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Full Length Article

Interactive Genotypic Influence of Durum Wheat and *Aegilops tauschii* on their Crossability and Fertility of Synthetic Amphiploids

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Abstract

Bread wheat (*Triticum aestivum* L., AABBDD) is an allohexaploid (AABBDD, 2n=6x=42) originated from hybridization which occurred about 8000 years BC between a cultivated tetraploid wheat (*T. turgidum* L.; AABB, 2n=4x=28) and the wild goat grass *Aegilops tauschii* (DD, 2n=2x=14). In order to synthesize bread wheat, crosses were carried out between seven genotypes of durum wheat and 13 ecotypes of *A. tauschii* of various origins. The production of synthetic hybrids was made with and without the implementation of embryo rescue technique. Four genotypes of durum wheat, carrying genes for the production of unreduced gametes, produced partially fertile hybrids after their cross with the ecotypes of *A. tauschii*. The percentage of grain germination harvested from these hybrids varied between 22.2 and 65.2%. Growth abnormalities were observed on hybrid plants. The barriers of incompatibility between the two species were manifested by leaf chlorosis and lethal necrosis on hybrid plants.

Keywords: *Triticum durum*; *Aegilops tauschii*; Embryo rescue; Hybrid chlorosis; Lethal necrosis

Introduction

Cultivated bread wheat, a hexaploid species with genomes A, B and D (2n = 42 chromosomes) is derived from a spontaneous hybridization between a tetraploid wheat *Triticum turgidum* (2n = 28 chromosomes, AB) and the wild grass *Aegilops tauschii* (2n = 14 chromosomes, DD). Bread wheat occurred between 7000 and 8000 BC in the common range of *A. tauschii* and tetraploid wheat, which extends from the Fertile Crescent area to the east to right up to the Chinese borders, to the west (Bonjean, 2001).

Independent hybridizations between different genotypes of tetraploid wheat and several forms of *A. tauschii* would have given great variability in wheat (Doussinault *et al.,* 2001). However, a limited number of parental genotypes of the two species would have contributed to the process of evolution of hexaploid wheat (Li *et al.,* 2014). Therefore, wheat genetic variability is decreased as compared to the variability of its two ancestors. Most of the genetic variability of tetraploid wheat with *A. tauschii* is expressed in the available wheat germplasm.

The evolution process of wheat has been reproduced artificially in order to increase the necessary selection variability. Several synthetic wheats were produced by hybridization between tetraploid wheat and the *A. tauschii* (Mujeeb-Kazi *et al.,* 1996; Matsuoka and Nasuda, 2004; Jones *et al.,* 2013). The exhaustion of the intraspecific genetic variability led wheat breeders to develop a new breeding strategy based on the exploitation of the interspecific variability. Indeed, the *A. tauschii*, wild wheat relative species, has important reservoir of genes of strong economic potential that control characters such as disease resistance, abiotic stress tolerance and quality of reserve proteins (Doussinault *et al.,* 2001).

The cross of tetraploid wheat with *A. tauschii* depends largely on the cross-ability of wheat genotype. Indeed, incompatibility barriers hinder the success of hybridization between the two species. Several growth abnormalities were observed on crosses of the variety of tetraploid wheat Langdon and accessions of *A. tauschii*. These abnormalities are manifested by hybrid chlorosis and necrosis (Mizuno *et al.,* 2010). Chlorosis, followed by an accelerated senescence of leaves, is often associated with powdery mildew resistance (Nakano *et al.,* 2015). Chlorosis of synthetic hybrids is controlled by the gene Hch1 located in the short arm of

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chromosome 7D of *Aegilops* (Kana *et al.*, 2015; Nakano *et al.*, 2015). The phenomenon of hybrid necrosis is controlled by two dominant genes Ne1 and Ne2 located on chromosomes 5B and 2B, respectively (Takumi and Mizuno, 2011).

Wheat breeding for adaptation to climate change is a priority area in many wheat production programs. The introduction of drought tolerance in cultivated varieties is considered critically important. The objective of this paper is to examine how the methods used and genotypic factors affect the production of new synthetic wheat.

