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Can Tritipyrum, a New Salt Tolerant Potential Amphiploid, Be a Successful Cereal Like Triticale?

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ABSTRACT

Soil affected by salt (NaCl) is a major problem worldwide and in areas with potential agriculture; lands in many countries are not enough to support crop production. The development of salt tolerant cultivars would be enhanced by better understanding of the genetic control of tolerance to salt stress. A new cereal, tritipyrum, a range of amphiploids between *Triticum spp.* and *Thinopyrum spp.* offers such a new chance. Those with the 6x construction (2n=6x=42, AABBE^{b}E^b) derived from *Triticum durum* (2n=4x=28, AABB) and *Thinopyrum bessarabicum* (2n=2x=14, E^{b}E^b) are of the potential to become a new high salt tolerant cereal crop. Tritipyrum is prone to problems similar to those exhibited by early triticales, e.g. chromosome instability and low fertility, which in that crop were eventually overcome by breeding. Other problems could be overcome through substitution of E^b genome chromosomes by D genome ones, and the feasibility of this has been assessed in the progenies of (6x tritipyrum) x (6x wheat) hybrids with the aid of fluorescent *in situ* hybridization (FISH). The cytological, morphological and agronomic studies of existing tritipyrum lines, including the effect of vernalization, were carried out, too. A novel multiple-pistil/seed characteristic of one original tritipyrum line has also been investigated and its genetic basis established. The results have shown that, first creation of substituted lines is feasible, and thus it could be a route for the elimination of undesirable traits. Second, improvement should be possible via selection for chromosomally stable lines, with increased fertility and yield. Third, it may also be possible to exploit the perennial habit and multi-tillering traits in a dual-purpose forage/grain crop. Fourth, the multiple-pistil/seed trait may be controlled by two recessive genes. Fifth, there is a high probability of having established the seven possible monosomic additions of *Th. bessarabicum* to *T.durum* for the first time.

Keywords: Amphiploid, Tritipyrum, Fluorescent *in situ* hybridization (FISH), Multiple-pistil/seed, *Triticum durum, Thinopyrum bessarabicum* , Salt tolerance.

INTRODUCTION

Based on different reports, it has been estimated that in the range of 3.4-9 million km^2 of lands are salt affected (Flowers & Yeo, 1988); more than one third of which can be found in Asian countries. In many of these countries, soil salinity and alkalinity have spread to such a degree that they have brought about severe problems for the national economies (Szabolcs, 1989). In Asia, the greatest extension of salt affected soils occurs in the former USSR, China, India, Pakistan and Iran (Dewan & Famouri, 1942). The genetic studies of salt tolerance in various species including sorghum, soy-

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bean and rice have shown that salt tolerance is controlled polygenetically and is quantitatively inherited (Azhar & McNeilly, 1988). Variation for salt tolerance in wheat has been reported (Quershi *et al.* 1980; Kingsbury & Epstein, 1984; Sayed, 1985) and there is some potential for improving the salt tolerance of wheat by conventional breeding. As wheat is the most important source of human nutrition, the study and ultimate improvement of salt tolerance in this crop serves the high priority. The development of crop varieties that combine high yield potential with high salt tolerance will not be an easy task. *Triticeae* contains several halophytic and perennial species, which are naturally adapted to saline environments. A number of these species, particularly members of the genus *Thinopyrum,* have been hybridized with wheat (Gorham *et al.* 1985, 1986) and some of the resulting progenies have been tested for salt tolerance (Storey *et al*., 1985) but successful incorporation of genes imparting salt tolerance to *T. aestivum* background has not been established yet, either due to the failure in obtaining fertile F_1 hybrids or due to difficulty in getting direct gene introgression by homoeologous recombination (Tomar *et al*., 1995). One of the most notable of these species is *Thinopyrum bessarabicum,* a littoral diploid grass native to the Crimea, Ukraine. It has been identified as a useful source of salt tolerance genes for transfer to wheat for the following reasons; 1-It is a diploid species and highly tolerant to prolonged exposure to 250 mM NaCl (Gorham *et al*., 1985), 2-The genes conferring tolerance to salt are expressed in a wheat genetic background (Gorham *et al*., 1986; Forster *et al.*, 1987), 3-Its chromosomes pair and presumably can combine with wheat homoeologous in the absence of chromosome 5B (Forster & Miller, 1985; King *et al*., 1993).

Amphiploids between wheat and its relatives have been of interest for many years as potential sources for wheat improvement and as potential crops in their own right, but only triticale (the amphiploid between wheat and rye) has so far become a new cereal crop (Gupta & Priyadarshan, 1982; Gregory, 1987). Tritordeum, the amphiploid between *Triticum durum* and *Hordeum chilense* is considered to possess potential as a novel crop species (Martin *et al.,* 1996). A similar amphiploid between *T. durum* and *Th. bessarabicum* might also be of potential, especially as *Th. bessarabicum* is known to exhibit tolerance to high levels of salt (Gorham *et al*., 1985). 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 quantum of work done in triticale is evident from several reviews written in recent years on different aspects of triticale and shows the importance of this first man-made cereal. There is no reason that the same success should not be achieved in the case of tritipyrum. Tritipyrum exhibits salt tolerance (King *et al.,* 1997) and data on the salt stress tolerance in an incomplete set of wheat-Th *bessarabicum* addition lines (Forster *et al*., 1988) and a set of *wheat-H. chilense* addition lines (Forster *et al*., 1990) revealed analogous effects of group 2 and 5 chromosomes. It is interesting to note that homoeologous group 5 chromosomes in the *Triticeae* appear to be responsible for tolerance to several abiotic stresses (Forster, 1990). Chromosome $5E^b$ was found to carry a dominant gene for salt tolerance (Forster *et al*., 1988).

