Bulgarian Journal of Agricultural Science, 22 (No 2) 2016, 262–266
Agricultural Academy

DEVELOPMENT AND CHARACTERIZATION OF INTERSPECIFIC HYBRIDS FROM HULLED X NAKED *TRITICUM* CROSSES IN STRESSED ENVIRONMENT

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Abstract

DASKALOVA, N., S. DONEVA and P. SPETSOV, 2016. Development and characterization of interspecific hybrids from hulled x naked *Triticum* crosses in stressed environment. *Bulg. J. Agric. Sci.,* 22: 262–266

This study is an attempt to produce hybrid plants from cross-combinations involving different *Triticum* species and hybrid forms under stressed conditions in polycarbonate greenhouse (temperature below 0°C in winter and high temperatures during pollination, fertilization and grain ripening). Materials included seven cross-combinations with participation of *T. monococcum* (three accessions), *T. sinskajae* (two), *T. dicoccum* (two) and three hybrid forms in which pedigree *T. boeoticum* was involved. From the 174 hybrid seeds obtained (averaged seed set of 42.2%), 86 (49.4%) germinated and 45 (25.9%) plants matured. Three crosses with different females and *T. sinskajae* acc.18397 and 18401 as males produced largest number of hybrids. All F_1 plants were fully sterile except the cross *T. monococcum* acc.45126 x *T. sinskajae* acc.18401. Two other crosses (*T. dicoccum* acc.45398 x TS18397 and 198-2F_3 x TS18397) yielded 17 seeds from plants treated with colchicine solution. Plants from the former cross were damaged by frost in December-January in tillering stage while those from the latter cross survived being younger, in 2–3 leaf phase. Two families were designated as amphiploids No.45-2 and 45-4 in C_1 generation, one of which born more seeds than the parental forms.

Key words: T. monococcum, *T. sinskajae*, *T. dicoccum*, amphiploid, seedset, stressed environment
Abbreviations: TS – *Triticum sinskajae*; A – amphiploid; TC – F_1 plants treated with colchicine solution

Introduction

Many interspecific hybrids in the genus *Triticum* have been created during the last 30 years. Wild wheats possess diverse genetic potential that provides high tolerance to biotic and abiotic stresses (Sang, 2009). In the country, only *Triticum boeoticum* (synonym *T. monococcum* ssp. *aegilopoides*) has been naturally spread. Goncharov et al. (2009) describes four diploid *Triticum* species: *T. urartu*, *T. boeoticum*, *T. monococcum* and *T. sinskajae*. The last is a mutant (naked form) found in einkorn *T. monococcum* (hulled wheat). Molecular studies indicate that *T. urartu* is the donor of genome A^u to polyploid wheats while *T. boeoticum* donated the genome A^b to einkorn wheat. Several amphiploids have been established involving *T. boeoticum* (Multani et al., 1988; Spetsov and Savov, 1992; Spetsov and Belchev, 2013). They manifest high resistance to fungi and tolerance to abiotic factors – traits that are very promising for wheat improvement (Plamenov et al., 2009; Megyeri et al., 2011). Einkorn production increases rapidly in the last years because of its high tocol and carotenoid contents, along with winter hardiness, drought tolerance, allelopathy and straw strength. Einkorn wheat (cultivated) is hulled wheat, a trait raising problems after harvesting. The production of synthetic AD is an effective and rapid way of introgressing desirable traits from related species into cultivated wheat (Goncharov et al., 2007). In most cases, the synthetics

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contain wild einkorn (*T. boeoticum*, A^bA^b), which has better crossability than that of cultivated einkorn (agronomically more valuable but less crossable).

The aim of the present work was to obtain hybrid seeds and plants from crossing different *Triticum* species, including some tetraploid hybrids, and to develop amphiploids in stressed environment (law and high temperatures), caused by polycarbonate greenhouse conditions during plant growth.

