been documented in species such as oilseed rape (*Brassica napus* L.) (Jørgensen and Andersen, 1994), maize (*Zea mays* L.) (Doebley, 1990), sorghum (*Sorghum halepense* (L.) Pers) (Arriola and Ellstrand, 1996), sunflower (*Helianthus annuus* L.) (Arias and Rieseberg, 1994) and sugarbeet (*Beta vulgaris* L.) (Bartsch and Pohl-Orf, 1996).

Transgenic wheat varieties are being developed and field-tested and probably in the next few years certified cultivars will be commercially available. Potential risks should be examined before their widespread adoption. Gene transfer between cultivated wheat *Triticum aestivum* L. and the weedy *Aegilops cylindrica* Host. is known to occur and scientific literature confirms that herbicide resistance genes can move from herbicide tolerant wheat to *Ae. cylindrica* through hybrids (Seefeldt *et al.*, 1998; Zemetra *et al.*, 1998; Guadagnuolo *et al.*, 2001; Wang *et al.*, 2001). Other wild *Aegilops* species like *Ae. geniculata* Roth., *Ae. biuncialis* Vis. and *Ae. triuncialis* L. also form natural intergeneric hybrids with bread wheat where they grow in sympathy (van Slageren, 1994; Loureiro *et al.*, 2006; Zaharieva and Monneveux, 2006), a phenomenon underlining the close genetic links of the two genera. Hybrids between *Ae. geniculata* and *Ae. triuncialis* and wheat have been found in several countries of Europe, mainly in Spain and France, while *Ae. biuncialis*-wheat natural hybrids have been described in Lebanon (van Slageren, 1994). These natural hybrids are highly sterile, although seeds may occasionally be found in *Ae. geniculata* hybrids (van Slageren, 1994;Loureiro *et al.*, 2008).

Crop-to-wild transgene transference may occur through recombination in homoeologous chromosome pairs, translocation, or chromosome retention (Schoenenberger *et al.*, 2006). Another possible transference mechanism is the formation of amphiploids. Spontaneous chromosome doubling usually results from unreduced female and male gametogenesis and the union of those unreduced gametes leads to the formation of a spontaneous amphiploid from an interspecific hybrid (Ramsey and Schemske, 2002). It is known that amphiploidy has played a fundamental role in the evolution in the Triticeae tribe: durum wheat *Triticum turgidum* L. probably evolved in one step as an amphiploid between the wild grasses *Ae. spelta* Tausch and *T. urartu* Tumanian ex Gandilyan; that durum wheat crossed naturally with *Aegilops tauschii* Coos, which resulted in the first hexaploid wheat, *T. spelta* L. (Stebbins, 1946; Maan and Sasakuma, 1977; Jauhar, 2003). In the amphiploids every chromosome of the original hybrids is represented twice and each chromosome has a homologous partner to pair at meiosis. Thus, meiosis is regular and fertile derivatives can be obtained from hybrids whose sterility was caused by chromosomal unbalance and irregularity (Bretagnolle and Thompson, 1995; Ramsey and Schemske, 1998; David *et al.*, 2004). In their review on hybridization between wheat and its relatives, Zaharieva and Monneveux (2006) mentioned that Tschermak and Bleier (1926) were the first to obtain an amphiploid species as a result of the spontaneous doubling of the chromosomes of a wheat hybrid produced by the cross of *T. turgidum dicoccoides* with *Ae. geniculata*. Amphiploidy is a mechanism that can also provide a valuable genetic resource for the introgression of desirable genes from alien species to cultivated wheats. Induced amphiploidy was indeed used to transfer *Ae. geniculata* chromosomes carrying disease resistance into bread wheat (Landjeva and Ganeva, 1998, 1999).

In nature, amphiploids could serve as an effective bridge for gene flow over the interspecific and intergeneric barriers. David *et al.* (2004) reported an estimated frequency of 10^-6 of spontaneous amphiploidy between the tetraploid wheat *T. turgidum* and *Ae. geniculata* in field sympatric populations, that was higher (10^-3) in nursery conditions and with different genotypes. Genomic *in situ* hybridization proved that fertile amphiploids had arisen through unreduced gametes and that some of them carried wheat-*Ae. geniculata* recombinant chromosomes.

The objective of this paper is to report the production and frequency of spontaneous amphiploids in the self-progenies of hybrids between hexaploid wheat *T. aestivum* and the *Aegilops* species *Ae. biuncialis*, *Ae. geniculata* and *Ae. triuncialis*.