The gene(s) conferring salt tolerance located on chromosome $5E^b$ from *Th. bessarabicum* has (have) greater effect when substituted for homoeologous wheat group 5 chromosomes than when present as an addition chromosome, as in the Chinese Spring $5E^b$ addition line. This characteristic of $5E^b$ chromosome demonstrates the potential for production of new salt-tolerant wheat varieties (King *et al*., 1996). The benefits of the R genome in triticale (AABBRR) over D genome in wheat (AABBDD), are that the R genome is superior to the D genome with respect to yield potential, disease resistance, tolerance to minor element deficiencies and toxicities and to phosphorus uptake efficiency (Varughese, 1996). Similarly in triti-

pyrum the E^b genome is superior to the D genome in terms of salt tolerance potential. Further breeding and selection may modify some of the negative traits. In the light of the studies of the substitution of D genome chromosomes into triticale it seems likely that similar substitution into tritipyrum could prove valuable. There are a number of detrimental characters in tritipyrum and beneficial ones in wheat that are obvious candidates for removal or insertion, respectively. The brittle rachis, which is almost certainly carried by $3E^b$, could be removed by substitution of $3E^b$ by 3D. Similarly the blue aleurone colour carried by $4E^b$ could be replaced by substitution with 4D; at the same time the *Rht2* semi-dwarfing gene could be inserted with the 4D. Bread making quality could be introduced by replacing $1E^b$ with 1D. On the other hand, substitution of $5E^b$ by 5D would be detrimental, as this would delete the salt tolerance.This could be a case for partial substitution in the form of Robertsonian translocation where only one arm of a chromosome is replaced (Hassani, 1998).

Alien chromosome addition lines, in which single pairs of homologous chromosomes from a related species are added to wheat, were first produced by O'Mara (1948), who succeeded in adding rye *(Secale cereal)* chromosomes to wheat by backcrossing a wheat-rye amphiploid (triticale) to wheat. Since that time chromosome addition lines have been obtained from a range of alien species (Sears, 1956; Kimber, 1967; Islam *et al*., 1978; Miller *et al*., 1982). The multiple pistil/seed characteristic occurs in rice and has been variously termed as multiple pistil (Parthasarathi, 1935), polygynous rice (Shen, 1933), or multiple seeded rice (Wickramasekera, 1939). This malformation proved to be under the control of three recessive genes in the progenies of a cross of two rice cultivars (Butany & Bhattacharyya, 1962) and monogenic recessive in crosses investigated by Ghose & Butany, 1952. This paper reports the recent preliminary investigations of tritipyrum potential, including the possibility of improvement by the substitution of D genome chromosomes for E^b ones, characterization of the alien E^b chromosomes in the possible (1-7, DD)E^bE^b substitution lines by FISH, morphological study of the existing tritipyrum lines in glass house, agronomic behavior in the first ever field trial and cytological studies for determination of the source of the chromosomes involved in aneuploidy. The genetic heritability of the novel multiple-pistil/seed characteristic of one original Tritipyrum line (Creso/*Th. bessarabicum)* and primary studies of the chromosomal location of the genes are discussed. To achieve the latter, the first ever attempt of conceiving and constructing a novel set of alien chromosome addition lines of *Th. bessarabicum* chromosomes to *T. durum* cv. Creso is also reported.

MATERIALS AND METHODS

1-Eight hexaploid tritipyrum (2n=6x=42, $AABBE^bE^b$) and two octaploid tritipyrum lines (2n=8x=56, $AABBDDE^bE^b$ and $AABBE_1E_1E_2E_2$). All Tritipyrum lines were produced in the Cereal Research Department at John Innes Center, UK (King *et al*., 1997, Fig. 2b). *2-Triticum aestivum* cvs. Chinese Spring (CS), Wembley, Axona and two breeding lines S_{2-4} and S_{6-4} (Fig. 2c). 3-*T. durum* cvs. Aziziah; Creso; Karim Langdon (4B) 4D, Macoun, Neodure, Stewart and *Th. bessarabicum* (Fig. 2a). 4-Genomic DNA's of tetraploid wheat cv. Creso (AABB), *T. aestivum* cv.CS and *Th. bessarabicum.*

Cytology and Cytogenetic Survey

In chromosome engineering studies both conventional Feulgen stained (Table 1) and FISH at meiotic metaphase I of Tritipyrum lines (Table 2) were utilized. The *in situ* hybridization followed the technique of Reader *et al.* (1994) with the addition of preblocking hybridization stage in which wheat DNA was hybridized to the template DNA for one hour prior to the normal hybridization.

Figure. 1. a) Diagram of production of monosomic *Th. bessarabicum* addition lines to *T. durum* wheat. b) Scheme for the production of substituted Tritipyrum.

