

Drought Resistance and Mitotic Instability of Tritipyrum Compared with Triticale and Bread Wheat

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Abstract

This study presents the first data on the drought resistance pattern of seven new synthetic 6x primary Tritipyrum amphiploid lines and evaluates their mitotic instability. The primary Tritipyrum lines were crossed with Iranian 6x bread wheat 'Navid' cultivar and their F₁ and F₂ progenies were obtained. Two experiments with complete randomized design were conducted under optimum and limited water conditions to evaluate Tritipyrum-derived genotypes for drought resistance in greenhouse. Under optimum water conditions, grain yield, numbers of grains per spike and harvest index of Tritipyrum-derived genotypes were significantly lower than bread wheat; however the differences were not significant under limited water conditions. These results showed the better responses of Tritipyrum-derived genotypes to drought conditions. Evaluation of leaf osmotic and water potentials and drought susceptibility index showed that drought resistance of Tritipyrum and F₁ genotypes was significantly higher than that of bread wheat and Triticale. Cytological investigations showed that Tritipyrum-derived genotypes aneuploidy was significantly higher than Triticale and bread wheat ($p < 0.05$). Mitotic instability in light grains (1000-grains weight < 30 gr) was significantly higher than heavy grains (1000-grains weight > 30 gr) in parental and F₂ genotypes ($p < 0.01$). Aneuploidy has showed a significant negative correlation with fertility, grain yield and 1000-grains weight in Tritipyrum genotypes. In general, Tritipyrum may complement the role of bread wheat in arid and semi-arid regions; but further breeding research is needed to overcome its mitotic instability.

Keywords: amphiploid, aneuploidy, drought susceptibility index

Introduction

Adverse environmental conditions and abiotic stresses are major limiting factors for wheat production in arid and semi-arid areas (Talebi *et al.*, 2009). Synthesis of 6x amphiploid cereals with higher tolerance against abiotic stresses is needed to supply the increasing world population with the required amount of wheat production (Kamyab *et al.*, 2009).

New cereal, primary Tritipyrum lines (2n=6x=42, AABBE^bE^b) is a synthetic amphiploid between *Triticum durum* (AABB) and *Thinopyrum bessarabicum* species (E^bE^b) in which the introduction of alien genetic material from *Thinopyrum* genus into wheat through wide crosses has provided increased genetic diversity and thereby an opportunity to improve traits such as drought and salinity tolerance and disease resistance (Allahdoo *et al.*, 2009; Chen Q, 2005; Shahsavand Hassani *et al.*, 2000).

Primary Tritipyrum lines, which was produced at John Innes Centre, Norwich, UK (Kamyab *et al.*, 2009; King *et al.*, 1997) is the third man made and salt tolerant synthetic crop after Triticale and Tritordeum, to be evaluated for conditions unsuitable for wheat growth, particularly, at the flowering stage (Shahsavand Hassani *et al.*, 2000). The genetic diversity and adaptation of wheat and Triticale have been studied quite extensively, but such assessments

are still far from enough in the case of primary Tritipyrum lines. Improved grain yield is a critical criterion which can be determined by measuring yield components and other closely associated characteristics (Kamyab *et al.*, 2009; Sharma *et al.*, 2003).

Having disadvantages such as mitotic instability, primary Tritipyrum lines still may not be considered as a commercial salt tolerant crop. Primary Tritipyrum lines tends to suffer problems similar to those of Triticale in its early days, such as chromosome instability and low fertility, which in Triticale were overcome by means of breeding practices (Shahsavand Hassani *et al.*, 1998; Shahsavand Hassani *et al.*, 2000).

The assessment of today's Tritipyrum situation in comparison with Triticale and the possible problems facing breeders in future is not an easy undertaking. The comprehensive and voluminous work performed on Triticale is manifested interview papers on different aspects of that plant, and shows the importance of this first man-made cereal. With due research, the primary Tritipyrum lines have the same potential to become a success story of another man made cereal (Shahsavand Hassani *et al.*, 2000).

The aim of this study was to determine the drought resistance and mitotic instability of a number of primary Tritipyrum lines and to evaluate bread wheat/ Tritipyrum progenies potential for drought resistance breeding in

bread wheat. This study presents the first data on the drought resistance pattern of primary 6x Tritipyrum amphiploid lines.

Materials and methods

The experiment was carried out in the green house and cytogenetic laboratory of department of agronomy and plant breeding, Shiraz University, Shiraz, Iran.