### Material and methods

Experiments were carried out at the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) experimental station in Madrid, Spain (40° 27’ North; 3º 44’ West).

*Ae. biuncialis*, *Ae. geniculata* and *Ae. triuncialis* are relatively easy to hybridize with wheat. *Aegilops × T. aes-
tivum hand crosses made during several years (from 2004 to 2007) yielded abundant F1 hybrid seeds, at rates from 30 to 70% of the pollinated flowers (Loureiro, 2005). The plant material employed in the experiments was the F2 seed progeny produced by selfing of the F1 hybrids between the wheat wild relatives Ae. geniculata (2n=4x=28 chromosomes, genomic constitution MMUU), Ae. biuncialis (2n=4x=28 chromosomes, UU MM) and Ae triuncialis (UUCC), and the T. aestivum (2n=6x=42, AABBDD) cultivars ‘Chinese Spring’ (CS), ‘Castan’ and ‘Astral’. Nomenclature of the wild Aegilops spp. is according to van Slageren (1994) and the genomic constitution is according to Kimber and Tsunewaki (1988). The F1 hybrids were sown each year in autumn in a greenhouse in which lateral walls were open and temperature and relative humidity were similar to outdoor conditions. There was no temperature or humidity control. Two to three days prior to anthesis, hybrid spikes from each plant were covered with cellophane bags to avoid any cross-pollination. The fertility of the hybrids was estimated as the number of seeds obtained per 100 spikelets after selfing. A Kruskal-Wallis test was carried out to determine significant differences between the F2 seed set in the different Aegilops-wheat combinations, followed by a Mann-Whitney U-test for multiple comparisons. A confidence level of 95% (p < 0.05) was considered significant.

Seeds collected on hybrids were placed on filter paper moistened with distilled water in 9-cm diameter Petri dishes and once germinated were subjected to cytological analysis to confirm amphiploidy. Root meristems for mitotic chromosome number counts were collected from each germinated seed 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 ready to be stained in Schiff reactive for 60 min after a 10-12 min hydrolysis at 60°C HCl 1N and squashed in a 1% Belling’s aceto-carmine solution prior to light microscopy observation.

The F2 plantlets were further grown to maturity in the greenhouse.

Results

A total of 182, 150 and 26 hybrids between hexaploid wheat and Ae. biuncialis, Ae. geniculata and Ae. triuncialis respectively, were studied. These F1 first-generation hybrids were pentaploids (2n=5x=35) (Figure 1A). F1 plants were grown to maturity in order to study the self-fertility. Some of the plants gave F2 seeds, with a percentage of F1 plants bearing at least one F2 seed that varied significantly in the distinct Aegilops-wheat combinations (Kruskal-Wallis test: H=24.06, p<0.001) (Table 1). The highest self-fertility was obtained for the Ae. triuncialis-wheat combination with frequencies of 1.82 seeds in 100 spikelets and percent averages of 48.14% F1 plants with F2 seeds. All the hybrids obtained between Ae. triuncialis and the wheat cultivar ‘Astral’ were fertile (Table 1), their fertility varied from 1.06 to 8.88 seeds in 100 spikelets among the five plants studied. The fertility of the hybrids with ‘Astral’ was significantly higher than the obtained with the other wheat cultivars (Mann-Whitney U tests, p < 0.05) that did not show differences among them (Mann-Whitney U tests, p > 0.05). Fertility was lower for Ae. biuncialis and Ae. geniculata.
wheat combinations with values of 0.09 (0.07-0.85) and 0.08 (0.05-0.16) seeds in 100 spikelets respectively, and without significant differences among these two combinations according to the Mann-Whitney U test ($p = 0.097$).

Not all the F$_2$ hybrid seeds obtained were germinated and used for amphiploidy study; some of them were conserved for subsequent studies (Table 1). F$_2$ seeds were mostly well formed with a germination rate of 89%, and all the germinated Aegilops × wheat F$_2$ hybrid seeds showed 70 chromosomes (Figure 1B). On the assumption that all the obtained F$_2$ seeds are amphiploids, the mean frequency of amphiploid formation (new decaploid F$_2$ plants per single pentaploid F$_1$ hybrid) would be of 0.049 for Ae. biuncialis, 0.047 for Ae. geniculata and 1.34 for Ae. triuncialis-wheat hybrid combinations.

