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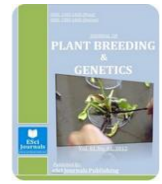

SOME CONSTRAINTS ON INTERSPECIFIC CROSSING OF DURUM WHEAT WITH *AEGILOPS TAUSCHII* ACCESSIONS SCREENED UNDER WATER-DEFICIT STRESS

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ABSTRACT

A total of 400 accessions of *Aegilops tauschii* Coss. (goat grass) collected from western Asia and the Caucasus were screened for the productive tillering capacity under rain-fed field conditions with the aim of developing new, synthetic hexaploid wheats having enhanced drought adaptation. Of these, 23 *A. tauschii* accessions were selected for interspecific crossing with two durum wheat varieties 'Belikh-2' and 'Jennah Khetifa'. Fifteen of the selected accessions were of Pakistani origin and exhibited early ear-emergence and low cross-compatibility, and five accessions were from Iran and Turkmenistan and exhibited high cross-compatibility. A wide variation among accessions in cross-compatibility might be related to their region of origin. Successful hybridization resulted in the formation of immature embryos, which are capable of regenerating to plants on culture medium. The *A. tauschii* accession ig 47219, of Turkmenistan origin, gave the highest frequency of embryos in crosses with both wheat varieties, but regeneration from the crosses with 'Belikh-2' failed due to the occurrence of hybrid necrosis. Thus, a high frequency of embryo production did not always result in the satisfactory development of hybrid plants. Treatment of the hybrid plants with colchicine was essential for the successful set of hexaploid seeds on the newly-synthesized plants. These constraints were discussed for the efficient development of new, synthetic hexaploid wheats.

Keywords: *Triticum aestivum*, *T. turgidum*, *Aegilops tauschii*, drought, synthetic wheat, cross-compatibility, hybrid necrosis, pre-breeding.

INTRODUCTION

In dry and rain-fed agricultural regions, many water-saving technologies have been developed for improving bread wheat (*Triticum aestivum* L.) production. These include tillage, mulching, adjusted planting density and crop rotation (Deng *et al.,* 2005). A further option under the Mediterranean environments with seasonal rainfall and terminal drought is the planting of "water-saving" wheat genotypes, in which water uptake by the roots is moderate and grain yield is relatively high (Mori *et al.,* 2011; Mori and Inagaki 2012). Different root systems have been also adapted to optimize the timing of water uptake from the soil depending on the drought-prone field environments (Manschadi *et al.,* 2006). The improvement of bread wheat germplasm for such drought adaptation increasingly requires developing new genetic resources as pre-breeding germplasm. Interspecific crossing that has not been failed during the process of wheat evolution is one of the approaches to enlarge the genetic variation of wheat germplasm.

Bread wheat can be artificially synthesized from interspecific crosses between durum wheat (*T. turgidum* L. ssp. *durum* (Desf.) Husn.) and *Aegilops tauschii* Coss.(common name; goat grass), and is providing an emerging genetic resource not only for increasing resistance to various biotic stresses but also to abiotic stresses such as to drought (Halloran *et al.,* 2008; Mujeeb-Kazi 1995; Sohail *et al.,* 2011; Trethowan and van Ginkel 2009). So far, a large number of synthetic wheat genotypes have been derived from cross combinations between durum wheat genotypes and *A. tauschii* accessions that enjoy high cross-compatibility, but are mostly irrespective of targeted-stress traits. (Matsuoka *et al.,* 2007; Zhang *et al.,* 2008; Takumi *et al.,* 2009; Ogbonnaya *et al.,* 2013). There is still insufficient

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information on the successful production of new, synthetic wheats having enhanced drought adaptation.

A large number of accessions of *A. tauschii* have been collected over an extensive area of western Asia and the Caucasus region, where hexaploid wheat is thought to have originated spontaneously some 8000 years ago from natural crosses of tetraploid wheat with diploid *A. tauschii* (Feldman 2001). More than 400 of these accessions have been characterized by the International Center for Agricultural Research in the Dry Areas (ICARDA) and some of these have already been used to develop pre-breeding wheat germplasm (Inagaki *et al.,* 2007; Valkoun 2001).

Seed sets successfully on durum wheat hybridized with *A. tauschii* to produce hybrid embryos (triploid) but these develop insufficient endosperm. It is possible to subject these embryos to artificial rescue and chromosome doubling by colchicine treatment to recover fertile hexaploid wheats. Cross-compatibility, plant regeneration and chromosome doubling are three factors for successful production of new, synthetic wheats.