Greenhouse trials

Primary Tritipyrum lines [Az/b; La/b; (Ka/b)(Cr/b), F₅; (Ma/b)(Cr/b); (St/b)(Cr/b), F₃; Ma/b, Cr/b] were crossed with Iranian bread wheat 'Navid' cultivar. The seeds of primary lines were selected based on previous studies in Iran (Kamyab *et al.*, 2009; Shahsavand Hasani *et al.*, 2000). Spikes of seven primary Tritipyrum lines (2n=6x=42, AABB^bE^b) were emasculated and then pollinated with pollen from Iranian bread wheat 'Navid' cultivar, and F₁ seeds of each cross were produced. The seven primary Tritipyrum lines, their F₁ progenies and two controls including promising Triticale 4118 line and Iranian bread wheat 'Navid' cultivar were sown in greenhouse at 5 kg pots in two experiments with completely randomized design with 3 replications and 3 samples in each experimental unit. The soil was loamy clay with a field capacity of 27%. The two experiments differed with respect to their irrigation regimes. Pots were irrigated twice a week to 20% (stress experiment) and 60% (non-stress experiment) of 100% soil water capacity, respectively. Grain yield per plant, 1000 grains weight (gr), biological yield (gr/plant), harvest index and fertility were measured. Fertility was expressed as the number of grains per spikelet measured on the main spike of plant with longest shoot. For drought resistance investigation, leaf water potential and osmotic potential were measured and drought susceptibility index was determined. Leaf water potential (ψ_w) was measured using a PMS pressure bomb (PMS Instrument Co., Corvallis, OR) at stem elongation, booting and flowering stages based on Zadoks's Code (Zadoks *et al.*, 1974) in both experiments. The youngest fully expanded leaf was detached and placed rapidly in a sample chamber and the pressure was recorded. Three randomly selected plants were used for each developmental stage. Measurements were completed between 13:00 and 15:00 o'clock. To measure osmotic potential (ψ_s), the youngest fully expanded leaf was used for each developmental stage. Leaves were placed in plastic bags and rapidly packed in a box in order to avoid water loss as evaporation from the sample, and were maintained at -15°C for five hours. The frozen samples were then thawed for approximately 30 minutes and the freezing point (T) of collected saps was measured using a digital thermometer (Model ET-2001). The osmotic potential was then calculated (Kramer, 1995) as $\psi_s = (-T / 1.86) \times 2.27$.

The drought susceptibility index (S) was determined by $S = [1 - (y_D / y_p)] / D$ equation (Fischer and Mourer,

1978), where y_D and y_p are the grain yields of each cultivar at stress and non-stress conditions, respectively; and $D = 1 - (Y_D / Y_p)$. Y_D and Y_p are average yields of all cultivars under stress and non-stress conditions, respectively.

Cytological investigations

For mitotic chromosome counting study, the primary Tritipyrum lines and their F₂ selfing progenies were evaluated in a complete randomized design. The Tritipyrum lines and promising Triticale line were divided into two classes based on their 1000-grains weight (less and higher than 30 gr). 'Navid' bread wheat cultivar was only classified into the heavy grain class (heavier than 30 gr). Grains were germinated in Petri dish and then planted in small pots and allowed to grow to a proper size for chromosome counting. The Chromosome number of all lines was counted in 5 metaphase plates by minor modification of the conventional root tip squash protocol (Oudjehih and Boukaboub, 2000; Oudjehih and Bentouati 2006; Tosun, 1999).

Statistical analysis

All data were subjected to analysis of variance using SPSS statistical software. Means were separated and compared using Duncan multiple range test (DMRT) at the 0.05 level of significance. Mean differences between light and heavy grains euploids were compared using least significant difference (LSD). Pearson correlation coefficient was calculated between variables (Assad, 1997).

Results and discussion

Drought resistance

The relative yield performance of all primary Tritipyrum, promising Triticale and bread wheat lines under drought stress and normal conditions is a common starting point in the selection of genotypes for use in breeding in dry environments (Byrne *et al.*, 1995; Rajaram and van Ginkel, 2001). Tritipyrum-derived genotypes and bread wheat did not differ significantly in respect to harvest index ($p > 0.05$), grain yield ($p > 0.05$) and biological yield ($p > 0.05$) under stress condition; however the differences were significant under non-stress conditions (Tab. 1 and 2). Tritipyrum-derived genotypes and Triticale did not differ significantly in respect to harvest index under stress conditions ($p > 0.05$). Biological yield and grain yield of some Tritipyrum-derived genotypes were significantly lower than Triticale under stress and non-stress conditions ($p < 0.05$). Under a particular pressure of environmental stress, cultivars with high yield potential produce less than certain cultivars that have lower yield potential but seem to be better adapted to stress (Blum, 2005). 1000 grains weight of Tritipyrum-derived genotypes was significantly lower than bread wheat and Triticale under stress and non-stress conditions ($p < 0.05$). Number of grains per spike of Tritipyrum-derived genotypes was significantly lower than bread wheat and Triticale under non-stress conditions

Tab. 1. Yield components of 6x Tritipyrum-derived genotypes compared with promising Triticale (4118) and bread wheat 'Navid' cultivar under normal irrigation conditions