Materials and Methods

Seven cross-combinations were made in the spring of 2013 in unregulated greenhouse, constructed from polycarbonate panels. Female parents had different chromosome numbers as some of them were tetraploid hybrids (195-1-1, 198-1 and 198-2 in F_3 generation) obtained by crossing hexaploid amphiploids 8BAP and 9BAP ($BBA^uA^uA^bA^b$) with durum varieties (Spetsov and Belchev, 2013). Other parents represented diploid and tetraploid accessions, two of which are local cultivated einkorn (ELD and ELV) (Table 1). All pollinators were diploids consisted of local *T. monococcum* form (hulled) and two *T. sinskajae* 18397 и 18401 accessions (naked). The tetraploid hybrid females (naked) were selected in F_2 for germination ability and winter hardiness in field conditions (Daskalova, 2015). Chinese Spring served as control for a spring genotype (lack of cold resistance).

Cross ability was expressed as the ratio of the number of seeds set to the total number of flowers pollinated. Germination occurred in petri dishes at 21–23°C and planted in pots (4 plants in a pot with 2.5 kg garden soil). About half of hybrid plants were treated with colchicine solution (0.05%). Amphiploid plants were analyzed on height and spike characteristics. Analysis of variance was carried out using a statistical program Assistat beta 7.7.

The sunny day temperature in the polycarbonate greenhouse surpassed with 15–20°C the temperature outside. The thermometer read more than 40°C in every sunny day when pollination, fertilization and embryo formation occurred in April-June. This was abnormal condition which negatively affected the plant growth and seed viability. Another stressed environment was the law temperatures (below 0°C) in winter when the plants are planted in December. In our experiment, law temperatures (–1 to –9) came suddenly at the end of December until 6th of January, 2015. During this time a part of amphiploid plants was destroyed by frost on a level with Chinese Spring and other spring type accessions.

Results and Discussion

The crossability in crosses varied from 7.1 до 59% (Figure 1), with medium of 42.2%. The highest seed set (59%) was obtained for *T. dicoccum* acc.45249 x TS18397, while the cross No.41 (198-1F_3 x TS18401) gave the lowest seed set (7.1%). Megyeri et al. (2011) achieved similar results of combinations durum x einkorn genotype ID140 and emmer x *T. turgidum* ssp. *durum* MVTD14-04, where the highest seed set percentage was > 20%. We used diploid species as pollinators and the seed set averaged of 42.2%, while Hadzhiivanova et al. (2009) had extremely low crossability of 0.9% in the cross durum var. Vazhod x *T. aegilopoides*.

The percentage of germinated F_1 seeds was 49.4% (Figure 1). Crosses No.41 and 43 had the best germinated seeds, followed by Nos.45, 47 and 48. All they showed germination > 50%. Hybrid necrosis 7-8 grades was observed after

Table 1

Designations, origin, genome formule of parental forms involved in crosses and their viable F_1 hybrids

Accession	Origin and species	GF	Cross design.	Cross-combinations	GS	PLAP	PLAT	MP
195-1-1F_3	9BAP x *T. durum* cv. Argonavt	2n = 28	41	198-1 F_3/TS18401	2	0	0	2
198-1 F_3	8BAP x *T. durum* cv. Progres	2n = 28	42	195-1-1 F_3/ ELV	1	0	0	1
198-2 F_3	8BAP x *T. durum* cv. Progres	2n = 28	43	45398/TS18397	39	12	2	25
45126	Syria, *T. monococcum*	A^bA^b	44	45249/TS18397	8	1	7	0
45249	Syria, *T. dicoccum*	BB A^uA^u	45	198-2 F_3/TS18397	17	1	1	15
45398	Syria, *T. dicoccum*	BB A^uA^u	47	45126/TS18401	7	1	4	2
ELV	Varna, *T. monococcum*	A^bA^b	48	ELD/TS18401	12	12	0	0
ELD	Varna, *T. monococcum*	A^bA^b		Total	86	27	14	45
TS18397	Germany, *T. sinskajae*	A^bA^b						
TS18401	Germany, *T. sinskajae*	A^bA^b						

9BAP (*T. dicoccum* x *T. monococcum*); 8BAP (*T. durum* var. Martondur 3 x *T. boeoticum* acc.110);
GF, genome formule and/or chromosome number; GS, germinated hybrid seeds; PLAP, no. plants lost after planting;
PLAT, no. plants lost after tillering; MP, no. plants matured.

