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Hybridization in Turkish *Aegilops* L. Species

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Abstract: Spontaneous hybridization in wild wheat species are frequently observed in natural flora of Turkey. The aim of this study is to investigate the incidence of hybridization in *Aegilops* L. species under natural conditions. *Aegilops* populations of 3 different ploidy level, collected from south east Turkey were sown in mixed plots with tetraploid and hexaploid wheat land races of the same origin. Harvested seed materials were sown in the following year to see incidence of hybridization. Out of 73 *Aegilops* entries, 16 of them consisting of 5 polyploid species gave hybrid individuals. Hybrids were characterized for 5 traits to check similarity between them. Significant differences and similarities were observed among the hybrids, in terms of the measured and observed characters. Results of the study suggest that introgression from the relatives into *Aegilops* species is quite possible. Another point to underline is many of the seed multiplication programs do not mind space isolation between self fertilizing species of wild and cultivated wheat species. Accomplishment of seed multiplication and rejuvenation studies may require some degree of space isolation to avoid contamination between populations.

Key words: Introgression, hybridization, wild wheat, *Aegilops*, characterization

INTRODUCTION

Wheat genus (both *Triticum* L. and *Aegilops* L.) has several species in different ploidy levels, namely diploid (2n = 14), tetraploid (2n = 28) and hexaploid (2n = 42). Number of wild wheat relatives is reported as 27 in the world, which 20 of them occur in Turkey (Kimber and Feldman, 1987). Those species include the primary parents *Triticum monococcum* L. sp. *aegilopoides* (Link) Thell., *T. urartu* Tum. ex. Gand., *Aegilops speltoides* Tausch., *T. turgidum* L. sp. *dicoccoides* (Körn. ex Asch. and Graebn.) and *A. tauschii* Coss..

Gene flow is transfer of genetic information between populations of the same or different species (Waines and Hegde, 2003) and have been taking place long before the start of agriculture. Cronquist (1988) defined it as gene leakage from one to another diluting the taxanomic distinction.

Jarvis and Hodgkin (1999) reported that being an ongoing process, introgression between crop cultivars and their wild relatives affected the genetic diversity of crops. In addition to this, introgressive hybridization played crucial role in the evolution of many crop species, especially the polyploids (Weissmann *et al*., 2005). Introgression can greatly enrich the gene pool of recipient species, increase its evolutionary potential and in extreme cases lead to speciation (Weissmann *et al*., 2005). Many cultivated plant species are derived from spontaneous introgressions. Typical example for interspecific hybridization is modern cultivated wheat. It is scientifically agreed that tetraploid wheat is a hybrid between *T. aegilopoides* or *T. urartu* (Dvorak, 1976; Valkoun *et al*., 1997) x *A. speltoides* (Dvorak, 1998) or some members from Sitopsis Section (Kimber and Feldman, 1987). Primary product of this hybridization at this stage is *T. dicoccoides* (Nevo *et al*., 2002). Hexaploid wheat is a hybrid of *T. dicoccoides* x *A. tauschii* (Kimber and Feldman, 1987; Waines, 1997).

Recent developments in genetic engineering especially introduction of Genetically Modified Organisms (GMO) into cultivation, developed considerable interest in the issue of gene transfer from cultivated plants to their wild relatives. Several risks have been reported to be associated with possible introgression from cultivated to the wild. The spread of transgenic herbicide resistance may result in establishment of undesired super weeds (Snow *et al*., 2002; Morrison *et al*., 2002; Waines and Hegde 2003). Conceivable end products of such transgene introgression are given terrifying names such as Frankenweeds and superweeds (Steward *et al*., 2003).

Weeds equipped with new resistance genes against risk factors are likely to compete better than the other species cohabiting the same environment. Under such circumstances it is inevitable that the so called superweed would change the botanical composition dramatically by withdrawing certain portion of the plant species sharing

the same habitat. The crops most likely to increase extinction risk by gene flow are those that are planted in new locations that bring them in the vicinity of wild relatives, thereby increasing the hybridization risk by proximity (Ellstrand, 2001). For example one can imagine a new variety that has increased salinity tolerance that can now be planted within the range of an endangered relative (Ellstrand, 2001). This is even more important in the centers of origin and/or diversity of cultivated crops where evolution is still under process.