6x amphiploides	Line/cultivar/progenies	Grain yield (gr/ plant)	Fertility (grain number / spikelet)	1000-grains weight (gr)	Grain no/spike	Biological yield (gr/plant)	Harvest index
Primary Tritipyrum lines	(Ka/b)(Cr/b), F ₅	2.93c	2.86d	32.87d	35.77cde	11.54cde	25.43e
	La/b	3.07bc	3.07bcd	33.7cd	34.56f	11.43def	26.81cde
	Az/b	3.03bc	2.9dc	33.74cd	35.07def	11.44def	26.52cde
	(Ma/b)(Cr/b)	3.13bc	3.03bcd	34.33cd	34.93ef	11.93b	26.26de
	(St/b)(Cr/b), F ₃	2.98c	3.5a	33.9cd	35.5cdef	11.76bcd	25.49e
	Ma/b	2.56d	3.07bcd	33.1d	36.17bc	11.63bcde	22f
	Cr/b	2.97c	3.26abc	34.74cd	35.97bcd	11.4def	26.31de
Triticale	4118	4.03a	3.4ab	45a	36.9a	12.47a	32.35ab
Bread wheat	'Navid'	4.23a	3.6a	41.5b	37.33a	12.8a	33.08a
Tritipyrum/bread wheat(F ₁)	(Ka/b)(Cr/b),F ₅ /Navid (F ₁)	3.27bc	2.1e	34.7cd	33.01g	11.24ef	29.06cd
	(La/b)/Navid(F ₁)	3.4b	1.83ef	34.71cd	32.09g	11.46def	29.65bc
	(Az/b)/Navid(F ₁)	3.1bc	2.07ef	33.6cd	32.5g	11.12f	27.9cde
	(Ma/b)(Cr/b)/Navid(F ₁)	3.23bc	2ef	33.97cd	32g	11.5def	28.12cde
	(St/b)(Cr/b), F ₃ /Navid(F ₁)	3.26bc	1.73f	33.57cd	31.01h	11.9bc	27.45cde
	(Ma/b)/Navid(F ₁)	3.03bc	2.04ef	35.26c	31.9gh	11.43def	26.53cde
	(Cr/b)/Navid(F ₁)	3.25bc	1.97ef	35.23c	32.3g	11.6bcde	28.16cde
	F test	2.6*	2.45*	2.53*	4.5**	4.64**	5.1**
	CV%	8.7	9.02	10.23	8.89	7.987	8.54

columns with the same letter(s) were not significantly different according to Duncan test at $p \leq 0.05$. * and ** significant at 0.05 and 0.01, respectively

Tab. 2. Yield components and drought susceptibility index of 6x Tritipyrum-derived genotypes compared with promising Triticale (4118) and bread wheat 'Navid' cultivar under limited water conditions

6x amphiploides	Line/cultivar/progenies	Grain yield (gr/ plant)	Fertility (grain number / spikelet)	1000-grains weight (gr)	Seed no/spike	Biologic Yield (gr/plant)	Harvest index	Drought susceptibility Index
Primary Tritipyrum lines	(Ka/b)(Cr/b), F ₅	2.07b	2.7cde	28.13de	a33.3	8.93bc	23.13a	0.425cd
	La/b	2.1b	2.63cde	26.5e	a33	9.03ab	23.28a	0.455bcd
	Az/b	2.27ab	3.16ab	27.98de	a32	8.83bc	25.65a	0.362cd
	(Ma/b)(Cr/b)	2.26ab	2.6cde	28.9cd	a32.1	8.96bc	25.26a	0.4cd
	(St/b)(Cr/b), F ₃	2.09b	3.3a	28.73cd	a32.9	8.67c	24.25a	0.431d
	Ma/b	2.33ab	2.93bc	28.78cd	a32	9.13ab	25.54a	0.321d
	Cr/b	2.17ab	2.6cde	28.6cd	a32.5	8.9bc	24.36a	0.402cd
Triticale	4118	2.4a	2.43e	38.8a	a33	9.3a	25.73a	0.584ab
Bread wheat	'Navid'	2.33ab	2.8cd	36.03b	a32	9.07ab	25.74a	0.649a
Tritipyrum/bread wheat(F ₁)	(Ka/b)(Cr/b),F ₅ /Navid (F ₁)	2.3ab	2.39e	30.4bc	33.1a	8.8bc	26.14a	0.427bcd
	(La/b)/Navid(F ₁)	2.16ab	2.46e	30.2c	32a	9.03ab	23.98a	0.521abc
	(Az/b)/Navid(F ₁)	2.4a	2.6cde	30.3c	31.5a	9.06ab	26.46a	0.324d
	(Ma/b)(Cr/b)/Navid(F ₁)	2.13ab	2.63cde	30.33c	32.1a	9.04ab	23.61a	0.491bc
	(St/b)(Cr/b), F ₃ /Navid(F ₁)	2.2ab	2.38e	30.13c	32.5a	8.94bc	24.68a	0.474bcd
	(Ma/b)/Navid(F ₁)	2.26ab	2.41e	29.73cd	33.3a	8.8bc	25.77a	0.362cd
	(Cr/b)/Navid(F ₁)	2.15ab	2.44e	30.13c	33.4a	8.92bc	24.07a	0.494bc
	F test	1.01 ^{ns}	2.33*	2.36*	1.32 ^{ns}	2.37*	0.99 ^{ns}	8.22**
	CV%	9.05	9.65	8.98	10.8	10.43	10.87	9.32

columns with the same letter(s) were not significantly different according to Duncan multiple range test at $p \leq 0.05$. * and ** significant at 0.05 and 0.01, respectively