The decaploid F$_2$ plants were grown in the greenhouse. The two F$_2$ Ae. biuncialis-wheat plants were not viable and died at the seedling stage. In the case of the Ae. geniculata and Ae. triuncialis-wheat hybrid plants, the phenotype of the F$_2$ amphiploids resembled that of the normal Aegilops × T. aestivum F$_1$ hybrids with regard to their leaf size, height and spike morphology (Figure 2). The plants showed a vigorous vegetative growth.

### Discussion

The hybrids between tetraploid Aegilops-species and hexaploid wheat are often considered self-sterile; however, seeds can occasionally be found (Maan and Sasakuma, 1977; van Slageren, 1994). This high sterility may be due to the expected highly disrupted meiosis in these F$_1$ hybrids, whose parental plants differ in chromosome number and in chromosome pairing homology due to the relative divergences between Aegilops genomes (U, C and M in the case of the species studied) and wheat genomes (A, B and D) (Sears, 1941). Structural differences in chromosomes between parents and cytoplasmic differences may also cause sterility in certain hybrids (Stebbins, 1950; Maan and Sasakuma, 1977).

Our data reveals certain degree of self-fertility in some Aegilops-wheat F$_1$ hybrid combinations. The most plausible explanation would be the fecundation of an unreduced female gamete (2n=5x=35) of an F$_1$ hybrid with another unreduced male gamete (2n=5x=35), resulting in a decaploid plant (2n=10x=70). As happens with the self-fertility of intergeneric hybrids, the formation of unreduced gametes was also considered to be infrequent in general on the assumption that the occurrence of 2n gametes

Table 1. Fertility of the three Aegilops × wheat F$_1$ hybrids by selfing and evidence of formation of amphiploids. The fertility of the hybrids was estimated as the number of seeds obtained per 100 spikelets after selfing

| Hybrid       | F$_1$ hybrids | Spikes | Spikelets | F$_2$ seed set | Fertility F$_2$ plants with F$_2$ seeds (%) | F$_2$ seed studied | Germinated F$_2$ seeds | Amphiploids |
|--------------|---------------|--------|-----------|----------------|---------------------------------------------|-------------------|------------------------|-------------|
| Ae. biuncialis × CS | 161           | 1488   | 8987      | 6              | 0.07                                        | 5 (3.10)          | 3                      | 1           |
| × Castan     | 14            | 118    | 765       | 1              | 0.13                                        | 1 (7.14)          | 1                      | 1           |
| × Degamit    | 7             | 53     | 234       | 2              | 0.85                                        | 1 (14.28)         | 1                      | 0           |
| Total        | 182           | 1659   | 9986      | 9              | 0.09                                        | 5                  | 2                      | 2           |
| Mean         |               |        |           |                | 8.17 ± 5.66                                 |                   |                        |             |
| Ae. geniculata × CS | 70            | 605    | 3959      | 2              | 0.05                                        | 2 (2.86)          | 1                      | 1           |
| × Castan     | 40            | 342    | 2529      | 4              | 0.16                                        | 4 (10.00)         | 2                      | 2           |
| × Degamit    | 40            | 332    | 1988      | 1              | 0.05                                        | 1 (2.50)          | 1                      | 1           |
| Total        | 150           | 1279   | 8476      | 7              | 0.08                                        | 4                  | 4                      | 4           |
| Mean         |               |        |           |                | 5.12 ± 4.23                                 |                   |                        |             |
| Ae. triuncialis × CS | 3             | 22     | 181       | 2              | 1.10                                        | 1 (33.33)         | 2                      | 1           |
| × Castan     | 18            | 129    | 1174      | 2              | 0.17                                        | 2 (11.11)         | 2                      | 2           |
| × Astral     | 5             | 72     | 570       | 31             | 5.44                                        | 5 (100.00)        | 15                     | 14          |
| Total        | 26            | 223    | 1925      | 35             | 1.82                                        | 19                 | 17                     | 17          |
| Mean         |               |        |           |                | 48.14 ± 46.26                               |                   |                        |             |
in plants is rare and sporadic. But contrary to this assumption, Harlan and de Wet (1975) showed that almost all plant species can produce 2n gametes in some frequencies and that all polyploids have originated through functional 2n gametes. Many of the intergeneric hybrids have disturbed chromosome pairing and produce high frequencies of 2n male and female gametes. Both phenomena have been reported to occur in the F₁ hybrids between T. turgidum and Ae. tauschii (Xu and Joppa, 1995). There is clear evidence that viable seeds are produced by functional 2n gametes in durum wheat haploids (Jauhar et al., 2000; Jauhar, 2003). Similarly, David et al. (2004) observed that fertile Ae. geniculata × T. turgidum durum hybrids always produced total or partial amphiploids in their offspring.