Several conditions are needed to be satisfied for gene flow to take place from cultivated to the wild. First condition is existence of the wild at proximity of the cultivated. It is a known fact that cultivated wheat self pollinates and blossoms even before heading time. In Turkey a wild relative of wheat *A. cylindrica* (4 n) is one of the major weeds in wheat field and they are also abundant at the sides of the cultivated areas and in disturbed habitats such as road sides and abandoned fields. Other polyploid wheat relatives found around the fields are; *A. triuncialis* (4 n), *A. biuncialis* (4 n), *A. ovata* (4 n), *A. columnaris* (4 n) in central and south eastern Turkey which are the major wheat growing areas. In addition to these species *A. triaristata* (4n, 6n), *A. crassa* (4 n, 6 n) and *A. juvenalis* (6 n) are frequently found within the vicinity of cultivated areas of south east Anatolia. Hanson *et al*. (2005) reported that the maximum distance, that gene flow occurs between wheat cultivars was reported as 42 m. Direction of the prevailing wind, temperature and humidity during pollination are another factors for increased gene flow.

Overlapped flowering time and duration are other factors for gene flow between cultivated and wild. It is generally the case that the wild wheat relatives flower and get mature earlier than the cultivated varieties. Heading time for wheat land races coming from areas with diverse climatic conditions were between 147-177 days after germination (Zencirci and Karagöz, 2005). Depending on the species, *Aegilops* species begin to flower on 6 May and anthesis continue until 30 May in Central part of Turkey (Kün, 1979). Similar to anthesis, maturity time ranges from 14 June till 13 July (Kün, 1979). In the same area, flowering time for wheat cultivars begin on 26 May and continues until 13 June (Karagöz and Zencirci, 2005). It implies that certain period of anthesis in cultivated and wild wheat species overlap.

Synchronized anthesis enable a suitable media for gene flow. In natural conditions anthesis is even more elongated in certain circumstances. Late coming rainfall promotes elongated anthesis period. It was also observed in Central Anatolia that *Aegilops cylindrica* populations may even continue anthesis until the end of July in wet and relatively cool summers. Plant density and competition between the individuals are other factors affecting the anthesis period of wild wheat relatives in natural flora. In absence of competition, plants tend do elongate anthesis.

Two of the tetraploid *Aegilops* species; *A. cylindrica* and *A. triuncialis* occur as weeds in almost all wheat production areas of Turkey. According to Hegde and Waines (2004), these species have been known to introgress occasionally with bread wheat when grown near wheat fields (Fig. 1). Among the diploid relatives, intermediate forms of *A. speltoides* sp. *ligustica* and *A. speltoides* sp. *aucheri* are frequently observed in nature.

Reporting several occurrence and combinations of female *Aegilops* x male *Aegilops* Van Slageren (1994)

Fig. 1: Left: Spontaneous hybrid of *Aegilops cyclindrica* from central Anatolia; center and right spontaneous hybrids from south east Turkey with unknown parents

underlined that, vast majority of the combinations are tetraploid x tetraploid.

MATERIALS AND METHODS

Materials of the experiment consisted of 73 *Aegilops* populations (Table 1) collected from Southeast Turkey in 1987 (Kiziltan *et al*., 1990) and during 1994-1995 (Karagöz, 1998). The accessions were multiplied first in 2001 at Research and Production Farm of Central Research Institute for Field Crops, Haymana, Ankara. Each plot consisted of 3 rows. Rows were 3.0 m long, distance between rows were 0.35 m and space between the blocks were 1.0 m. Sowing rate for wild wheat was 30 seeds row^{-1}. Sides of the plots were sown durum (tetraploid) and bread (hexaploid) wheat land races. Landrace material was of the same origin as the wild relatives. Sowing rate of the land races was 10 g row^{-1}. Wild wheat plots were harvested manually at the time of maturity and spikes were threshed by means of a mechanical barley awn thresher. In the second year of the experiment, wild wheat material was sown once again in 5 row plots. Row length and width as well as the sowing rate were the same as the previous year. The plots were not replicated.