Materials and Methods

Plant Material

Durum Wheat: Seven varieties of durum wheat of Tunisian origin (Jnah Khouttifa, Agili, Biskri, Ouji, Bayadha, Mahmoudi, Chile) were used as female parent in crosses with *A. tauschii* (Table 1). Seeding of durum wheat material was conducted in the field one month after sowing the ecotypes of *A. tauschii* to synchronize the heading of both species. Plants were irrigated and weeded manually. Plant fertilization consisted of the contribution of 6.7 $g.m^{-2}$ of phosphorus in the form of triple superphosphate before sowing and 8 $g.m^{-2}$ of nitrogen in the form of ammonium nitrate: 4 $g.m^{-2}$ at the emergence stage and 4 $g.m^{-2}$ at the tillering stage.

***Aegilops tauschii*:** Thirteen *A. tauschii* ecotypes, originating from West Asia region, were provided by ICARDA and used as male parent. These ecotypes were selected for their drought tolerance. A staggered seeding was realized in 20 cm-diameter pots containing a culture substrate made up of two-thirds of sand and one-third of peat. The plants were irrigated and the mineral fertilization was the same as that supplied to the varieties of durum wheat.

Embryo Rescue

About 18 to 20 days after the pollination of the varieties of durum wheat by the ecotypes of *A. tauschii*, the embryos were rescued by *in vitro* culture. The caryopses were harvested, then sterilized with sodium hypochlorite solution at 2% for 15 min; rinsed three times with sterile distilled water under a laminar flow hood. The embryos were extracted at aseptic conditions and put in culture in 100 mm × 15 mm Petri dishes containing the Gamborg B5 culture medium added with 30 $g.L^{-1}$ of sucrose and 8 $g.L^{-1}$ of agarose. The embryos were grown in darkness at 25°C. From the appearance of the coleoptiles and roots, new plants were transferred to the light in an incubator set at 25°C with a photoperiod of 16 h a day until the formation of leaves and vigorous roots. Then, plants were transferred in pots containing a mixture of sand and peat to continue their growth until the harvest of grains.

Results

Production of Hybrids by Embryo Rescue

Formation of embryos and plant regeneration: The seed-set rates were very variable from one cross to another and fluctuated between 0 and 24.7% (Table 2). The highest seed-set rates were observed in crosses with the variety 'Jnah Khouttifa' that showed a good cross affinity with the ecotypes of *A. tauschii* 10-09 and 10-27 originating from Pakistan and Syria. The good compatibility of the durum wheat variety ‘Agili’ with the Ecotype 10-28, native of Iran, gave a rate of 17.3% of caryopses formation. The cross compatibility was low and even zero in the crosses 'Bayadha × Ecotype 10-26’ and 'Biskri × Ecotype 10-23’. The germination rate of embryos was also variable between crosses and varied from 0 to 100%. Many successful crosses led to high embryo germination rates. Most germinated embryos were able to continue their growth in the B5 medium and developed whole plants (Fig. 1). However, the embryos resulting from the cross 'Agili × Ecotype 10-11’ presented growth abnormalities and have not led to plant formation.

Abnormalities in hybrid plants: After the embryos were cultured in B5 medium, several growth abnormalities in embryos and hybrid plants were noticed. These irregularities were demonstrated firstly by the growth disorder of the embryo (Fig. 2a) and the absence of stem formation (Fig. 2b and c), and secondly by severe necrosis leading to the death of plants. Chlorosis of hybrid plants was observed after their transfer in pots and during tillering stage (Fig. 3b). The phenomenon of plant chlorosis has led gradually to the death of intergeneric hybrids. Out of a total of 96 hybrid plants, 90 plants showed hybrid chlorosis while growing in the pots. Only 6 plants resulting from the crosses ' *durum wheat* Jnah Khouttifa × *A. tauschii* 10-9’, ' *durum wheat* Chili × *A. tauschii* 10-1’, ' *durum wheat* Mahmoudi × *A. tauschii* 10-11’ and '*durum wheat* Agili × *A. tauschii* 10-28’ formed spikes and have reached the maturation stage (Table 3). Plants resulting from other crosses showed severe necrosis and died during the *in vitro* phase or later after their transfer in the pots (Fig. 4b). Lethal necrotic symptoms were also observed on plants after they were transferred in pots and at different development stages (Fig. 4c).