($p < 0.01$); however it was similar to that of bread wheat under stress conditions ($p > 0.05$). Fertility of some Tritipyrum-derived genotypes was significantly higher than Triticale and bread wheat under limited water conditions ($p < 0.05$). The results were in agreement with several stud-

ies that indicated grains per spike and spikes per square meter were the yield components most sensitive to drought; while grain weight remains relatively stable (Guinta *et al.*, 1993; Zhong and Rajaram, 1994). Bread wheat and Triticale yield components was more sensitive to drought

Tab. 3. Leaf water potential (ψ_s) of 6x Tritipyrum-derived genotypes compared with promising Triticale (4118) and bread wheat 'Navid' cultivar in different growth stages under non-stress and drought stress conditions

6x amphiploides	Line/cultivar/progenies	Non-stress conditions			Stress conditions		
		Stem elongation	Booting	Flowering	Stem elongation	Booting	Flowering
Primary Tritipyrum lines	(Ka/b)(Cr/b), F ₅	-0.593d	-1.75d	-2.11h	-2.4cd	-3.11e	-4.193g
	La/b	-0.59d	-1.743d	-2.09gh	-2.13cd	-3.14e	-4.2g
	Az/b	-0.598d	-1.737c	-2.08fgh	-2.1c	-3.11e	-4.15f
	(Ma/b)(Cr/b)	-0.591d	-1.727d	-2.07fgh	-2.33d	-3.1e	-4.18fg
	(St/b)(Cr/b), F ₃	-0.61d	-1.76d	-2.04f	-2.12cd	-3.13e	-4.176fg
	Ma/b	-0.603d	-1.747d	-2.057fg	-2.15cd	-3.12e	-4.21g
	Cr/b	-0.601d	-1.74d	-2.05f	-2.11c	-3.1e	-4.18fg
Triticale	4118	-0.43b	-1.26b	-1.42b	-1.42a	-2.82b	-3.87b
Bread wheat	'Navid'	-0.337a	-1.07a	-1.24a	-1.32a	-2.46a	-3.62a
Tritipyrum/bread wheat (F ₁)	(Ka/b)(Cr/b), F ₃ /Navid (F ₁)	-0.49c	-1.513c	-1.617c	-1.67b	-2.93d	-3.65de
	(La/b)/Navid(F ₁)	-0.48c	-1.53c	-1.647cd	-1.73b	-2.94d	-3.92cd
	(Az/b)/Navid(F ₁)	-0.46bc	-1.53c	-1.69e	-1.83b	-2.87c	-3.95de
	(Ma/b)(Cr/b)/Navid(F ₁)	-0.46bc	-1.523c	-1.7e	-1.8b	-2.9cd	-3.99e
	(St/b)(Cr/b), F ₃ /Navid(F ₁)	-0.487c	-1.54c	-1.673de	-1.81b	-2.89c	-3.96de
	(Ma/b)/Navid(F ₁)	-0.47c	-1.547c	-1.68de	-1.79b	-2.94d	-3.9bc
	(Cr/b)/Navid(F ₁)	-0.473c	-1.5c	-1.68de	-1.8b	-2.903cd	-3.92cd
F test		4.2**	2.42*	6.3**	5.43**	4.17**	7.21**
CV%		9.86	8.43	9.78	9.45	10.06	8.52

Columns with the same letter(s) were not significantly different according to Duncan test at $p \leq 0.05$. * and ** significant at 0.05 and 0.01, respectively

Tab. 4. Leaf osmotic potential of 6x Tritipyrum-derived genotypes compared with promising Triticale (4118) and bread wheat 'Navid' cultivar at different developmental stages under non-stress and drought stress conditions