During the heading time, wild wheat populations were observed for presence of hybrid plants. Number of hybrid plants in each plot were recorded. Spikes of hybrids were harvested individually. Number of spikes per hybrid plants, number of spikelets per spike, length of spikes and awns (mm), length and width of spikelets (mm), glume color (white, white-cream, cream-white, cream, brown, black) and glume hairiness (naked, slightly hairy, hairy, densely hairy) were recorded. Measurements were taken by means of calipers. Statistical analysis was performed on 10 randomly selected spikes of each hybrids (Table 2). Similarities between the hybrids were tested by means of Multivariate Analysis using the above mentioned traits.

RESULTS AND DISCUSSION

Among the 73 populations tested, 16 of them consisting of 5 species gave hybrid individuals (Table 1).

Table 1: Species, ploidy level, number accessions sown for each species and number of accessions with hybrid plants

Species	Ploidy level	No. of populations	No. of populations with hybrids
A. umbellulata Zhuk.	2 n	14	0
A. biuncialis Vis.	4 n	15	8
A. columnaris Zhuk.	4 n	16	3
A. triuncialis L.	4 n	20	3
A. triaristata Willd.	(4 n, 6 n)	1	1
A. ovata L.	4 n	4	1
A. crassa Boiss.	(4 n, 6 n)	1	0
A. vavilovi (Zhuk.) Chennav.	6 n	2	0

All the hybrids were sterile. Hybridization was only observed in polyploid species. As it was suggested by Van Slageren (1994), vast majority of the combinations are tetraploid X tetraploid.

Significant differences among the hybrids were observed in terms of the measured and observed characters (Fig. 3-5). Differences were determined even among the individuals of the same populations. Since hybridization was not performed in controlled conditions, it is not possible to speak about the paternal material. However observed diversity among the hybrid plants of the same populations suggest that they might have been hybridized by male plants of different origin.

Populations formed three major clusters in terms of the similarities based on measured characteristics (Fig. 2). Out of 11 hybrid individuals of *A. biuncialis*, 9 of them took place in the same cluster. The other 2 hybrids namely, *A. BIUN. I-4* and *A. BIUN. VII-1* were located in cluster 2 and 3 respectively. While 3 hybrids of population *A. BIUN. I* took place in cluster 1, fourth individual of the same population (*A. BIUN. I-4*) was in cluster 2. Dissimilarity was probably due to the fact that it was hybridized by a different male than the other three. All the three populations of *A. columnaris* were located in 3 of the clusters. Conversely all the three individuals of *A. ovata* were placed in the same

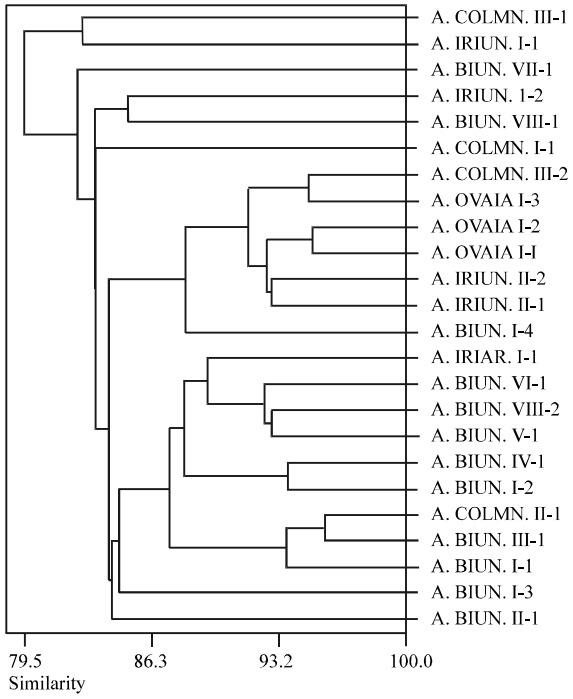

Fig. 2: Similarity dendogram based on measured characters

Table 2: Spike length (mm), awn length (mm), No. of spikelets per spike, length and width of spikelets (mm) of the species, accession numbers and given population numbers