Production of Hybrids without Embryo Rescue

Crosses with durum wheat genotypes 'Jnah Khouttifa', 'Chili', 'Mahmoudi' and 'Agili' produced mature seeds containing viable embryos. Sowing of these seeds resulted in plants that did not show hybrid necrosis (Table 4). Using the embryo rescue technique, these crosses produced viable hybrid plants. However, the compatibility of the durum wheat varieties 'Bayadha', 'Ouji' and 'Biskri' with *Aegilops* ecotypes was poor and crosses did not produce seeds.

Table 1: Crosses between durum wheat and *A. tauschii*

Variety of durum wheat (female parent)	Ecotype of *A. tauschii* (male parent)		
	Ecotype	ICARDA N°	Origin
Jnah Khouttifa	10-06	46668	Pakistan
	10-09	46673	Pakistan
	10-26	48502	Turkmenistan
	10-27	47259	Syria
Agili	10-11	46905	Turkey
	10-22	46890	Afghanistan
	10-28	48677	Iran
Mahmoudi	10-11	46905	Turkey
	10-02	46640	Pakistan
	10-28	48677	Iran
Chili	10-1	46638	Pakistan
	10-26	48502	Turkmenistan
	10-27	47259	Syria
Bayadha	10-24	47219	Turkmenistan
	10-26	48502	Turkmenistan
	10-27	47259	Syria
Ouji	10-11	46905	Turkey
	10-24	47219	Turkmenistan
	10-28	48677	Iran
Biskri	10-23	46919	Iran
	10-28	48677	Iran

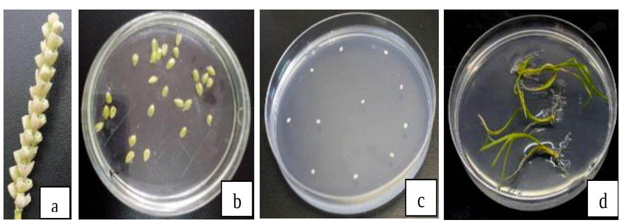

Fig. 1: Culture of embryos and regeneration of hybrid plants from the Jnah Khouttifa × *A. tauschii* 10-6 crossing: a) spike containing 16-days caryopses, b) caryopses from a pollinated spike, c) embryos culture in B5 medium, d) hybrid regenerated plants

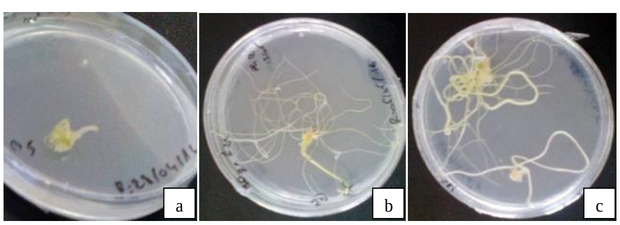

Fig. 2: Growth abnormalities in durum wheat × *A. tauschii* crosses: a) absence of coleoptile and root development, b) cessation of coleoptile development with development of roots, c) root development without development of the coleoptile

By the embryo rescue technique, these crosses resulted in plants that lost their viability, because of the phenomenon of lethal hybrid necrosis.

Discussion

Long straw durum wheat varieties were used as female parent in crosses with *A. tauschii* ecotypes to synthesize

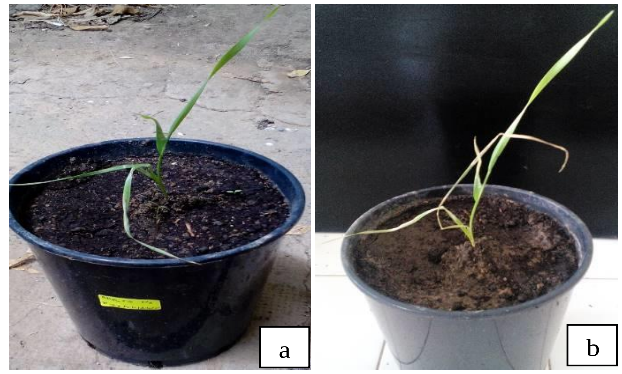

Fig. 3: Chlorosis of hybrid plants from durum wheat × *A. tauschii* crosses: a) normal plant, b) plant chlorosis