Figure. 2. a) From left to right: *T. durum* cvs. Aziziah; Creso; Karim; Langdon (4B)4D, Macoun, Neodure, Stewart and *Th. Bessarabicum.* b) From left to right 6x Tritipyrum Az/b, Cr/b, La/b, La (4B)4D, Ka/b, Ma/b, Ne/b, St/b and 8x Tritipyrum, CS/b, St/*Th. junceiforme.*c) From right to left *T. aestivum* cvs. Chinese Spring, Wembley, Axona and two breeding lines $S_{2\text{-}4}$ and $S_{6\text{-}4}$ d) The Tritipyrum section of the field trail, note the lateness of the lines as compared with the surrounding wheat crop. e) Brittle rachis of Tritipyrum. f) Perennial habit of Tritipyrum.

Table 1. Mean chromosome pairing of individual plants of Tritipyrum genotypes by the Feulgen staining technique (ranges in parenthesis).

Tritipyrum lines	PMC	Univ.	Bivalents			Thy.	Quadriv.	Arm assoc.	Pairing frequency
			Rods	Rings	Total				
Az/b	30	1.20	6.97	13.43	20.40	-	-	33.83	0.81
		(0-4)	(0-17)	(4-20)	(17-21)				
	25	1.36	5.96	14.36	20.32	-	-	34.68	0.83
		(0-6)	(0-14)	(11-20)	(18-21)				
Cr/b	30							33.33	0.79
		2.20	4.47	15.43	19.90	-	-		
		(0-16)	(1-11)	(7-17)	(13-21)				
	37	4.00	5.78	13.22	19.00	-	-	32.22	0.77
		(0-12)	(1-10)	(13-21)	(14-21)				
La/b	27					0.04	-	33.60	0.80
		2.52	5.76	13.92	19.68	(0-1)			
		(0-14)	(0-12)	(7-21)	(11-21)				
	24	1.04	4.52	15.96	20.48	-	-	36.44	0.87
		(0-4)	(2-9)	(11-19)	(17-21)				
La (4B)4D/b	30	2.17	4.69	15.02	19.71	0.06	0.06	33.73	0.80
		(0-8)	(0-12)	(8-18)	(17-21)	(0-1)	(0-1)		
	30	3.23	6.00	13.37	19.37	-	-	32.74	0.78
		(0-8)	(0-10)	(9-17)	(16-21)				
Ka/b	30	2.60	4.92	15.78	20.70	-	-	36.48	0.87
		(0-8)	(3-11)	(7-17)	(15-21)				
Ma/b	23	3.82	6.26	12.83	19.09	-	-	31.92	0.76
		(0-14)	(1-14)	(4-9)	(14-21)				
Ne/b	30	2.83	5.14	14.36	19.50	0.04	-	33.86	0.81
		(0-6)	(1-8)	(7-20)	(14-21)	(0-1)			
	27	3.89	5.89	13.16	19.05	-	-	32.21	0.77
		(0-9)	(2-11)	(10-18)	(15-21)				
St/b	30					0.06	-	33.32	0.79
		3.30	5.20	14.06	19.26	(0-1)			
		(0-7)	(2-10)	(11-17)	(18-21)				
	30	3.43	5.26	14.03	19.29	-	-	33.32	0.79
		(0-8)	(2-10)	(10-18)	(16-21)				
CS/b	25								
		5.16	8.38	16.68	25.06	0.03	0.16	41.74	0.75
		(2-7)	(4-12)	(12-21)	(22-28)	(0-1)	(0-2)		
	30	2.60	8.15	18.37	26.52	0.03	0.07	44.89	0.80
		(0-6)	(4-13)	(12-20)	(22-28)	(0-1)	(0-2)		
St/Th.j	19					0.05	0.11	43.4	0.78
		4.89	7.12	18.14	2526	(0-1)	(0-1)		
		(4-8)	(3-14)	*(10-25)*	(19-28)				
Analysis of variance									
Ms between 6x lines	7df	1.30^{ns}	0.52^{ns}	0.87^{ns}	0.36^{ns}	---	---	2.61^{ns}	0.0016^{ns}
Ms within 6x lines	7df	0.64	0.55	1.17	0.15	---	---	1.15	0.0006
Ms between all lines	9df	1.73^{ns}	2.06^{*}	4.11	10.15^{**}	---	---	27.61^{**}	0.0014^{ns}
Ms within all lines	9df	1.02	0.47	1.21	0.28	---	---	1.69	0.0007

ns,*,** Non.significant, significant at P=0.05 and P=0.01, respectively.

Greenbouse Test

Six 42-chromosome seedlings of each line were selected from among 20 seedlings and transplanted into plastic pots. Three were

Table 2. Univalents and rod bivalents of E^b and AB genomes of Tritipyrum lines

Genotype	Univalents		Row total	Rod bivalents		Row total
	E^b	AB		E^b	AB	
Cr/b	1.12 (1.27)	0.81 (0.66)	1.93	1.35 (1.44)	2.38 (2.25)	3.73
La/b	1.53 (1.51)	0.77 (0.79)	2.30	2.20 (2.05)	3.12 (3.21)	5.32
La(4B) 4D/b	1.31 (1.23)	0.56 (0.64)	1.87	1.62 (1.63)	2.60 (2.58)	4.26
Ka/b	1.54 (1.56)	0.81 (0.80)	2.35	1.59 (1.65)	2.68 (2.57)	4.27
St/b	2.30 (2.24)	1.11 (1.17)	3.41	1.86 (1.86)	2.96 (2.90)	4.82
Column total	7.80	4.06		8.62	13.47	
Grand total			11.86			22.36
χ^2			0.058^{**}		$.006^{**}$	

Expected values in parenthesis

**Significant P=0.01

grown under natural day length ranging from 12-17 hours during spring/summer 1995 in the greenhouse. At the same time, the other three seedlings were vernalized at 7.5^0C with an eight-hour photoperiod for six weeks with normal light density. After vernalization, they were transferred to the greenhouse and grown under the same conditions as the unvernalized plants for the duration of the experiment, using a completely randomized design consisting of three replicates. Each set of three vernalized and unvernalized plants were scored for a number of traits; percent fertility, spikelet number and total grain of the first and second floret of each spikelet were scored and analysis of variance was carried out (Table 3).