This study aims to examine how these factors affect the production of new synthetic wheats with drought adaptation. A total of 400 accessions of *A. tauschii* were screened for productive tillering capacity under water-deficit field conditions. The selected *A. tauschii* accessions were then crossed with two durum wheat varieties. Artificial rescue of the immature hybrid embryos and colchicine treatment of the hybrid plants developed for chromosome doubling were also examined.

MATERIALS AND METHODS

Screening of *A. tauschii* accessions under water-deficit field conditions: Two sets of 200 accessions of *A. tauschii* were planted in late November, one set in each of the two cropping seasons 2008 and 2010 at the ICARDA Experiment Station Station (Latitude; 36°01'N, Longitude; 36°56'E, Altitude; 291 m), Tel Hadya, near Aleppo, Syria. A total of 400 accessions of *A. tauschii* used for screening are grouped by their origins in Table 1. The largest group had 69 accessions from Iran.

Field experiments in each year were performed in an alpha design (20 accessions x 10 blocks) with two replications. Each plot consisted of two rows 100 cm long and 30 cm apart, and each plot was surrounded by two rows of a winter wheat variety to reduce growth competition between accessions. Seeding rate was 100 spikelets (approximately 200 grains) per plot. Total annual rainfall during the two cropping seasons was 223 mm in 2008 and 287 mm in 2010, whereas the long-term annual average is 338 mm. Growth was accelerated by above-average temperatures during the spring of 2010.

For characterization of the wild species, the following five traits were recorded; growth habit, number of days to ear emergence, number of spikes per plant, plant height, and spike length. The number of spikes per plant and visual greenness of leaves after ear emergence were used as screening criteria.

Crossing of durum wheat and selected *A. tauschii* accessions: Two durum wheat varieties 'Belikh-2' and 'Jennah Khetifa' were used as female parents. 'Belikh-2' is an improved, short-stature variety in Syria and Lebanon. It is highly cross-compatible with both *Hordeum bulbosum* L. and maize (*Zea mays* L.) in wheat haploid production through preferential chromosome elimination (Inagaki and Tahir 1995). 'Belikh-2' is also promising in tolerance to saline soil (Katerji *et al.,* 2005). 'Jennah Khetifa' is a Morocco-Tunisia landrace of tall stature, having high cross-compatibility with maize (Sourour *et al.,* 2011), high penetrability of roots into dry and hard soil (Kubo *et al.,* 2007) and relatively high tolerance to salinity (Chahine *et al.,* 2013). According to information from GRIS (Genetic Resources Information System for Wheat and Triticale, http://wheatpedigree.net/), 'Belikh-2' relates genetically to 'Jennah Khetifa' in their pedigrees with a very low coefficient of parentage (at least 0.0625). A total of 23 accessions of *A. tauschii* selected previously under water-deficit stress were used as male (pollen) parents. Plant materials were grown with potted soil from December to May in a naturally-lit greenhouse with relatively short day-length and temperatures controlled to 23/15°C (day/night) and relative humidity controlled in the range of 60–90%.

Following a technical manual for wide hybridization for wheat doubled-haploid production (Inagaki 2003), durum wheat spikes were emasculated and pollinated with fresh pollen of *A. tauschii* accessions. No plant growth regulator was applied before or after pollination. Four spikes (as replications) per cross combination were used. All crossing procedures were conducted by one person to keep skill levels as uniform as possible. Numbers of seeds set were counted 10 days after pollination. Frequency (%) of seed set per 100 florets pollinated was calculated to assess cross-compatibility and this value was angularly transformed for statistical analysis based on the binomial distribution.

Regeneration of hybrid plants: At 12–16 days after pollination, immature embryos were aseptically excised from developed seeds and transferred to Murashige-Skoog culture medium supplemented with 20 g·L^{-1} sucrose and 6 g·L^{-1} agarose in plastic dishes. The incubation conditions were 25/15°C (day/night) with 12-hour day-length and 10,000 lux fluorescent light intensity. Regenerated plants with fully-developed roots and leaves were transferred to potted soil for further growth. The numbers of plants developing normally or withering in necrosis were counted per cross combination. Plants developed with 2–3 tillers were immersed in colchicine solution containing 1 g·L^{-1} colchicine, 20 mL·L^{-1} dimethyl sulfoxide and 10 drops·L^{-1} Tween-20 for five hours at room temperature. Treated plants were again transferred to potted soil following thorough washing with tap water overnight. Half of the plants obtained from a highly cross-compatible combination were not treated with colchicine solution as control. Potted plants were grown in a growth chamber with 20/10°C (day/night), 12-hour day-length and 30,000 lux fluorescent light intensity. The numbers of seeds set on the treated plants were counted.