6x amphiploides	Line/cultivar/progenies	Non-stress conditions			Stress conditions		
		Stem elongation	Booting	Flowering	Stem elongation	Booting	Flowering
Primary Tritipyrum lines	(Ka/b)(Cr/b), F ₅	-2.38f	-3.01f	-3.65e	-3.017d	-3.71g	-4.63i
	La/b	-2.37f	-2.98f	-3.62e	-2.99d	-3.66ef	-4.59gh
	Az/b	-2.39f	-3.01f	-3.63e	-2.97d	-3.68efg	-4.6hi
	(Ma/b)(Cr/b)	-2.36f	-2.98f	-3.61e	-2.98d	-3.65e	-4.58gh
	(St/b)(Cr/b), F ₃	-2.37f	-2.97f	-3.6e	-2.96d	-3.7fg	-4.52f
	Ma/b	-2.37f	-3.02f	-3.58e	-2.95d	-3.68efg	-4.56g
	Cr/b	-2.38f	-2.99f	-3.59e	-3.02d	-3.69efg	-4.57gh
Triticale	4118	-1.46b	-2.41b	-2.7b	-2.51b	-3.24b	-4.02b
Bread wheat	'Navid'	-0.96a	-1.91a	-2.07a	-1.98a	-3.79a	-3.61a
Tritipyrum/bread wheat (F ₁)	Ka/b*Cr/b(F ₃)*Navid(F ₁)	-2.11de	-2.6d	-2.94cd	-2.74c	-3.49d	-4.29de
	La/b*Navid(F ₁)	-2.09cd	-2.64de	-2.98d	-2.73c	-3.43c	-4.29de
	Az/b*Navid(F ₁)	-2.07c	-2.66de	-2.97d	-2.75c	-3.46cd	-4.26cde
	Ma/b*Cr/b*Navid(F ₁)	-2.1cde	-2.7e	-2.93cd	-2.74c	-3.44c	-4.27cde
	St/b*Cr/b (F ₃)*Navid(F ₁)	-2.13e	-2.51c	-2.93cd	-2.73c	-3.44c	-4.257cde
	Ma/b*Navid(F ₁)	-2.09cd	-2.61de	-2.84c	-2.69c	-3.42c	-4.253cd
	Cr/b*Navid(F ₁)	-2.11de	-2.59cd	-2.92cd	-2.71c	-3.44c	-4.247c
F test		**	*	**	*	**	**
CV%		7.94	8.71	8.93	9.01	10.31	7.87

Columns with the same letter(s) were not significantly different according to Duncan test at $p \leq 0.05$. * and ** significant at 0.05 and 0.01, respectively

stress than that of Tritipyrum-derived genotypes. Yield and yield-related traits under stress were independent of yield and yield-related traits under non-stress conditions (Talebi *et al.*, 2009). For most cereals grown under water-limited conditions the crossover occurs at a yield level of around 2-3 t/ha, which is approximately one-third of the yield potential (Blum, 2005).

Tritipyrum-derived genotypes showed lower drought susceptibility indices than bread wheat ($p < 0.01$). Drought susceptibility indices of some Tritipyrum-derived genotypes had no significant difference with Triticale (Tab. 2). Susceptible cultivars on average have higher drought susceptibility indices than intermediate and resistant cultivars (Amiri and Assad, 2005).

Tab. 5. Mean comparison between euploid plants in light grain (1000-grains weight < 30 gr) and heavy grain (1000-grains weight > 30 gr) genotypes. Aneuploidy percentage in both grain weight classes is showed as well (r=3 with 60 seedlings per replication)

6x amphiploides	Line/cultivar/progenies	Heavy grains Euploid seedling number (2n=42)	Heavy grain Aneuploidy (%)	Light grains Euploid seedling number (2n=42)	Light grains Aneuploidy (%)	Mean differences between light and heavy grain's Euploids (LSD)
Primary Tritipyrum lines	(Ka/b)(Cr/b), F ₅	47.3def	21.2	44.67 bc	25.6	2.63**
	La/b	49.5bcde	17.5	46.3 b	22.8	3.2**
	Az/b	51bcd	15.0	46.7 b	22.2	4.3**
	(Ma/b)(Cr/b)	49bcde	18.3	45 bc	25.0	4**
	(St/b)(Cr/b), F ₃	50bcde	16.7	46.3 b	22.8	3.7**
	Ma/b	51.67bc	14.0	45.6 b	24.0	6.07**
	Cr/b	52.7b	12.2	46.7 b	22.2	6**
Triticale	4118	58a	5.0	54 a	5.0	4**
Bread wheat	'Navid'	60a	0.0	-	-	-
Tritipyrum/bread wheat (F ₂)	(Ka/b)(Cr/b), F ₅ /Navid (F ₂)	45f	25.0	40.7 e	32.2	4.3**
	(La/b)/Navid(F ₂)	47.3def	21.2	42.3cde	29.5	5**
	(Az/b)/Navid(F ₂)	48.3cdef	19.5	41de	31.7	7.3**
	(Ma/b)(Cr/b)/Navid(F ₂)	47ef	21.7	42cde	30.0	5**
	(St/b)(Cr/b), F ₃ /Navid(F ₂)	48.6cdef	19.0	41de	31.7	7.6**
	(Ma/b)/Navid(F ₂)	50.3bcde	16.3	42.3cde	29.5	8**
	(Cr/b)/Navid(F ₂)	49.3bcde	17.8	44bcd	26.7	5.3**
F test	**	-	**	-	-	-
CV%	6.5	-	7.1	-	-	-

Columns with the same letter(s) were not significantly different according to Duncan test at p≤0.05. * and ** significant at 0.05 and 0.01, respectively