Field Trial

A small-scale trial field was sown and divided into sections, one section containing the tritipyrum and the other containing the parents as a randomized block design with four replications. A range of morphological and agronomical traits were measured or counted on each plot (Table 4). For protein content and grain hardiness 5-10 g of mixed grain from each plot was milled by a Cyclotec 1093 Sample Mill, 3.5g of the flour of each sample being analyzed by an Oxford QN near infrared analyzer.Analysis of variance was applied.

The Production of Substituted lines

To produce substituted Tritipyrum, spikes of each 42-chromosome (6x) line of Tritipyrum were emasculated, then pollinated with pollen from the 6x wheat cultivars (Fig.lb). For selecting, the 42-chromosome F_2 seedlings from the F_1 hybrids, the mitotic chromosome preparation technique was made. To identify the number of E^b chromosomes of F_2 and BC_1 plants via FISH, the meiotic chromosome preparations were made following the method of Hutchinson *et al.* (1980). Total genomic DNA *in situ* hybridization (GISH) was carried out as described by Schwarzacher *et al.* (1992) and Reader *et al.* (1994), with the addition of a preblocking step (Hassani, 1998). The chromosome constitution of possible substituted Tritipyrum plants of the F_2 and the BC_1 progenies were assessed by scoring the number of E^b chromosomes present in meiotic cells at metaphase I for individual fertile plants (Table 5).

Novel Multiple-pistil/seed

Genetic Inheritance of Multiple Pistils

Table 3. Mean height, tiller number, maturity and fertility of tritipyrum under unvernalized and vernalized conditions

Tritipyrum lines	Height (cm)		Tiller no.		Maturity[a]		Bagged fertility %		Unbagged fertility %	
	Unver.	Ver.	Unver.	Ver.	Unver.	Ver.	Unver.	Ver.	Unver.	Ver.
Az/b	119.3±32	118.7±4.6	25±2.94	9.7±1.70	54.7±11.9	61.3±3.7	45.5±7.93	55.4±14.3	63.4±14.8	54.3±20.7
Cr/b	103±10.9	89.7±1.5	26.3±2.4	20.3±1.7	64.0±4.7	54.0±1.0	14.2±3.06	18.8±12.7	34.8±7.14	48.3±9.21
La/b	145±4.59	16.3±13.8	23.3±3.2	24±4.71	66.3±10.1	60.3±1.3	39.1±7.53	13.2±11.3	42.9±14.6	20.6±2.63
La (4B)4D/b	149±2.56	14.8±2.2	21.7±2.7	17.7±1.5	67.3±7.5	48.0±1.0	17.8±16.5	39.2±6.34	35.6±21.2	62.1±14.4
Ka/b	72±7.69	91.0±2.56	11±3.97	9.0±0.59	65.0±2.1	57.3±43	29.5±15.4	35.7±11.3	35.2±25.9	19.3±11.6
Ma/b	115±14.1	126±2.35	26.7±4.1	13.7±2.8	52.0±1.6	58.3±1.7	30.0±15.6	59.1±1.06	36.2±25.0	50.9±6.94
Ne/b	161±5.79	143±3.24	27.7±3.8	13±2.04	56.7±5.9	52.7±1.3	49.9±19.9	63.1±1.80	20.7±9.31	67.0±5.16
St/b	148±5.89	127.3±7.5	25±2.56	11.7±2.1	65.0±5.1	52.7±1.3	25.1±13.1	48.4±9.13	39.4±15.8	18.1±2.69
CS/b	131±2.70	116.3±6.01	26.7±2.8	14.7±2.7	54.3±2.7	42.7±2.7	37.0±13.3	20.4±18.9	37.0±1.88	26.5±12.0
St/*Th.j.*	130±23.9	94.0±7.71	28.3±3.4	21.3±2.6	54.0±3.3	53.7±4.7	16.4±8.76	13.3±11.4	29.1±20.3	13.5±13.5
Mean	127.5	117.4	24.17	15.50	59.93	54.93	30.44	35.59	42.18	38.29
Grand Mean	122.45		19.84		57.43		33.02		40.24	
LSD5% for Genotype	6.9		2.37		4.07		9.66		11.94	
LSD5% for (Treatment)	1.38		0.47		0.81		1.93		2.39	
LSD5% for (Genotype x Treatment)	13.81		4.73		8.14		19.32		23.89	

[a] Days from ear emergence to ripening.