Species	Accession No.	Population No	Hyb. No	Spike length (mm)	Awn length (mm)	No of spiklets spike	Spikelet length (mm)	Spikelet width (mm)	Glume color	Glume haireness
A. biuncialis	TUR00021	A.BIUN.I	1	59.50±2.76	64.70±1.45	7.00±0.21	14.00±0.93	4.40±0.22	White-cream	Naked
			2	61.90±3.48	57.20±3.10	6.70±0.26	16.00±0.21	4.70±1.53	Brown-cream	Naked
			3	64.80±5.41	70.70±4.39	5.80±0.13	16.70±0.70	5.30±0.15	Cream-brown	Very hairy
			4	72.80±2.86	63.70±2.84	7.30±0.30	15.90±0.53	4.70±0.21	White-cream	Naked
	TUR00002	A.BIUN.II	1	69.70±2.47	49.20±1.81	7.50±0.27	15.30±0.82	4.90±0.18	Brown	Sl. hairy
	TUR00047	A.BIUN.III	1	57.25±2.66	62.25±3.00	6.00±0.38	14.63±0.73	4.88±0.23	Cream-brown	Sl. hairy
	TUR00102	A.BIUN.IV	1	64.78±3.40	56.33±4.24	6.33±0.29	14.22±0.40	5.11±0.35	Black	Sl. hairy
	TUR00184	A.BIUN.V	1	57.50±1.95	52.40±1.93	7.40±0.34	15.20±0.29	4.90±0.23	Cream	Sl. hairy
	TUR00385	A.BIUN.VI	1	50.60±2.03	55.70±6.79	5.60±0.22	14.20±0.49	4.50±0.22	Cream	Naked
	TUR01406	A.BIUN.VII	1	51.00±1.69	83.10±1.74	5.80±0.25	14.90±0.57	5.20±0.13	Cream	Sl. hairy
	TUR01712	A.BIUN.VIII	1	50.30±1.62	70.40±1.54	5.90±0.28	13.90±0.53	5.00±0.15	Black	Sl. hairy
			2	54.00±3.24	54.20±5.02	6.80±0.25	15.80±0.65	4.80±0.20	Black	Sl. hairy
A. triaristata	TUR00227	A.TRIAR.I	1	61.00±2.12	48.80±3.22	8.40±0.24	12.20±0.49	5.00±0.00	Black	Black
A. triuncialis	TUR00257	A.TRIUN.I	1	89.75±3.04	45.25±1.32	9.50±0.50	14.00±1.68	4.50±0.29	Cream	Black
A. triuncialis	TUR00597	A.TRIUN.II	1	56.70±3.04	75.20±1.32	6.10±0.50	16.00±1.68	5.50±0.29	Cream	Sl. hairy
A. triuncialis	TUR00569	A.TRIUN.III	1	79.00±2.56	59.80±3.71	9.40±0.34	15.60±0.72	3.60±0.27	White	Naked
			2	81.80±5.90	59.60±2.79	8.90±0.55	18.30±0.45	4.50±0.17	Brown	Naked
A. ovata	TUR00376	A.OVATA I	1	84.70±4.06	55.00±2.37	9.50±0.50	17.00±0.58	3.90±0.23	Black	Sl. hairy
			2	83.90±4.75	57.20±2.71	9.60±0.31	15.70±0.52	4.70±0.15	Black	Hairy
			3	75.25±3.53	57.13±1.36	9.00±0.46	15.13±0.61	4.25±0.25	Black	Hairy
A. columnaris	TUR00814	A.CLMN.I	1	76.33±4.26	44.00±3.61	10.67±0.88	17.00±0.58	6.33±0.33	Black	Hairy
A. columnaris	TUR00936	A.CLMN.II	1	56.80±4.40	62.00±1.79	5.80±0.37	17.00±0.45	4.80±0.20	Brown	Sl. hairy
A. columnaris	TUR01000	A.CLMN.III	1	96.40±4.78	51.50±2.26	10.90±0.50	16.90±0.46	4.80±0.20	White-cream	Sl. hairy
			2	73.00±2.48	57.25±5.31	8.25±0.25	16.75±0.63	4.75±0.25	Brown	Sl. hairy