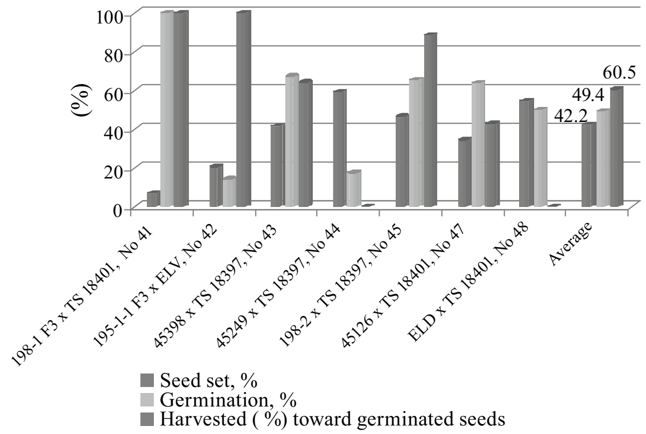

Fig. 1. Seed set (crossability), germination and harvested F_1 plants in greenhouse

2–3 leaf seedlings to tillering, and 3–4 grades after tillering to heading (Figure 2) according to Hermsen (1963a,b). It is proved that necrosis genes are active during cool weather and law temperature. This specific effect can be overcome in controlled regime between 28 и 35°C (Takumi and Mizuno, 2011; Mizuno et al., 2011). All plants from the cross No.48 showed heavy necrotic symptoms and died at the end of tillering, followed by cross No.44 with 100% plants lost after tillering to maturity (Figure 2). Female parents of these crosses are cultivated einkorn acc. ELD and emmer 45249, and *T. sinskajae* accessions as pollinators. In the cross No.45 the percentage of lost plants1+2 was quite low (5.9 and 6.3%, respectively). No necrosis was observed in Nos.41 and 42.

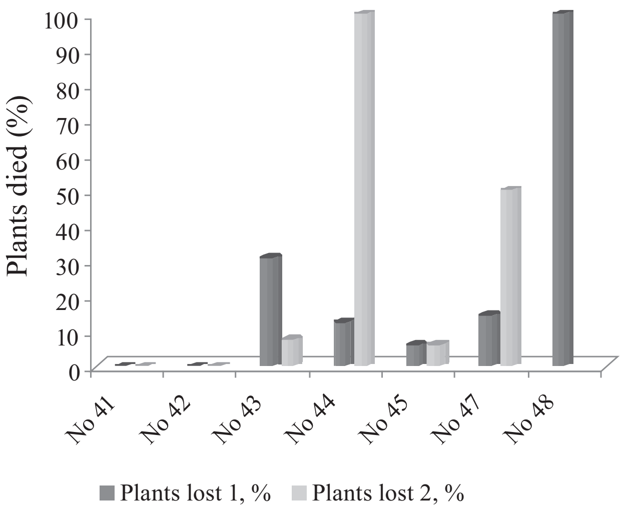

Fig. 2. Development and comparison of F_1 plants lost (died seedlings) after planting to tillering (1) and after this stage to maturity (2)

All F_1 plants, not treated with colchicine, were sterile, except those from the cross No.47. The sterility was very high in the group of TC plants too, from 71.4 to 100%. The best seed set was formed in TC plants of the cross No.45 (28.6%) (Figure 3). Valkoun (2001) employed no embryo-rescue technique to produce F_1 plants between durum wheats and *T. boeoticum*, *T. urartu* and *T. dicoccoides*. He obtained AAB triploids with diploid wheats as pollinators, which were 100% sterile. Fricano et al. (2014) obtained almost-sterile F_1 plants when the seed-bearing parent was *T. monococcum*. We could overcome this problem by only treated hybrids with colchicine solution.