The presence or absence of multiple pistils and seeds were screened in the florets of immature and mature spikes of Creso/*Th. bessarabicum* (Cr/b), all the durum wheat parents of the Tritipyrum lines and *Th. bessarabicum.* Segregation of the characters was studied in the progenies of (Cr/b) x (Karim/*Th. bessarabicum=Ka/b),* Macoun/*Th. bessarabicum* (Ma/b) and Stewart/*Th. bessarabicum* (St/b) crosses (Table 6). All individual plants at flowering and maturity were carefully checked for multiple pistils or seeds. The Chi-Square analysis for the fixed-ratio dihybrid hypothesis, i.e. one multiple pistil: fifteen single pistils, was performed (Gomez & Gomez, 1984).

Production of Monosomic *T. durum-Th. bessarabicum* addition lines

After emasculation, the selected 42-chromosome seedlings of Cr/b were pollinated with the pollen of Creso (Fig. 1a). The resulting amphiploid F'_1s (2n=5x =35; AABBE[b]) were grown. The mitotic and meiotic studies of (Cr/b)/Creso progenies and FISH were carried out. The 29-chromosome plants were selected from the self-pollinated progenies and checked for the presence of multiple pistils and seeds in the vegetative and mature stages of growth, respectively. The 29-chromosome plants in F_3 (Fig. 5g) and F_4 (Fig. 5d) progenies were classified into seven morphologically distinct groups.

RESULTS AND DISCUSSION

Tritipyrum is a new amphiploid combination between wheat *(Triticum* spp.) and *Thinopyrum* spp., and to date, there hav only been three scientific publications, two of which are the work of the author of this paper. In the first paper, all produced Triti-

Table 4. Mean of morphgical characters of tritipyrum and wheat cultivars.

Genotype	Survival %	Height (cm)	Tiller number	Spikelet number	Fertility %	Fmgrgmce (day)	1000-Grain weight (g)	Protein %	Grain Hardness
Az/b	58.8±5.5	49.3±1.6	1.3±0.3	6.5±0.7	57.2±3.0	238.5±0.7	22.1±1.7	15.4±0.3	59.6±2.7
Crr/b	61.3±6.3	58.0±3.3	6.8±2.8	10.0±1.6	60.9±6.1	239.0±1.6	38.4±4.8	15.7±02	60.4±2.0
La/b	58.3±7.7	99.3±2.5	13.3±1.6	14.8±0.9	75.3±4.7	240.0±0.4	46.4±1.0	16.3±0.3	62.1±0.8
La(4B)4D/b	76.3±8.3	92.3±1.9	13.8±2.3	15.5±0.4	69.3±3.5	238.3±1.1	48.2±1.0	17.8±0.5	53.0±3.9
Ka/b	51.3±2.4	59.3±2.3	14.8±3.3	12.7±1.5	63.5±5.2	242.2±1.4	59.4±7.2	15.3±0.3	62.2±0.7
Ma//b	61.3±4.3	81.5±1.9	10.0±0.9	15.0±0.3	61.2±7.7	240.3±0.3	41.9±0.5	16.6±0.3	57.0±2.5
Ne/b	62.5±7.2	101.8±2.1	13.7±1.1	16.5±0.0	59.1±12	240.0±0.4	46.3±0.8	16.7±0.2	60.2±1.5
St/b	65.0±5.4	99.8±3.1	14.0±0.6	15.5±0.7	60.2±8.4	239.7±1.7	43.5±1.5	17.8±0.3	56.7±2.6
Cs/b	53.8±5.2	77.8±1.3	10.5±2.2	19.0±1.1	63.2±2.9	236.5±0.7	37.1±0.6	16.7±0.3	58.2±2.6
St/*Th.j*	63.8±4.3	99.0±1.2	13.0±0.9	17.0±1.7	35.3±2.4	250.3±2.5	49.1±4.5	18.0±0.3	54.1±1.9
Az	31.3±2.4	65.3±2.5	5.5±0.9	14.3±1.0	84.5±8.9	236.5±1.6	42.7±2.6	16.5±0.4	34.6±32
Cr	41.3±3.8	60.8±±4.2	7.5±5.3	14.5±0.5	90.1±4.4	238.3±0.3	50.8±0.7	16.0±0.4	38.7±3.5
La	43.8±4.3	101.8±2.0	18.5±2.0	20.5±1.0	90.6±3.2	238.0±0.9	49.8±2.1	16.3±0.2	38.5±1.6
Ka	32.5±3.3	55.0±2.6	5.0±1.2	12.5±0.9	78.2±14	237.5±1.2	46.1±5.5	15.5±0.3	39.8±2.4
Ma	47.5±6.3	81.5±2.5	7.8±0.9	17.8±0.3	85.2±4.0	236.8±0.8	51.6±1.0	17.2±0.1	39.2±1.8
Ne	40.0±2.0	62.8±0.9	10.0±1	17.5±1.1	92.1±1.4	239.7±0.6	59.3±5.4	17.2±0.4	37.7±2.1
St	41.3±2.4	101.5±2.3	13.5±2.6	19.0±0.3	93.4±0.6	237.3±1.1	52.4±0.5	16.8±0.4	39.5±2.8
CS	57.5±12.0	75.3±2.3	9.8±2.2	19.5±0.6	90.6±1.8	233.8±1.8	38.2±0.9	13.9±0.4	60.5±0.6
Grand Mean	52.6	78.3	10.5	15.44	72.78	239	45.72	16.41	50.67
CV%	21.0	5.95	34.4	11.99	17.47	0.99	12.85	3.83	9.45
LSD (Block)	7.40	3.12	2.41	1.24	8.52	1.57	3.94	0.42	3.21
LSD (Genotype)	15.7	6.62	5.11	2.61	18.07	3.33	8.35	0.89	6.81

pyrum lines, which were tested in the hydroponic culture, exhibited good salt tolerance (King *et al*., 1997). For example, the Neodur/*Th. bessarabicum* and Langdon/*Th. bessarabicum* lines exhibited 27.8 and 38.9%, survival rates, respectively, in 250 mM NaCI, whereas both Neodure and Langdon wheat parents died. This paper reports the results in some aspects of this new third man-made cereal as follows.