RESULTS

Selection of *A. tauschii* accessions under water-deficit field conditions: The number of *A. tauschii* accessions that produced spikes was 181 accessions in 2008 and 127 accessions in 2010 under water-deficit conditions because some accessions having a winter growth-habit did not reach ear emergence. Figure 1 shows the relationship between days to ear emergence and number of spikes per plant for each of the accessions used in 2008 and 2010. It was found that early ear-emergence is associated with increased numbers of spikes per plant under water-deficit conditions (r=-0.712** for 2008, r=-0.672** for 2010). The accessions from Pakistan showed early ear-emergence and a wide variation in numbers of spikes per plant while the largest group, from Iran, had wide variations in both traits.

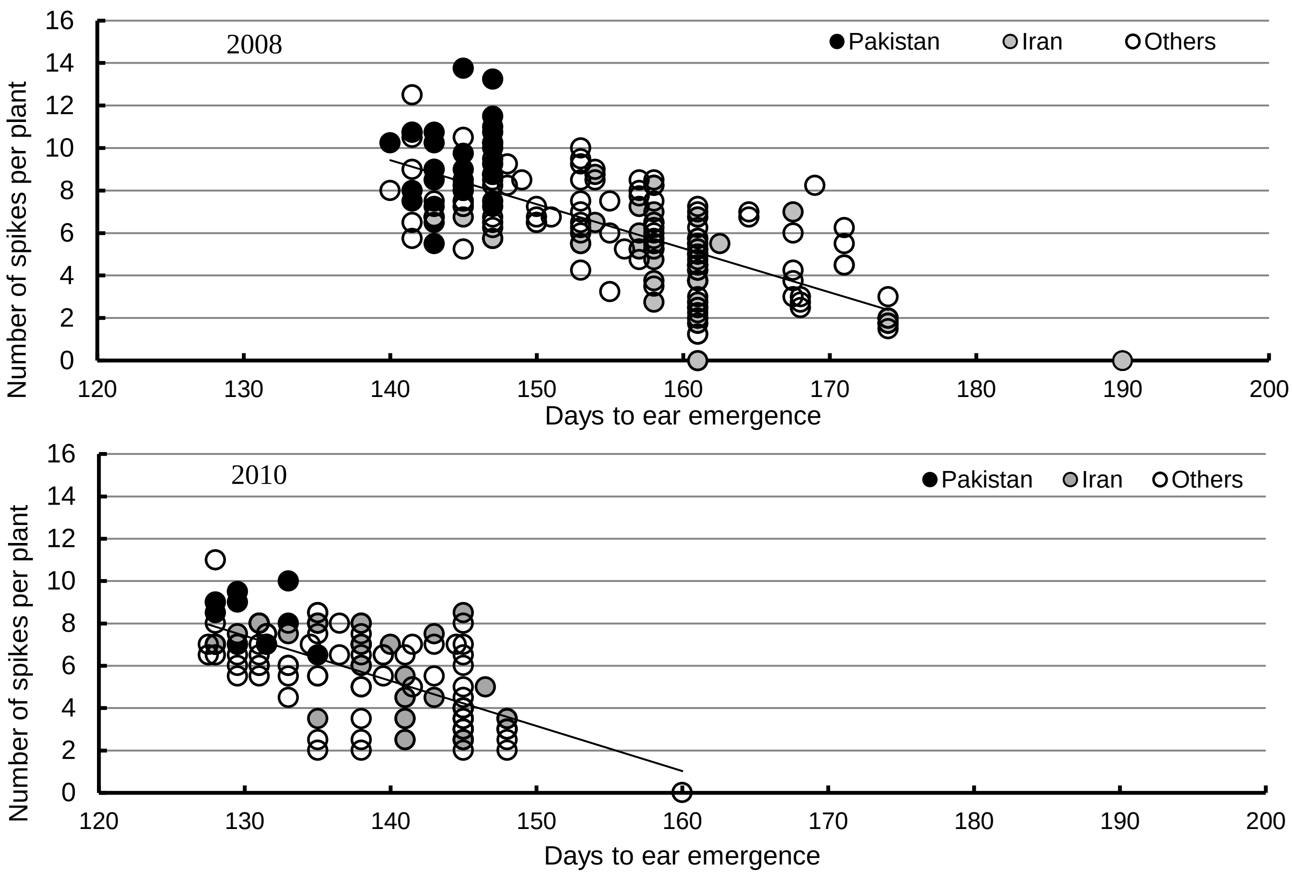

Figure 1. Relationships between days to ear emergence and number of spikes per plant for 181 accessions of *A. tauschii* in 2008 ((r=-0.712**) and for and 127 accessions in 2010 (r=-0.672**). Accessions with no spikes are given the estimated days to ear emergence.