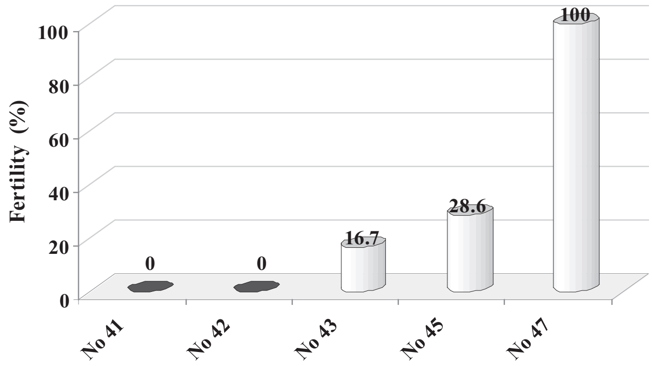

Fig. 3. Seed set in hybrids from five cross-combinations (Nos.43 and 45 are F_1 plants treated with colchicine at tillering)

In autumn of 2014, seeds in C_1 from two crosses (Nos. 43 and 45) and their parents were normally germinated in petri dishes. Plants of the latter cross were planting later, in the middle of December. During 8 days (30.12.2014 –6.01.2015), they were subjected to law temperatures (Figure 4). Plants of No.43 died, although they were in tillering stage. AD45 was divided in two families (–2 and –4 C_1) in which by one plant was lost. In total, 12 plants reached maturity: 9 from No.45-2 and 3 of No.45-4. Parents of this cross did not suffer from the cold conditions, too. The survival of AD45 could be explained with the influence of female parent (198-2 F_3), selected for winter hardiness earlier in the field.

Differences on four traits (VIS, EL, SN и GN) between the AD and its parents were found (Table 2). The two families differed in between only on grain number per main spike. The amphiploid expressed plant height (86.7–87.6 cm) of intermediate value, but formed longer ears than both parents. The naked einkorn TS18397 born numerical spikelets but almost a half of them were sterile (data not shown). Plants of AD45-2 produced the highest seed set (45 seeds per ear in average), followed by 198-2F_5 and AD45-4 (Table 2). In the stressed greenhouse environment the einkorn TS18397 yielded the least number of grains (16.6 seeds). Pauk et al.

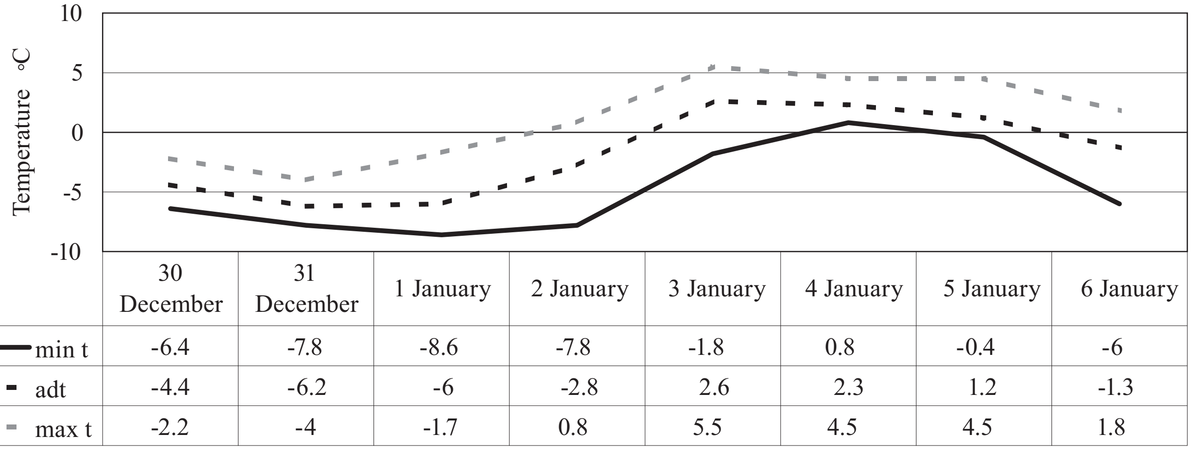

Fig. 4. Temperature values for eight days at the end of 2014 and beginning of 2015 (adt = average daily temperature)

Table 2
Expression of four traits in AD45 and its parents in unregulated polycarbonate greenhouse