Cytology and Cytogenetic Survey

Chromosome counts of progenies of 42-chromosome hexaploid Tritipyrums exhibited a considerable amount of instability in the form of aneuploidy (45.3 %) with a proportion of plants having lost chromosomes (40-41), and some having addition chromosomes (43-44). In the 56-chromosome octaploid genotypes, the aneuploidy was even higher (62.5 %) with the chromosome number ranging from 52 to 60 (Table 1). King *et al.* (1997) reported an overall mean of 17.80 bivalents for 6x tritipyrum in the early generation of tritipyrum, but in this study it was 19.05. This suggests that after relatively few selfing generations the pairing behavior has already improved. A very low rate of multivalents was found in some of the lines. This could be due to homoeologous pairing between A, B and E^b suggesting that the E^b genome may have the capacity to partly suppress the activity of the *Ph1* locus of chromosome 5B of wheat, which normally prevents homoeologous pairings (Miller *et al.,* 1998). FISH, using total genomic DNA of the alien *Th. bessarabicum* species as a probe (GISH), was a valuable technique for identifying the E^b chromosomes in tritipyrum genotypes (Table 2, Fig. 4a-b). Although the random univalent and rod bivalent ratio was expected to be 1 (E^b): 2 (A+B) and I (E^bE^b): 2 (AA+BB) respectively, but ratios of 2 (E^b): 1 (A+B) and 1 (E^bE^b): 1.5 (AA+BB), respectively, were found and confirmed by Chi-Square analysis. These results show that although the E^b chromosomes play a major part in the pairing failure and meiotic instability of tritipyrums, the A and B genome chromosomes, must not be ignored, as univalent and rod bivalent chromosomes occur in both E^b and AB genomes

Table 5. The E^b chromosome constitution at meiosis of the Tritipyrum x 6x wheat F_2 and Tritipyrum/6x wheat x Tritipyrum backcrosses.

F_2 Plants	No. of E^b chromosomes			Back cross plants	No. of E^b chromosomes		
	Univ.	Biv.	Total		Univ.	Biv.	Total
1	5	3	11	1	3	4	11
2	4	3	10	2	3	4	11
3	1	4	9	3	3	3	9
4	3	3	9	4	4	2	8
5	6	1	8	5	2	3	8
6	4	2	8	6	6	1	8
7	2	3	8	7	4	2	8
8	2	3	8	8	5	1	7
9	3	2	7	9	3	2	7
10	1	3	7	l0	4	1	6
11	3	2	7	11	3	1	5
12	4	1	6	12	5	0	5
13	2	2	6	13	3	1	5
14	3	1	5	14	3	1	5
15	5	0	5	-	-	-	-
16	2	1	4	-	-	-	-
17	1	1	3	-	-	-	-
18	2	0	2	-	-	-	-
Mean	3	2	6	-	3.8	1.7	8

(Table 2). The level of *in situ* cross hybridization also indicated that there is a relatively close genetic relationship between the, A, B and E^b genomes of Tritipyrum.

Greenhouse Test

The morphologies of the glass-house grown Tritipyrum lines were predominantly wheat-like. They had relatively low fertility and showed perennial habit (Fig. 2f), ears with a brittle rachis (Fig. 2e), with the exception of CS/*Th. bessarabicum* line, a range of tiller production and variation in plant and spike morphology in both vernalized and unvernalized conditions (Table 3), which are in agreement with King *et al.* (1997). Despite the involvement of a single accession of *Th. bessarabicum,* these observations suggest that improvement is possible by breeding and selection within the A and B genome complements. All lines had relatively the same range of late maturity under both conditions (Table 3), therefore; the lack of vernalization is not a major factor in the late maturity of Tritipyrum.

Field Trial

The results of the Tritipyrum lines alongside their wheat parents demonstrated that the Langdon (4B)4D/*Th. bessarabicum* [La (4B/4D)/b] line showed the best overall performance. It had the greatest survival rate of plants, the second highest fertility level, and was the first to produce ears. Only Chinese Spring/*Th. bessarabicum* was earlier. It had the third highest 1000-grain weight, the third highest tiller number, and the second highest protein content, in comparison with the other Tritipyrums including the normal Langdon/*Th. bessarabicum* (La/b) line (Ta-

Table 6. Segregation ratio for multiple pistil in F_2 progeny. a) Small population of Cr/b x Ks/b. b) Large population of Cr/b x Ks/b, Ma/b and Sub.