Tab. 6. Distribution of Monosomic, nullisomic and Trisomic seedlings among light and heavy grains within the hexaploid Tritipyrum genotypes and their F2 progenies compared with Triticale and bread wheat (r=3 with 60 seedlings per replication)

6x amphiploides	Line/cultivar/progenies	Heavy Grains			Light Grains		
		Monosomic seedling (2n=41)	Trisomic seedling (2n=43)	Nulisomic seedling (2n=40)	Monosomic seedling (2n=41)	Trisomic seedling (2n=43)	Nulisomic seedling (2n=40)
Primary Tritipyrum lines	(Ka/b)(Cr/b), F ₅	6.3abc	1.3ab	4.3abc	9.3cde	1.7 ab	3.7bcd
	La/b	5bcdef	1.3ab	3.3bc	7.3e	1.3 b	3.5bcd
	Az/b	4defg	0.7abc	3.25bc	7.27 e	1 b	3.61bcd
	(Ma/b)(Cr/b)	4.3cdefg	1.27ab	4abc	8.4 de	1.67ab	4bc
	(St/b)(Cr/b), F ₃	4.7bcdefg	1.32ab	3.33bc	8.34 de	1.27 b	3cd
	Ma/b	3.7efg	1abc	3.27bc	8.36 de	1.01 b	3.3bcd
	Cr/b	3fg	0.68abc	3c	9 cde	1.32 b	2.27d
Triticale	4118	2.66g	0.33bc	0d	2.66 f	0.33c	0e
Bread wheat	'Navid'	0 i	0 c	0d	-	-	-
Tritipyrum/bread wheat (F ₂)	(Ka/b)(Cr/b), F ₅ /Navid (F ₂)	7.3 a	1.35ab	5.3a	11.7 ab	1.26 b	5.7a
	(La/b)/Navid(F ₂)	5.3abcde	1.7a	4abc	10 bcd	3 a	3cd
	(Az/b)/Navid(F ₂)	6abcd	0.67abc	4.7abc	12.4 a	1.8 ab	4.59ab
	(Ma/b)(Cr/b)/Navid(F ₂)	6.7ab	0.33bc	5ab	11 abc	1.71 ab	4.3abc
	(St/b)(Cr/b), F ₃ /Navid(F ₂)	5.5abcde	1abc	4abc	11.7 ab	2.3 ab	4.3abc
	(Ma/b)/Navid(F ₂)	4.3cdefg	1abc	3.28bc	10 bcd	2.27 ab	4.32abc
	(Cr/b)/Navid(F ₂)	4.7bcdefg	1.7a	4abc	10.67 abc	1 b	3.7bcd
F test	**	*	**	*	*	*	
CV%	6.5	9.23	9.74	8.2	9.3	9.55	

Columns with the same letter(s) were not significantly different according to Duncan test at p≤0.05. * and ** significant at 0.05 and 0.01, respectively

Tritipyrum parental genotypes showed significantly lower ψ_w values than F₁ genotypes, and F₁ genotypes showed significantly lower ψ_w values than Triticale and

bread wheat at three developmental stages (Tab. 3). Drought resistant genotypes showed lower ψ_w values compared with sensitive genotypes (Amiri and Assad, 2005;

Tab. 7. Simple correlation coefficients between light and heavy grains aneuploidy, fertility, 1000 grains weight and grain yield of Tritipyrum-derived genotypes

Traits	Light grains aneuploidy (%)	Heavy grains aneuploidy (%)	Fertility	1000-grains weight (gr)
Light grains aneuploidy (%)	1			
Heavy grains aneuploidy (%)	0.935(**)	1		
Fertility	-0.765(**)	-0.681(**)	1	
1000-grains weight (gr)	-0.879(**)	-0.846(**)	0.426(**)	1
Grain yield (gr / plant)	-0.706(**)	-0.615(**)	0.662(**)	0.909(**)

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed)

Barlow *et al.*, 1980; Keim and Kronstad, 1981; Moustafa *et al.*, 1996).

The trend of variation in leaf osmotic potential (ψ_s) values in different growth stages for all genotypes was similar to that of ψ_w (Tab. 4). Drought resistant genotypes showed lower ψ_s values compared with sensitive genotypes (Amiri and Assad, 2005; Blum, 1989; Musick *et al.*, 1994).

Mitotic stability

Analysis of variance indicated that the effect of grain weight on mitotic chromosome stability was significant. There was a significant difference ($p < 0.01$) between euploid plants means within light and heavy grains of Tritipyrum derived genotypes and Triticale (Tab. 5). The distribution of monosomic, trisomic and nullisomic plants among light- and heavy-grain genotypes was provided in Tab. 6. Other types of aneuploid seedlings were rarely observed. Similar observations have been reported in Triticale (Akgun and Altindal, 2010; Tosun *et al.*, 2003). Euploid seedlings means among light grains of all Tritipyrum-derived genotypes (parental Tritipyrum lines and F_2 progenies) were significantly lower than Triticale. Euploid seedlings means among light grains of four F_2 progenies were significantly ($p < 0.05$) lower than their parental genotypes. Chromosome number investigation of the light-grain class showed that Tritipyrum-derived genotypes were significantly more mitotically instable than Triticale and four F_2 progenies were significantly more mitotically instable than their parental Tritipyrum genotypes.