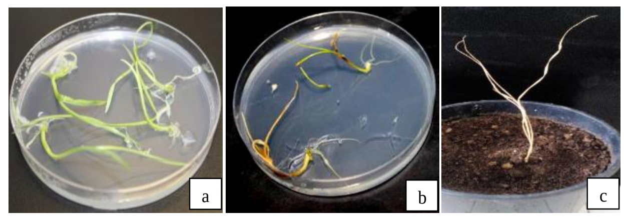

Fig. 4: Lethal necrosis on hybrid plants (durum wheat × *A. tauschii*): a) normal plants, b) severely necrotic plants, c) lethal necrosis on potted plant

hexaploid wheat. The female genotype played a major role in the success of crossing and obtaining hybrid plants. Indeed, cross compatibility of genotypes of tetraploid wheat with ecotypes of *A. tauschii* made it possible to differentiate two groups of genotypes.

A first group of female genotypes formed of the varieties 'Jnah Khouttifa', 'Chili', 'Mahmoudi' and 'Agili', was characterized by good compatibility with *A. tauschii* ecotypes and their crosses delivered hybrid plants. These plants were obtained either by embryo rescue technique or without *in vitro* culture technique. The ability of certain genotypes of tetraploid wheat, such as 'Jnah Khouttifa', 'Chili', 'Mahmoudi' and 'Agili' to produce fertile hybrid plants without needing embryo rescue and treatment with colchicine is a particularly stimulating phenomenon. The hybrid plants were partially fertile since they produced 1 to 3 grains per spike. This partial fertility excludes any somatic origin of resulting plants. The hybrid, which is a fertile and stable amphiploid, would result from the fusion of a male and a female unreduced gametes (Jauhar, 2007). The frequency of unreduced gametes was low, which explains the partial fertility of amphiploid spikes. The ability to induce the formation of unreduced gametes in synthetic hybrids of durum wheat × *A. tauschii* is genetically controlled. Certain genotypes of tetraploid wheat have genes that induce the formation of unreduced gametes in synthetic hybrids (Zhang *et al.*, 2010).

Table 2: Seed-set rate and regenerated hybrid plants by embryo rescue

Variety of durum wheat	Ecotype of *A. tauschii*	Number of pollinated flowers	Seed-set rate	Rate of germinated embryos	Rate of regenerated plants
Jnah khouttifa	10-06	124	2.4	99	89.5 (17)
	10-09	210	24.7	89	81.5 (17)
	10-26	68	4.5	60	80 (4)
	10-27	210	11.9	92	85.7 (27)
Agili	10-11	148	6.7	50	0
	10-22	272	6.2	80	60 (6)
	10-28	150	17.3	66	93.3 (14)
Mahmoudi	10-11	206	3.9	60	40 (2)
	10-02	36	0.0	0	0
	10-28	394	8.9	0	0
Chili	10-1	334	2.1	100	100 (2)
	10-26	168	2.4	100	100 (2)
	10-27	150	0.7	0	0
Bayadha	10-24	410	1.7	86	57.1 (4)
	10-26	224	0.0	0	0
	10-27	194	10.8	89	36.8 (7)
Ouji	10-11	86	1.1	0	0
	10-24	218	0.4	0	0
	10-28	360	0	0	0
Biskri	10-23	456	0.0	0	0
	10-28	164	1.2	50	100 (2)
LSD			6.7	8.3	7.5

( ): Number of regenerated plants
LSD: little significant difference at threshold 5%

Table 3: Number of plants obtained by rescuing embryos that reached maturation stage

Cross variety of durum wheat × *A. tauschii* ecotype	Number of plants transferred in pots	Number of plants with fertile spikes
Jnah Khouttifa × 10-9	17	2
Chili × 10-1	2	1
Mahmoudi × 10-11	2	1
Agili × 10-28	12	2
Total	35	6