In the case of our Ae. triuncialis-wheat F₁ hybrids from ‘Astral’ wheat, the F₂ seed set was quite elevated and all plants obtained were fertile. The number of spikes and spikelets per plant was greater in these hybrids, which could contribute to their higher fertility. This wheat genotype effect is also a factor that has an influence on the frequency of 2n gamete formation (Maan and Sasakuma, 1977; Bretagnolle and Thompson, 1995; Ramsey and Schemske, 2002). However, the Ae. triuncialis-wheat F₁ hybrid plants flowered later due to the Aegilops parent’s life cycle and suffered higher temperatures during their meiosis, so it cannot be excluded that this increase in their fertility may be the result of the effects of those temperatures which can also induce meiotic abnormalities promoting unreduced gamete production (Sax, 1937; Ramsey and Schemske, 1998).

Hybridization rates between wheat and Ae. biuncialis and Ae. geniculata under semi-natural conditions are around 0.3% (Loureiro et al., 2007) while the frequencies of amphiploid formation obtained in this study vary from 3.7 to 28.5 per 100 F₁ hybrids studied between Ae. biuncialis and wheat and from 2.5 to 10 amphiploids for Ae. geniculata-wheat hybrids, depending on the Aegilops-wheat parental combination. Thus, finding spontaneous amphiploids in nature would not be completely exceptional. In the case of Ae. triuncialis, although the frequency of hybridization with wheat under natural conditions is unknown, it is a matter of concern that between 11 and 620 amphiploids may be formed per 100 F₁ hybrids. As stated above, fertility rates were significantly higher with ‘Astral’ and these data on amphiploid formation cannot be generalized for all Ae. triuncialis-wheat hybrid combinations.

However, the production of unreduced gamettes and the formation of a new polyploid is only one step towards the establishment of this polyploid, which must be competitive in order to persist. Even if the fertility and/or viability of F₁s were low, these traits often increase in each successive hybrid generation (Rieseberg, 1997). Therefore if the F₁ sets results in viable seed at all, this will provide a second generation of hybrids that might be more fertile. These will breed, among themselves and with their parents, and might provide a third hybrid generation comprising more and fitter individuals than those of the second one. Once gene dispersal has occurred, it is important to understand whether these genes will persist and establish in the natural populations of free-living relatives; since

Figure 2. Spikes of A) Ae. geniculata (left), F₁ hybrid between Ae. geniculata and T. aestivum ‘Castan’, F₂ amphiploid obtained by selfing the F₁ hybrid and ‘Castan’ (right) and B) Ae. triuncialis (left), F₁ hybrid between Ae. triuncialis and T. aestivum ‘Astral’, F₂ amphiploid obtained by selfing the F₁ hybrid and ‘Astral’ (right).
there is a lack of information it follows that more studies are necessary.

Herbicide-resistant wheat, primarily glyphosate tolerant wheat (Blackshaw and Harker, 2002; Zhou et al., 2003), has the potential to improve the efficiency of weed management. The risk of gene transfer to wild and weedy relatives that grow in sympathy and with overlapping flowering times should be studied, in order to evaluate the likelihood that such an event occurs. Ae. geniculata, Ae. bicornis and Ae. triuncialis are colonizing species with the capacity to develop large stands, up to many hectares, that could increase their invasiveness under the selection pressure of the herbicide if they acquire the herbicide resistance gene. The risk of introgression of the wheat transgenes into the genome of these Aegilops species is related to the occurrence of meiotic recombination during chromosome pairing in the meiosis of the F1 hybrids. The introgression may be achieved either by hybridization with transgenic wheat and recurrent backcrossing with the Aegilops parent or by the “bridge” of the amphiploids. This second route of spontaneous amphiploid production, via doubling the chromosomes can permit a good bivalent pairing and overcome the sterility of the F1 hybrids. In the case of Ae. geniculata-wheat, the F2 fertility varied between 0 and 36 seeds in 100 spikelets among combinations, with one Ae. geniculata × ‘Castan’ F2 plant that reached up to 66.7 seeds in 100 spikelets (Loureiro et al., 2008), while the F2 amphiploids between Ae. triuncialis and wheat are also fertile and, indeed, plants producing up to 11 F1 grains can be found (Loureiro, unpublished). In both cases F3 seeds were well-developed.