A total of 23 accessions of *A. tauschii* were selected for productive tillering capacity from the 400 accessions examined for the two cropping seasons, as presented in Table 1. They had different origins; 15 accessions were from Pakistan, three from Turkmenistan, two from Iran, one from Afghanistan, one from Syria and one from Turkey.

Crosses of two durum wheat varieties with selected accessions of *Ae. tauschii*: Some of the durum wheat florets pollinated with *A. tauschii* developed seeds and embryos. Seeds developed from inter-specific crosses were smaller than self-pollinated seeds, and had poorly-developed endosperms (Figure 2a). Mean frequencies of seed set in crosses of the two durum wheat varieties 'Belikh-2' and 'Jennah Khetifa' with *A. tauschii* accessions are given in Table 2, with the least significant difference (LSD) at 5% probability between the means after angular transformation. Both durum wheat varieties showed large variations in cross-compatibility among the *A. tauschii* accessions. In the 23 crosses with 'Belikh-2', 17 accessions were cross-compatible. All five accessions from Turkmenistan and Iran showed high seed-set frequencies (9.5-17.8%). All fifteen accessions from Pakistan had relatively low seed-set frequencies (<5%). Statistical analysis showed that the seed-set frequencies of the best four accessions from Turkmenistan and Iran were significantly higher than those of the Pakistani accessions.

Table 1. Numbers of *A. tauschii* accessions examined and selected under the water-deficit stress conditions of 2008 and 2010.

Sr.	Origin	2008		2010		Totals	
		Examined	Selected	Examined	Selected	Examined	Selected
1	Afghanistan	7	0	9	1	16	1
2	Armenia	12	0	28	0	40	0
3	Azerbaijan	16	0	40	0	56	0
4	China	12	0	4	0	16	0
5	Georgia	1	0	0	0	1	0
6	India	1	0	0	0	1	0
7	Iran	41	1	28	1	69	2
8	Kazakhstan	4	0	3	0	7	0
9	Kyrgyzstan	0	0	1	0	1	0
10	Pakistan	31	11	9	4	40	15
11	Russia	10	0	15	0	25	0
12	Syria	3	1	3	0	6	1
13	Tajikistan	9	0	8	0	17	0
14	Turkmenistan	23	1	29	2	52	3
15	Turkey	18	1	14	0	32	1
16	Uzbekistan	9	0	5	0	14	0
17	Unknown	3	0	4	0	7	0
	Total	200	15	200	8	400	23

Plant regeneration from immature hybrid embryos: Immature hybrid embryos were smaller than those from self-pollinated seeds but vigorously regenerated to plants with roots and leaves on the culture medium (Figure 2b). All 20 plants regenerating from a cross of 'Belikh-2' x *A. tauschii* accession ig 47219 expressed necrotic symptoms at an early growth stage and failed to develop further. The overall frequency of plant regeneration from 135 embryos of the other crosses was 95.6%. Colchicine treatment slowed growth due to the recovery period from treatment damage. In a comparison of treatment effect using plant materials from a cross of 'Jennah Khetifa' x *A. tauschii* ig 47219, 16 treated plants produced 79 spikes with 50 grains on 14 plants whereas 16 untreated (control) plants produced 200 spikes with only one grain on one plant. Colchicine treatment was clearly essential to the reliable set of hexaploid seeds of synthetic wheat.

Table 2. Mean frequencies (%) of seeds set in crosses of two durum wheat varieties with 23 accessions of *Ae. tauschii*