Fig. 3: *A. biuncialis* hybrids. Left TUR 00002 (*A. BIUN. II-1*). center TUR 01712 (*A. BIUN. VIII-1,2*), right TUR 00047 (*A. BIUN. III-1*)

cluster and similarity between them was very high. This may suggest that all of the *A. ovata* hybrids were hybridized by the same paternal material.

This hybridization study suggests that gene exchange between wheat relatives is quite possible in nature. Although our findings are not supported by molecular evidences, significant diversity was observed within the hybrid plants. This implies that several of the wild relatives were introgressed with wheat land races to form hybrid individuals.

All the hybrid individuals we observed were the tetraploid ones. This finding is in harmony with Van Slageren (1994). It was reported by Kimber and Feldman (1987) that wild relatives of wheat tend to act as maternal material in hybridization process, which was the case in this study.

Despite the great progress achieved in preventing transgene escape, most gene containment strategies have their weaknesses and in no case have these methodologies been field-tested and/or been shown to

Fig. 4: Left *A. triaristata* hybrid TUR 00227 (*A. TRIAR. I-1*); Center and right *A.triuncialis* hybrids TUR 00252 (*A. TRIUN. I-1*), TUR 00569 (*A. TRIUN. II-1* and *2*)

Fig. 5: Left: *A. ovata* hybrid TUR 00376 (*A. OVATA I-1*); center and right *A. columnaris* hybrids TUR 00936 (*A. CLMN. II-1*) and TUR 00814 (*A. CLMN. I-1*)

be 100% effective (Chapman and Burke, 2006). Keeping in mind the possibility of introgressive hybridisation, one should be cautious with introducing exotic or GMO material to the areas of center of diversity such as south east Turkey. Introduction of alien genes may result in orienting wheat evolution in an unexpected direction. In many places, *Aegilops* habitats are side by side with wheat fields in Turkey, that the distance is much shorter than the distance for gene flow to take place between wheat cultivars (Hanson *et al*., 2005).

Many of the seed multiplication programs do not mind spatial isolation between self fertilizing species of wild and cultivated wheat species. Our study indicated that gene flow occurs in wheat genus. Accomplishment of seed multiplication and rejuvenation studies may require some degree of isolation. If it can not be applied to all species at least polyploid *Aegilops* species can be isolated to avoid contamination of populations.

REFERENCES

Chapman, M.A. and J.M. Burke, 2006. Letting the gene out of the bottle: The population genetics of genetically modified crops. New Phytol., 170: 429-443.

Cronquist, A., 1988. The Evolution and Classification of Flowering Plants. The New York Botanical Garden, Bronx, USA.

Dvorak, J., 1976. The relationships between the genome of *Triticum urartu* and the A and B genomes of *Triticum aestivum.* Can. J. Genet. Cytol., 18: 371-377.

Dvorak, J., 1998. Genome analysis in the *Triticum-Aegilops alliance*. In: Proceedings of the 9th International Wheat Genetics Symposium, Slinkard, A.E. (Ed.)., University of Saskatchewan, Canada, pp: 8-11.

Ellstrand, N.C., 2001. When transgenes wander, should we worry? Plant Physiol., 125: 1543-1545.

Hanson, B.D., C.A. Mallory-Smith, B. Shafii, D.C. Thill and R.S. Zemetra, 2005. Pollen mediated gene flow from blue aleurone wheat to other wheat cultivars. Crop Sci., 45: 1610-1617.