Further studies of the recombination in the meiosis of the amphiploid hybrids are essential in order to determine whether a transgene may be transferred to the wild genome. In addition, the possible fertility of these amphiploids increases the likelihood of them becoming a new species in which a transgene would be maintained easily. Data on amphiploid frequency and fertility will without doubt be useful in assessing the potential risks of future transgenic wheat cultivars.

Acknowledgements

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

References

ARIA D M., RIESEBERG L.H., 1994. Gene flow between cultivated and wild sunflower. Theor Appl Genet 89, 655-660. doi:10.1007/BF00223700.

ARRIOLA P.E., ELLSTRAND N.C., 1996. Crop-to-weed gene flow in the genus Sorghum (Poaceae): spontaneous interspecific hybridization between johnsongrass, Sorghum halepense, and crop sorghum, S. bicolor. Am J Bot 83, 1153-1160. doi:10.2307/2446198.

BARTSCH D., POHL-ORF M., 1996. Ecological aspects of transgenic sugar beet: transfer and expression of herbicide resistance in hybrids with wild beets. Euphytica 91, 55-58. doi:10.1007/BF00035276.

BLACKSHAW R.E., HARKER K.N., 2002. Selective weed control with glyphosate in glyphosate-resistant spring wheat (Triticum aestivum). Weed Tech 16, 885-892. doi:10.1614/0890-037X(2002)016[0885:SWCWGI]2.0.CO;2.

BRETAGNOLLE F., THOMPSON J.D., 1995. Gametes with somatic chromosome number: mechanism of their formation and role in the evolution of autopolyploid plants. New Phytol 129, 1-22. doi:10.1111/j.1469-8137.1995.tb03005.x.

DARMENCY H., 1994. The impact of hybrids between genetically modified crop plants and their related species – introgression and weedyness. Mol Ecol 3, 37-40. doi:10.1111/j.1365-294X.1994.tb00305.x.

DARMAINCY H., 1994. The impact of hybrids between genetically modified crop plants and their related species – introgression and weedyness. Mol Ecol 3, 37-40. doi:10.1111/j.1365-294X.1994.tb00305.x.

DOEBLEY J., 1990. Molecular evidence for gene flow among Zea species. BioSci 40, 443-448. doi:10.2307/1311391.

GUADAGNUOLO R., SAVOYA-BIANCHI D., FELBER F., 2001. Gene flow from wheat (Triticum aestivum L.) to jointed goatgrass (Aegilops cylindrica Host), as revealed by RAPD and microsatellite markers. Theor Appl Genet 103, 1-8. doi:10.1007/s001220100636.

HARLAN J.R., DE WET J.M.J., 1975. On Ø. Winge and a prayer: the origins of polyploidy. Bot Rev 41, 361-390. doi:10.1007/BF02860830.

JAUHAR P.P., 2003. Formation of 2n gametes in durum wheat haploids: sexual polyploidization. Euphytica 133, 81-94. doi:10.1023/A:1025692422665.

JAUHAR P.P., DO RAMACI-ALTUNTEPE M., PETERSON T.S., ALMOUSELEM A.B., 2000. Seedset on synthetic haploids of durum wheat: cytological and molecular investigations. Crop Sci 40, 1742-1749.
Jørgensen R.B., Andersen B., 1994. Spontaneous hybridisation between oilseed rape (Brassica napus) and weedy B. campestris (Brassicaceae): A risk of growing genetically modified oilseed rape. Am J Bot 81, 1620-1626. doi:10.2307/2445340.

Kimber G., Tsunewaki K., 1988. Genome symbols and plasma types in the wheat group. 7th Intnl. Wheat Genet. Symp. (Miller T.E. and Koebner R.M.D., eds), Bath Press, Bath, Avon, Cambridge, England, U.K., pp. 1209-1210.

Landjeva S., Ganeva G., 1998. Transfer Aegilops ovata chromosomes into bread wheat. Proc. 9th EWAC Conference, Viterbo, Italy, 16-19 June 1997. Newsletter 29-33.

Landjeva S., Ganeva G., 1999. Identification of Aegilops ovata chromosomes added to wheat (Triticum aestivum L.) genome. Cereal Res Commun 27, 55-61.

Loureiro I., 2005. Estudio del riesgo potencial agrícola y medioambiental del cultivo de trigo tolerante a herbicidas. Doctoral thesis. Universidad Complutense, Madrid. [In Spanish].