Entry number	ICARDA ig number	Origin	'Belikh-2'		'Jennah Khetifa'	
			Mean	Angular transformed	Mean	Angular transformed
1	47219	Turkmenistan	17.8	24.9	32.2	34.4
2	46919	Iran	14.3	21.9	21.1	27.2
4	48502	Turkmenistan	11.9	19.3	1.9	4.0
3	48677	Iran	11.5	19.5	13.5	21.3
5	135534	Turkmenistan	9.5	17.9	0.0	0.0
6	46655	Pakistan	4.7	10.5	0.0	0.0
7	46656	Pakistan	4.3	10.3	--	--
8	46672	Pakistan	3.8	9.6	0.0	0.0
9	46889	Pakistan	2.9	7.0	2.9	5.0
10	46670	Pakistan	2.9	6.9	0.7	2.3
11	46646	Pakistan	2.8	8.3	0.8	2.6
12	46905	Turkey	2.6	8.1	0.6	2.3
13	46652	Pakistan	1.9	4.0	0.0	0.0
14	47236	Pakistan	1.9	4.0	--	--
15	46638	Pakistan	1.0	2.8	0.0	0.0
16	46673	Pakistan	1.0	2.8	--	--
17	47232	Pakistan	1.0	2.8	--	--
18	46640	Pakistan	0.0	0.0	--	--
19	46641	Pakistan	0.0	0.0	--	--
20	46668	Pakistan	0.0	0.0	2.6	6.5
21	46677	Pakistan	0.0	0.0	--	--
22	46890	Afghanistan	0.0	0.0	--	--
23	47259	Syria	0.0	0.0	1.0	2.8
	LSD (0.05)			7.73		7.92

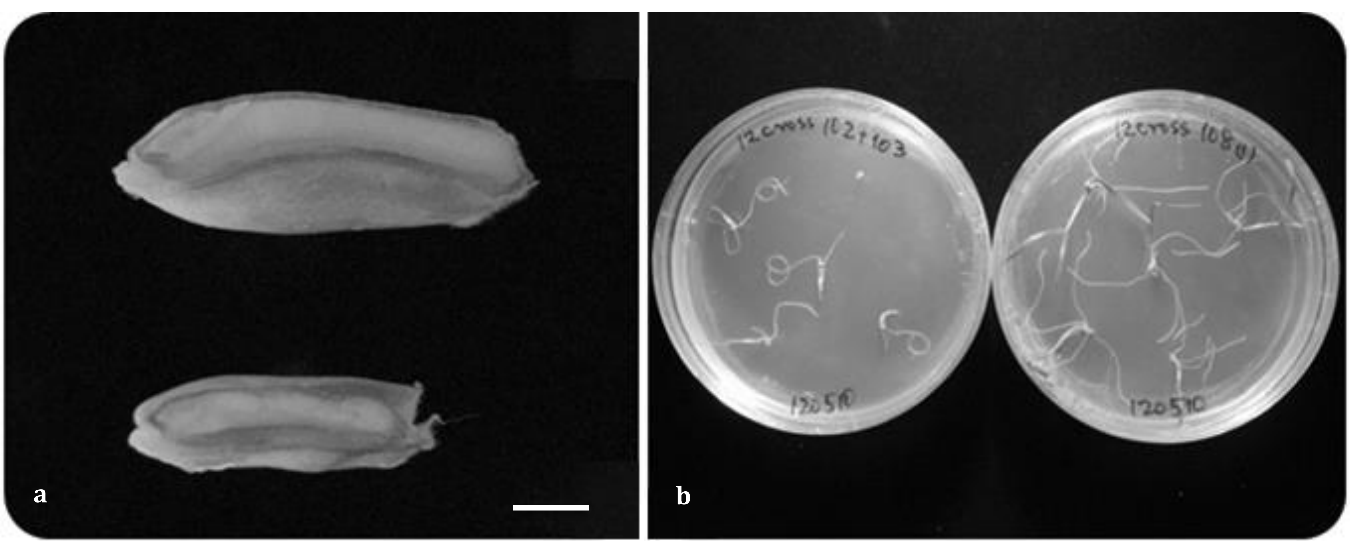

Figure 2. Seed development and plant regeneration in crosses of durum wheat with *Ae. tauschii*. (a) Seeds set on durum wheat 'Belikh-2' in self-pollination (above) and in cross with *A. tauschii* accession ig 47219 (below) at 14 days after pollination (bar 2 mm). (b) Plants regenerating from immature embryos at 21 days after incubation, showing necrosis symptoms in cross of 'Belikh-2' x *A. tauschii* ig 47219 (left) and normal growth in cross of 'Jennah Khetifa' x *A. tauschii* ig 47219 (right).