Euploid seedlings means among heavy grains of all Tritipyrum-derived genotypes (parental Tritipyrum genotypes and F_2 progenies) were significantly lower than Triticale and bread wheat as control. Euploid seedlings means among heavy grains of F_2 progenies showed no significant difference with their parental Tritipyrum genotypes. These data showed that grain weight positively affected chromosome stability in segregation generations (Akgun and Altindal, 2010; Oudjehih and Boukaboub, 2000; Suarez and Favret, 1986; Tosun *et al.*, 2003; Tsuchiya, 1973).

Fertility, 1000 grains weight and grain yield of Tritipyrum-derived genotypes have shown a significant negative correlation (Tab. 7) with mitotic instability (Elneskog Staam and Marker, 2002). Tritipyrum-derived genotypes' fertility had a significant positive correlation with their 1000-grains weight and grain yield (Kamyab *et al.*, 2009; Shahsavand Hassani *et al.*, 2000).

Chromosome counts of 42-chromosome hexaploid Tritipyrum-derived genotypes exhibited a considerable amount (12-32.1%) of instability in the form of aneuploidy. The results were in agreement with Shahsavand and Hassani *et al.* (2000); (1997), Siahshar *et al.* (2011), and King *et al.* (1997). It has been suggested that the screening of larger and heavier grains could give a higher frequency of euploids and grain weight significantly affected mitotic chromosome stability in segregation generations. Similar investigations in other crops displayed a close relationship between grain characteristics and aneuploid frequency (Akgun and Altindal, 2010; Oudjehih and Boukaboub, 2000; Suarez and Favret, 1986; Tosun *et al.*, 2003; Tsuchiya, 1973).

Conclusions

F_1 genotypes from 6x primary Tritipyrum/6x bread wheat showed acceptable yield components under drought stress and non-drought stress conditions compared with 6x primary Tritipyrum lines (Tab. 1 and 2). Also mitotic stability of F_2 heavy grains was significantly similar to 6x primary Tritipyrum lines (Tab. 5). Leaf water and osmotic potential and drought susceptibility index indicated better drought resistance of F_1 genotypes than that of their bread wheat parent 'Navid' cultivar. Data showed that hybridization between 6x primary Tritipyrum lines and bread wheat creates unique and novel, promising generations for wheat drought resistance selection and breeding. This study indicated that the yield of primary Tritipyrum lines was equal to that of Iranian bread wheat cultivar 'Navid' and promising Triticale 4118 line under limited water conditions. In general primary Tritipyrum lines may complement the role of bread wheat in arid and semi-arid regions (Kamyab *et al.*, 2009; Shahsavand Hassani *et al.*, 2006) and is a good genetic resource for wheat breeding (Allahdoo *et al.*, 2009; Chen Q, 2005; Shahsavand Hassani *et al.*, 2000); but more breeding research is needed to overcome its mitotic instability by producing the secondary Tritipyrum genotypes via crossing the primary lines with bread wheat cultivar such as 'Navid'.