Progeny	Observed plants withpistil		Expected plants withpistil on 3:1		χ^2	Expected plants withpistil on l5:l		χ^2	P value
	Single	Multiple	Single	Multiple		Single	Multiple		
a	13	2	11.25	3.75	0.56^{**}	14.063	0.938	0.36^{**}	0.01
b	92	5	72.75	24.25	19.3^{ns}	90.938	6.063	0.056^{**}	0.01
a+b	105	7	84	28	20.1^{ns}	105	7	0.038^{**}	0.01

ns, ** Non-significant, significant p=0.01 respectively.

ble 4). Therefore, it could be concluded that the removal of chromosome 4B and/or the substitution of 4D has a pronounced beneficial effect and could be an important factor in future Tritipyrum breeding.Variation in survival (Table4) indicated that in contrast to the wheat parents, which were all of spring habits, the Tritipyrums had greater winter hardiness and possessed a potential as a winter-sown crop, presumably as a result of genes of the E^b genome. All Tritipyrums showed relatively low levels of fertility in comparison with the wheat parents (Table 4). This may be due to chromosome instability at meiosis, although there is no evidence of a correlation between fertility and variation in chromosome pairing (King *et al.*, 1997). Therefore, other factors in E^b genome may be responsible for this phenomenon. Ear emergence can be used as an indicator of the maturity behavior of Tritipyrum in comparison with the wheat parents. All Tritipyrum lines had later ear emergence than their wheat parents (Fig. 2d). The 8x St/*Th. junceiforme* showed the greatest delay in ear emergence, presumably due to the presence of the two *Thinopyrum* genomes (Hassani *et al*., 1998).

The highest protein amount result, that of the 8x Tritipyrum involving *Th. junceiforme* probably partly results from the generally accepted negative correlation between yield and protein content, but may also indicate a potential of this genotype for introducing into Tritipyrum variation for this character from within the E genomes. The large variation for all the characters studied shows there is a considerable potential for improvement of Tritipyrum as a new cereal. In this respect, the development of Tritipyrum has parallel aspects with the development of triticale, which in early stages, it also showed similar variations, but breeding and continuing selection, especially within the wheat components produced a successful new cereal to the world farming system.

The Production of Substituted Tritipyrum

In spite of considerable variation between the Tritipyrum lines, the morphology of F_1 hybrids was predominantly wheat-like. Pollen sterility was observed in all hybrid combinations ranging from 1.9% (Cr/b x Wembley) to 63.1% (La/b x Wembley). Seven of the unpaired chromosomes could be identified as E^b chromosomes by FISH on meiotic preparations in F_1 progeny (Fig. 4c-d). The *in situ* results on mitotic preparations also confirmed the presence of seven E^b chromosomes (Fig. 4e). Due to the large number of univalents in F'_1s a high range of aneuploidy and cytological segregation was, as expected, observed in the F_2 generation. A considerable range of segregation was also observed in terms of plant morphology (Fig. 3a-b). There was also a high level of sterility, production of back tillers and late maturity. From the large population of F_2 self-generation the seed production was mainly from those involving [La (4B)4D/b, Ma/b and Ne/b] x 6x Wembley and La (4B)4D/b x S_{6-4} combinations. This suggests that the choice of wheat parent, both primary

tetraploid and secondary hexaploid, is important if the problem of hybrid necrosis is to be avoided (Fig. 3e). Wembley apparently is a better parent than $S_{6\text{-}4}$. Lines with specific *T. durum* backgrounds, especially La (4B)4D, showed higher performance, indicating that the initial 4x wheat parent is also important. These effects suggest that variation in both 4x and 6x wheat could play a part in improving Tritipyrum (Hassani *et al*., 1998).

The mean ratio of fertile to sterile plants in F_3 (1.5:1) and the BC_1-F_2 (1.83: 1) progenies in comparison with F_2 (0.41:1) and BC_1 (0.55:1) generations were very much higher, respectively. This is presumably the result of higher chromosomal stability in F_3 and BC_1-F_2 in comparison with F_2 and BC_1 generations, respectively (Hassani, 1998). The GISH on meiotic slides from fertile F_2 and BC_1 plants ranging (2-1l) and (5-11) of E^b chromosomes show that it is possible to produce Tritipyrum with variable numbers of E^b and D genome chromosomes (Table 5, Fig. 4f-g), and that FISH is a useful technique for determining the number of E^b chromosomes present. To recognize E^b chromosomes from wheat ones (A, B and D genome chromosomes) various probe, hybridization and prehybridization mixtures with different fluorochrome labeled nucleotides were tried. The best differentiation results were achieved when dCTP nucleotide labeled with Texas Red fluorochrome was used following preblocking by total DNA of Chinese Spring wheat. GISH also showed the presence of *T. durum*-*Th. bessarabicum* Robertsonian translocated chromosomes (Fig. 4h). The 1BL.1RS wheat/rye translocated chromosome is an example of this type of chromosome and is widespread in European cultivated wheat varieties, where it initially conferred resistance to certain foliar diseases. Although this type of chromosome may have a far from ideal, large amount of unwanted and possibly detrimental alien genetic material,it may nevertheless, be useful for Tritipyrum, by permitting the substitution of a single chromosome arm. Such translocated chromosomes may also have value as a means of introducing *Th. bessarabicum* characters into wheat. The low level of fertility and crossability means that a considerable effort will be required to produce sufficient progeny to produce specific combinations of E^b and D genome chromosomes. Further investigations by molecular cytogenetics and molecular markers along with morphological selection will be required in subsequent generations to establish stable genotypes with varying E^b and D genome chromosome substitutions and desirable phenotypes. However, this study supports the feasibility of substituted Tritipyrum.