Hegde, S.G. and J.G. Waines, 2004. Hybridization and introgression between bread wheat and wild and weedy relatives in North Am. Crop Sci., 44: 1145-1155.

Jarvis, D. I. and T. Hodgkin, 1999. Wild relatives and crop cultivars: Detecting natural introgression and farmer selection of new genetic combinations in agroecosystems. Mol. Ecol., 8: 159-173.

Karagöz, A., 1998. *In situ* conservation of plant genetic resources in Ceylanpınar State Farm. In The Proceedings of the International Symposium on *In Situ* Conservation of Plant Genetic Diversity Zencirci, N., Z. Kaya, Y. Anikster and W.T. Adams (Eds.), CRIFC, Ankara, pp: 87-92.

Karagöz, A. and N. Zencirci, 2005. Variation in wheat (*Triticum* sp.) land races from different altitudes of three regions of Turkey. Genetic Resou. Crop. Evol., 52: 775-785.

Kimber, G. and M. Feldman, 1987. Wild wheat. An introduction. Special Report 353. College of Agriculture, University of Missouri-Columbia.

Kiziltan, M., A. Karagöz and J.G. Waines, 1990. Plant expedition research from south eastern Anatolia (Atatürk Dam) C7 square. Wheat Information Service, 70: 15-28.

Kün, E., 1979. Investigation on some important characteristics of wild wheats (*Aegilops* L.) of Turkey, in relation to wheat breeding. Ankara University, Agricultural Faculty, Publ. No: 685, Scientific Research and Investigation: 400, Ankara.

Morrison, L.A., O. Riera-Lizarazu, L. Cremieux and C.A. Mallory-Smith, 2002. Jointed goatgrass (*Aegilops cylindrica* Host) x Wheat (*Triticum aestivum* L.) Hybrids: Hybridization dynamics in oregon wheat fields. Crop Sci., 42: 1863-1872.

Nevo, E., A.B. Korol, A. Beiles and T. Fahima, 2002. Evolution of Wild Emer and Wheat Improvement. Springer-Verlag, Berlin Heidelberg.

Snow, A.A., D. Pilson, L.H. Rieseberg and H.M. Alexander, 2002. Ecological Effects of Pest Resistance Genes That Disperse into Weed Populations. In Proceedings of the 7th International Symposium on The Biosafety of Genetically Modified Organisms, October 2002 Beijing (http://www. worldbiosafety.net/).

Steward, Jr. C.N., D.H. Matthew and S.L. Warwick, 2003. Transgene introgressions from genetically modified crops to their wild relatives. http://www.nature.com/reviews/genetics. October 2003, 4:806-817.

Valkoun, J., J.G. Waines and J. Konopka, 1997. Current Geographical Distribution and Habitat of Wild Wheats and Barley. Proceedings of Harlan Symposium. 10-14 May Aleppo. Damania *et al.* (Eds.). Part 6. www.ipgri.cgiar.org/Publications/HTML Publications/

Van Slageren, M.W., 1994. Wild wheats: A monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. and Spach) Eig (Poaceae). Wageningen Agricultural University Papers 94-7, Wageningen, The Netherlands.

Waines, J.G., 1997. *In situ* Conservation of Wild Relatives of Crop Plants in Relation to Their History. Proceedings of Harlan Symposium. 10-14 May Aleppo. Damania *et al.* (Eds.). Part 6. www.ipgri.cgiar.org/Publications/HTML Publications/

Waines, J.G. and S.G. Hegde, 2003. Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. Crop Sci., 43: 451-463.

Weissmann, S., M. Feldman and J. Gressel, 2005. Sequence evidence for sporadic intergeneric DNA introgression from wheat into a wild *Aegilops* species. Molecular Biol. Evol., 22: 2055-2062.

Zencirci, N. and A. Karagöz, 2005. Effect of developmental stages length on yield and some quality traits of Turkish durum wheat (*T. turgidum* L. Conv. *durum* (Desf.) M K.) land races. Genetic Resou. Crop Evol., 52: 765-774.