Table 4: Germination of durum wheat × *A. tauschii* hybrids

Genotype of durum wheat	Ecotype of *A. tauschii*	Pollinated flowers	Harvested seeds	Developed plants	Germination (%)
Jnah Khouttifa	10-6	248	20	14	70
	10-9	170	38	26	68.4
	10-26	192	0	0	0
	10-27	206	11	5	45.4
	Total	816	69	45	65.2
Chili	10-1	300	5	1	20
	10-26	152	2	0	0
	10-27	56	2	1	50
	Total	508	9	2	22.2
Mahmoudi	10-2	28	0		0
	10-11	164	3	2	66.7
	10-28	394	35	7	20.0
	Total	586	38	9	23.7
Agili	10-11	192	7	7	100
	10-22	226	7		0
	10-28	76	11		0
	Total	494	25	7	28.0
Bayadha	10-24	314	0	0	0
	10-26	184	0	0	0
	10-27	116	1	0	0
	Total	614	1	0	0
Ouji	10-11	120	0	0	0
	10-24	204	0	0	0
	10-28	448	0	0	0
	Total	772	0	0	0
Biskri	10-23	444	0	0	0
	10-28	156	0	0	0
	Total	600	0	0	0

Highly fertile hybrids were produced by crossing the durum variety 'Langdon' with *A. tauschii* (Matsuoka and Nasuda, 2004; Zhang *et al.*, 2010). The formation of unreduced gametes results from the inhibition of the pairing of chromosomes of durum wheat with the major Ph1 gene located on chromosome 5B (Jauhar, 2003). The genotypes of durum wheat 'Jnah Khouttifa', 'Chili', 'Mahmoudi' and 'Agili' became carriers of genes which induced the formation of unreduced gametes in synthetic hybrids. They also became carriers of the Ph1 gene that controls chromosome pairing. The phenomenon of formation of unreduced gametes, by which plant fertility is naturally restored, was reported in durum wheat haploids produced by durum wheat × maize crosses (Jauhar, 2003) and durum wheat × *A. tauschii* hybrids (Jauhar, 2007; Zhang *et al.*, 2010; Inagaki *et al.*, 2014). The incompatibility barriers, encountered generally in interspecific crosses, were pronounced and did not interfere with the formation of the interspecific hybrid in this group of genotypes.

The second group is composed of the genotypes 'Bayadha', 'Ouji' and 'Biskri', which showed poor compatibility with the ecotypes of *A. tauschii*. These genotypes are not carriers of genes, inducing the formation of unreduced gametes. Indeed, the crosses realized with these varieties did not give any hybrid plant without the implementation of the embryo rescue technique. The barriers of incompatibility were very visible and prevented the formation of hybrids. Indeed, the inhibition of hybridization between durum wheat and *A. tauschii* is genetically controlled. The use of substitution lines of the durum variety 'Langdon' as female parent, Zhang *et al.* (2008) proved that the compatibility of tetraploid wheat with *A. tauschii* is controlled by two genes located on chromosomes 7A and 4B, which also control the inhibition of hybridization between durum wheat and maize (Inagaki *et al.*, 1998). Implementation of the embryo rescue technique resulting from the crossing of this group of varieties with *A. tauschii* ecotypes allowed the production of hybrid plants whose growth was inhibited and which lost their viability due to the lethal hybrid necrosis. The observed necrosis on synthetic hybrids was severe and lethal. Matsuoka *et al.* (2007) identified different types of hybrid necrosis associated with the geographic distribution of *A. tauschii*. Genes associated with hybrid necrosis were recently mapped on chromosomes 2B, 5B (Chu *et al.*, 2006) and 7D (Mizuno *et al.*, 2010). Nevertheless, the relationship between these genes and the lethal necrosis of the hybrids described in this study remains unknown.

Conclusion

The cross compatibility between durum wheat and *A. tauschii* was largely influenced by the genotype of durum wheat. Chlorosis and lethal necrosis engendered two barriers of incompatibility between the two species. The parental genotypes of durum wheat under study belong to two different compatibility groups. Genotypes having good crossing compatibility with *A. tauschii* ecotypes produced fertile hybrids with and without the use of embryo rescue technique. A second group of genotypes characterized by poor cross compatibility did not produce synthetic hybrids due to lethal necrosis.

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