Loureiro I., Escorial M.C., García-Baudín J.M., Chueca M.C., 2006. Evidence of natural hybridization between Aegilops geniculata and wheat under field conditions in Central Spain. Environ Biosaf Res 5, 105-109. doi:10.1051/ebi:2006020.

Loureiro I., Escorial M.C., García-Baudín J.M., Chueca M.C., 2007. Hybridization between wheat (Triticum aestivum) and the wild species Aegilops geniculata and A. biuncialis under experimental field conditions. Agric Ecosyst Environ 120, 384-390. doi:10.1016/j.agee.2006.10.015.

Loureiro I., Escorial M.C., García-Baudín J.M., Chueca M.C., 2008. Importance of the hybridization and the fertility of the hybrids between wheat and Aegilops geniculata in the herbicide resistance transfer. Weed Res 48, 561-570. doi:10.1111/j.1365-3180.2008.00659.x.

Maan S.S., Sasakuma T., 1977. Fertility of amphihaploids in Triticinae. J Hered 68, 87-94.

Ramsey J.D., Schemske D.W., 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annu Rev Ecol Syst 29, 467-501. doi:10.1146/annurev.ecolsys.29.1.467.

Ramsey J.D., Schemske D.W., 2002. Neopolyploidy in flowering plants. Annu Rev Ecol Syst 33, 589-639. doi:10.1146/annurev.ecolsys.33.010802.150437.

Rieseberg L.H., 1997. Hybrid origin of plant species. Annu Rev Ecol Syst 28, 359-389. doi:10.1146/annurev.ecolsys.28.1.359.

Sax K., 1937. Effect of variations in temperature on nuclear and cell division in Tradescantia. Am J Bot 24, 218-225. doi:10.2307/2436408.

Schoenenberger N., Guadagnuolo R., Savoabianchi D., Küpfner P., Felber F., 2006. Molecular analysis, cytogenetics and fertility of introgression lines from transgenic wheat to Aegilops cylindrica Host. Genetics 174, 2061-2070. doi:10.1534 genetics.106.058529.

Sears E.R., 1941. Chromosome pairing and fertility in hybrids and amphihaploids in the Triticeae. Missouri Agric Exp Sta Res Bull 337, 1-20.

Seefeldt S.S., Zemetra R., Young F.L., Jones S.S., 1998. Production of herbicide resistant jointed goatgrass (Aegilops cylindrica) × wheat (Triticum aestivum) hybrids in the field by natural hybridization. Weed Sci 46, 632-634.

Stebbins G.L., 1946. Plant evolution through amphiplody. Ecology 26, 420-421. doi:10.2307/1931668.

Stebbins G.L., 1950. Variation and evolution in plants. Columbia University Press. New York.

Tschermak E.V., Bleier H., 1926. Über fruchtbare Aegilops-Weizenbastarde. Berichte der Deutschen Botanischen Gesellschaft 44, 110–132. [In German].

Van Slageren M.W., 1994. Wild wheats: a monograph of Aegilops L. and Amblyopyrum (Jaub. & Spach.) Eig (Poaceae). Wageningen Agricultural University and ICA-DA, Wageningen.

Wang Z., Zemetra R.S., Hansen J., Mallory-Smith C.A., 2001. The fertility of wheat x jointed goatgrass hybrid and its backcross progenies. Weed Sci 49, 340-345. doi:10.1614/0043-1745(2001)049[0340:TFOWJG]2.0.CO;2.

Xu S.J., Joppa L.R., 1995. Mechanisms and inheritance of first division restitution in hybrids of wheat, rye and Aegilops squarrosa. Genome 38, 607-615.

Zaharieva M., Monneveux P., 2006. Spontaneous hybridization between bread wheat (Triticum aestivum L.) and its relatives in Europe. Crop Sci 46, 512-527. doi:10.2135/cropsci2005.0023.

Zemetra R.S., Hansen J., Mallory-Smith C.A., 1998. Potential for gene transfer between wheat (Triticum aestivum) and jointed goatgrass (Aegilops cylindrica). Weed Sci 46, 313-317.

Zhou H., Berg J.D., Blank S.E., Chay C.A., Chen G., Eskelsen S.R., Fry J.E., Hoit, U.H., Isakson P.J., Lawton M.B., Metz S.G., Rempele C.B., Ryerson D.K., Sansone A.P., Shook A.L., Starke R.J., Tichota J.M., Valentini S.A., 2003. Field efficacy assessment of transgenic Roundup Ready wheat. Crop Sci 43, 1072-1075.