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References

- Akgun I, Altindal N (2010). Relationship among Aneuploidy, Germination rate and seed shriveling in 6X-Triticals. *Turkish J Field Crops* 15(1):25-28.
- Allahdoo M, Siahisar BA, Hassani HS, Pour AK, Nazhad NM (2009). The identification of E^b chromosome in Tritipyrum Primary lines using random and semi random primers. *Trakia J Sci* 4:1-6.
- Amiri Fahliani M, Assad MT (2005). Evaluation of Three Physiological Traits for Selecting Drought Resistant Wheat Genotypes. *J Agric Sci Technol* 7:81-87.
- Assad MT (1997). *Agricultural field experiments design and analysis*. Shiraz University Press, 543 p.
- Barlow EWR, Lee JW, Munns R, Smart MG (1980). Water Relations of Developing Wheat Grain. *Aust J Plant Physiol* 7:519-525.
- Blum A (1989). Osmotic Adjustment and Growth of Barley Genotypes under Drought Stress. *Crop Sci* 29:230-233.
- Blum A (2005). Drought resistance, water-use efficiency, and yield potential-are they compatible, dissonant, or mutually exclusive? *Aus J Agric Res* 56:1159-1168.
- Byrne PF, Bolanos J, Edmeades GO, Eaton DL (1995). Gains from selection under drought versus multi location testing in related tropical maize populations. *Crop Sci* 35:63-69.
- Chen Q (2005). Detection of alien chromatin introgression from Thinopyrum into Wheat using S genomic DNA as a probe-A landmark approach for Thinopyrum genomic research. *Cytogenetic Genome Res* 109:350-359.
- Elneskog Staam P, Marker A (2002). Chromosome composition, stability and fertility of Allopolyploid between Triticum turgidum var. cartholicum and Thinopyrum junceiforme. *Hereditas* 136:59-65.
- Fischer RA, Maurer R (1978). Drought Resistance in Spring Wheat Cultivars I: Grain Yield Responses. *Aust J Agr Res* 29:897-912.
- Guinta FR, Motzo R, Deidda M (1993). Effect of drought on yield and yield components of durum wheat and Triticale in a Mediterranean environment. *Field Crop Res* 33:399-409.
- Kamyab M, Hassani HS, Tohidinejad E (2009). Agronomic behavior of a new cereal (Tritipyrum: AABBE^bE^b) compared with modern Triticale and Iranian bread wheat cultivars. *Plant Ecophysiol* (2):69-80.
- Keim DL, Kronstad WE (1981). Drought response of winter wheat cultivars grown under field stress conditions. *Crop Sci* 21:11-15.
- King IP, Law CN, Cant KA, Orford SE, Reader SM, Miller TE (1997). Tritipyrum, a potential new salt-tolerant cereal. *Plant Breed* 116:127-132.
- Kramer PJ and Boyer JS (1995). *Water relations of plant and soils*. Ed. Academic Press, New York.
- Oudjehih B, Boukaboub A (2000). Cytogenetic study of Triticale. *Agricultures* 9:519-523.
- Oudjehih B, Bentouati A (2006). Chromosome numbers of the 59 species of Eucalyptus L'Herit. (*Myrtaceae*). *Caryologia* 59(3):207-212.
- Moustafa MA, Boersma L, Kronstad WE (1996). Response of four Spring Wheat Cultivars to Drought Stress. *Crop Sci* 36:982-986.
- Musick JT, Jones OR, Stewart BA, Dusek DA (1994). Water-yield relationships for irrigated and dryland wheat in the U.S. Southern plains. *J Agron* 86:980-986.
- Rajaram S, Van Ginkle M (2001). A History of Wheat Breeding, p. 579-604. In: Bonjean AP, Angus WJ (Eds). *World Wheat Book*. Lavoisier Publishing, Paris, France.
- Shahsavand Hassani H, Caligari PDS, Miller TE (1997). Production of secondary Tritipyrum: Assessment of chromosome constitution by in situ hybridization. Proc. 3rd. Inter Symp "New genetical approaches to crop improvement". Tando Jam, Pakistan.
- Shahsavand Hassani H (1998). Development and cytogenetic studies of a potential new salt tolerant cereal, Tritipyrum. Ph.D. Thesis, The University of Reading, UK.
- Shahsavand Hassani H, King IP, Reader SM, Caligari PDS, Miller TE (1998). An assessment of Tritipyrum, a new potential cereal with salt tolerance. 8th. Inter. Wheat genet. Symp, Saskatoon, Canada.
- Shahsavand Hassani H, King IP, Reader SM, Caligari PDS, Miller TE (2000). Can Tritipyrum, a new salt tolerant potential amphiploid, be a successful cereal like Triticale? *J Agric Sci Technol* 2:177-195.
- Shahsavand Hassani H, Reader SM, Miller TE (2006). Agronomical and adoption characters of Tritipyrum lines in comparison with Triticale and Iranian wheat. *Asian J Plant Sci* 5(3):553-558.
- Sharma SN, Sain RS, Sharma RK (2003). The genetic control of the flag leaf length in normal and late sown durum wheat. *J Agric Sci* 141:323-331.
- Siahisar BA, Maryam A, Hassani HS (2011). Genetic variation among and within Tritipyrum (*Thinopyrum bessarabicum* × *Triticum durum*) lines using PCR-based molecular markers. *Cien Inv Agr* 38(1):127-135.
- Suarez EY, Favret EA (1986). Aneuploidy as an explanation of high values of phenotypic variability in commercial wheat varieties. *Cereal Res Comm* 14(3):229-235.
- Talebi R, Fayaz F, Naji AM (2009). Effective selection criteria for assessing drought stress tolerance in durum wheat (*Triticum durum* Desf.). *Gen Appl Plant Physiol* 35:64-74.
- Tosun M (1999). Karyotype analysis in hexaploid Triticale. *Turkish J Agric For* 23:943-949.
- Tosun M, Halilo-Lu K, Pinar SM, Sasöz S (2003). Test weight, kernel shriveling, and aneuploidy frequency in Triticale. *New Zealand J Agric Res* 46:27-30.
- Tsuchiya T (1973). Frequency of euploids in different seed size classes of hexaploid Triticale. *Euphytica* 22(3):592-599.
- Zadoks JC, Chang TT, Konzak CF (1974). A decimal code for the growth stages of cereals. *Weed Res* 14:415-421.
- Zhong-hu H, Rajaram S (1994). Differential responses of bread wheat characters to high temperature. *Euphytica* 72:197-203.