DISCUSSION

A total of 400 accessions of *A. tauschii* were screened under drought conditions for the productive tillering capacity. As the number of spikes per plant is strongly correlated with days to ear emergence, the result was a selection of those having early ear-emergence. All accessions of Pakistani origin had a spring growth-habit and showed early ear-emergence but this population also showed considerable variation in the number of spikes per plant. The total of 23 accessions selected for further study included 15 from Pakistan. Under field conditions at the Tel Hadya field station, wheat plants generally receives sufficient winter rainfall to support the early growth stages but stored soil moisture is insufficient for the later growth stages. Such was the case in both the 2008 and 2010 seasons. Under these conditions, early ear-emergence genotypes have an advantage in that they usually escape or avoid a terminal drought and so produce more spikes. This probably accounts for the selection of many early ear-emergence accessions of Pakistani origin. Selection of the early-maturing accessions under these field conditions is critical for enhancing drought adaptation in the Mediterranean environments even if it might result from drought escape or avoidance.

Cross-compatibility depends strongly on the genetic background of the parents. The study shows that with *A. tauschii* accessions from Iran and neighboring Turkmenistan as the pollen parent, high cross-compatibilities resulted, whereas accessions from Pakistan exhibited low cross-compatibilities. This suggests a high variability among accessions in cross-compatibility with durum wheat depending upon their origins. Matsuoka *et al.* (2007) reported that many Iranian accessions were highly cross-compatible and produced fertile triploid plants in crosses with durum wheat. As a result, they suggested that the natural synthesis of hexaploid wheat probably arose in northern Iran from spontaneous hybridization between durum wheat and *A. tauschii* plants growing close by (Matsuoka *et al*., 2008). Using chromosome-substitution lines of the durum wheat 'Langdon' as the female parent, Zhang *et al.* (2008) reported that cross-compatibility was genetically controlled by loci on chromosomes 7A and 4B. The same chromosomes of durum wheat also controlled cross-compatibility with maize (Inagaki *et al*., 1998). The application of plant growth regulators may enhance cross-compatibility, but this requires further research to identify effective agents.

Successful hybridization of durum and *A. tauschii* results in the formation of well-developed embryos but with poorly-developed endosperm. These embryos are capable of regenerating to plants on culture medium. In crosses with *A. tauschii* ig 47219, both durum wheat varieties 'Belikh-2' and 'Jennah Khetifa' gave high frequencies of seeds (embryos). None of the embryos from the cross of 'Belikh-2' x *A. tauschii* ig 47219 regenerated to plants whereas the other cross of 'Jennah Khetifa' x *A. tauschii* ig 47219 was successful in developing hybrid plants. Matsuoka *et al.* (2007) reported different levels of hybrid necrosis that also revealed geographical distributions among *A. tauschii* accessions. Some loci associated with hybrid necrosis have recently been mapped on chromosomes 2B and 5B (Chu *et al*., 2006) and on 7D (Mizuno *et al*., 2010). The hybrid necrosis found in this study was lethal and the association with these identified loci remains unknown. A high frequency of seeds (embryos) produced in interspecific crosses did not always result in the development of hybrid plants. It is possible that use of durum wheat genotypes of different pedigrees will allow the avoidance of hybrid necrosis in some crosses. In addition, the use of the specific durum wheat genotype 'Langdon' made embryo rescue and colchicine treatment unnecessary because triploid hybrid plants were regenerated without embryo rescue and spontaneously fertile to set hexaploid seeds (Matsuoka and Nasuda, 2004; Zhang *et al*., 2010). However, the results obtained from this study suggested that procedures of embryo rescue and colchicine treatment were clearly essential to the successful production of synthetic hexaploid wheats as a means of alien genetic transfer.

This study remains the cytological as well as morphological examinations of newly-synthesized wheat genotypes, because they may exhibit a variation in chromosome number (Niwa *et al*., 2010). It is also required to examine their drought adaptation in further experiments in relation to the transfer of traits from *Ae. tauschii*. Sohail *et al.* (2011) reported that no morphological or physiological traits on drought tolerance in the synthetic wheat genotypes were significantly correlated with the corresponding traits of their parental *A. tauschii* accessions under drought conditions. It may suggest unforeseen and complex expression of drought tolerance in the hexaploid form of newly-synthesized wheat.

Most of the *A. tauschii* accessions selected under water-deficit stress were of Pakistani origin and had early maturity. These accessions also exhibited low cross-compatibilities whereas both the Iranian and Turkmenistan accessions selected had high cross-compatibility. This suggests a wide variation in cross-compatibility among durum wheat genotypes depending on their origins. Technical information on cross-compatibility, plant regeneration and chromosome doubling obtained in new interspecific crossing of durum wheat with selected accessions of *A. tauschii* will help to increase the efficiency of synthetic hexaploid wheat production with the aim of enhancing drought adaptation.

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