Amphi-ploid/ Parents	Plants, No	VIS	F	SMD	EL	F	SMD	SN	F	SMD	GN	F	SMD
45-2 C_1	9	87.6 b	146.2**	2.31	11.0 a	24.6 **	0.42	26.7 b	66.8**	1.14	45.3a	12.9**	3.5
45-4 C_1	3	86.7 b	P<0.0001		10.7 a	P<0.0001		27.7 b	P<0.0001		29.3bc	P<0.0001	
198-2 P1	10	65.9 c			8.0 b			16.3 c			33.9 b		
18397 P2	5	129.6 a			7.0 b			37.0 a			16.6 c		

VIS – plant height; EL – ear length; SN – spikelet number; GN – grain number; all traits were measured on the main ear; F – statistics of the test; SMD – significative minimum difference through Tukey test; ** – significative at a level of 1% of probability ($p < 0.01$)

Table 3
Analysis of variance for grain number per main ear in AD45 C_1 and its parents grown in polycarbonate greenhouse

Amphiploid	No. plants	No. ears in plant	Mean	Parents	No. plants	No. ears in plant	Mean
AD45-2 C_1	1	5	36.8 abcde	198-2 F_5	1	3	29.0 abcdef
	3	3	28.7 abcdef		2	3	27.0 abcdef
	4	2	37.5 abcde		3	2	4.5 f
	5	2	35.5 abcdef		4	3	33.7 abcdef
	6	2	40.0 abcde		5	3	22.7 abcdef
	7	3	35.0 abcdef		6	3	14.3 cef
	8	2	44.0 ab		7	2	43.0 abc
	9	4	44.0 ab		8	3	25.7 abcdef
	10	4	41.8 abcd		9	2	41.0 abcde
AD45-4 C_1	1	4	34.3 abcdef		10	2	46.0 a
	2	4	7.8 f	TS18397	1	3	14.3 cef
	3	4	27.0 abcdef		2	6	10.8 ef
					3	2	16.5 acdef
					4	2	10.5 ef
					5	2	6.0 f

$F = 7.4925$ **; $p < 0.0001$

(2011) studied 25 varieties of winter wheat under different environments at 11 locations in three countries in Central Europe. Hybrid variety Hyland showed high grain yield with good adaptability to humid conditions. High temperature and precipitation deficiency were extreme weather circumstances with maximum influence on crop production and fertility in greenhouse. Knowledge should be acquired on various plant defence mechanisms and their physiological and biochemical backgrounds. Changes in thousand kernel weight were a good indicator of the sensitivity of wheat lines to biotic and abiotic stresses. Small changes indicate adaptation to different field/greenhouse and weather conditions (Blum, 2005).

The comparative analysis found some differences among the AD plants, and between them and its parents (Table 3). Least seed set was produced in AD45-4 (plant no. 2 with 7.8 grains), plant no.3 of 198-2(F_5) and no.5 of TS18397, which formed between 4 and 6 seeds per ear. One plant (45-4-2) was morphologically different from the rest 11 and developed typical traits of TS18397. It exhibited brown colored glumes and owns, setting one seed in spikelet. About one third of normally developed spikelets per spike did not generate seeds that are characteristic for the naked einkorn wheat, grown in stressed environment.

Conclusions

The production of hybrid F_1 seeds and plants were achieved in *Triticum* x *Triticum* crosses in greenhouse stressed environment (low temperatures in winter and heat conditions in April-June). From the 174 hybrid seeds obtained, 86 (49.4%) germinated and 45 (25.9%) plants matured. Three crosses with different tetraploid females and *T. sinskajae* acc.18397 as a pollinator produced largest number of hybrids. All F_1 plants were sterile except from the hulled einkorn x naked einkorn cross. Plants from emmer x naked einkorn crosses were treated with colchicine solution to produce C_1 seeds. AD plants from the cross *T. dicoccum* acc.45398 x TS acc.18397 damaged by frost in winter, while those from 198-2F_3 x TS acc.18397 survived and yielded two families, one of which born more seeds in C_2 generation than the parental forms, grown in stressed environment.

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Received November, 18, 2015; accepted for printing February, 19, 2016