Novel Multiple pistil/seed

Genetic Inheritance of Multiple Pistils

King *et al.* (1997) reported the occurrence of multiple seeds in the Creso/*Th. bessarabicum* Tritipyrum line. This appears to be the first report of this phenomenon in the *Triticeae* with no indication of the mechanism. Pistillody in which the anthers become converted or partially converted to pistils is known in wheat (Sears, 1954). This condition is produced by the absence of a gene on chromosome 6B, and rarely gives rise to multiple seeds within a floret. In the Creso/*Th.bessarabicum,* the number of pistils (gynoecia) in each floret varies from one to three with single pistil florets predominating in each spike of individual plants. The number of stamens remains unchanged. Observation of both *Th. bessarabicum* and *T. durum* showed that neither had multiple pistils and the phenomenon is not a post-fertilization event as Cr/b has multiple pistils in pre-anthesis (Fig.5a-b). The possibility that plants from multiple seed florets were haploid was considered, but no baploid plants were found from pairs of seeds from multiple seed florets of Cr/b and Cr/b x Ka/b. However, aneuploid differences did occur within their florets, which prove the pre-pollination mechanism of multiple seed formation if the multiple seeds arose from a

post-pollination event, seed within a single floret would be expected to have identical chromosome numbers (Hassam, 1998). If the multiple pistil trait is the result of two recessive genes (Table 6), one in each parent, then it follows that there is variation for the genes in the tetraploid wheats as the trait only occurred in the Creso based line, and possibly in the Ma/b line which had a few multiple seeds in the early generation (Miller, Pers. Comm.). The variation is less likely to be from *Th*. *bessarabicum* as a single accession was used as the parent for all of the lines (King *et al*., 1997). The trait does not appear to be environmentally affected because it occurs both under glass house and field conditions.The possibility of a specific Creso cytoplasmic effect may also warrant consideration, although in general all *T*. *durum* cultivars would be expected to have similar cytoplasm (Hassani *et al*., 1998).

Production of Monosomic *T. durum-Th. bessarabicum* addition lines

In order to determine the chromosomal location of the trait a novel set of addition lines of the chromosomes of *Th. bessarabicum* to *T*. *durum* Creso were produced (Fig. 1a). Some of the F_1 plants had *35* chromosomes but the majority did not, presumably as a result of aneuploid female gametes in the Cr/b. The 35-chromosome plants exhibited 14 bivalents and seven univalents (Fig. 5e). The FISH study showed that, as expected, the 14 bivalents were from A and B genomes and the seven univalents from the E^b genome (Fig. 5f). The 29-chromosome F_2 (Fig. 5c) and F_3 plants (Fig. 5h*)* were selected, by chromosome counting, as potential *T. durum-Th. bessarabicum* addition lines. At maturity, these were classified into seven morphologically distinct groups (Fig. 5g&d). These results indicate a high probability of having established the seven possible monosomic additions of *Th. bessarabicum* to *T*. *durum.*Although the multiple pistil trait showed heritability through several generations of the Cr/b Tritipyrum and in the hybrid progenies, but there was no evidence for its presence in any of the 29-chromosome monosomic additions. This could be due to the need for the gene(s) to be present in two doses before expression occurs. If this is the case, it will be necessary to obtain 30-chromosome disomic additions to identify its chromosomal location. Alternatively, if the trait results from genes on more than one *Th*. *bessarabicum* chromosomes, e.g. two chromosomes, then single additions will not locate it. In this case multiple addition lines will be required to find the cbromosomal location of the genes (Hassani, 1998). Although seven individual addition lines have not been categorically identified, it seems likely that the seven morphological groups equate with the seven different chromosomes. Chromosome $4E^b$ is certainly present in group B on the evidence of the presence of seeds with blue aleurone, a character carried by chromosome $4E^b$ and group F, on the basis of its narrow ears, probably carries $2E^b$ (Forster *et al.*, 1988). Further characterization, however, including RFLP analysis is required before it can be claimed with certainty that a complete set of seven distinct addition lines has been established.

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Figure. 3. Morphological variation of Tritipyrum x wheat F_2 plants a) Vegetative stage. b) At maturity. c and d) A range of (Tritipyrum x wheat) x Tritipyrum BC_1-F_1 and BC_1-F_2 plants at maturity. e) Mature plants of Tritipyrum x wheat F_1 hybrids. Left: four necrotic plants of Tritipyrum genotypes x 6x wheat Axona. Right: four normal plants of Tritipyrum genotypes x 6x wheat Wembley.

Figure. 4. a) A metaphase I cell of meiotic preparation of Creso/*Th*. *bessarabicum* stained with DAPI, showing two rod bivalent and nineteen ring bivalent, undifferentiated chromosomes. b) Meiotic preparation of *Creso/Th*. *bessarabicum* showing one E^b rod bivalent and six E^b ring bivalents. c) GISH on a meiotic preperation of a Tritipyrum x wheat F_1 hybrid. A metaphase I cell stained with DAPI, showing 14 undifferentiated univalent chromosomes of the E^b and D genomes. d) The same cell, the seven E^b univalent fluoresce (bright red). e) GISH on mitotic cells of Tritipyrum x wheat F_1 hybrids. A mitotic preparation of the St/b x S_{6-4} hybrid showing seven univalent chromosomes of the E^b genome (Single filter). f) A pollen mother cell at Metaphase I of a Tritipyrum x Wembley F_2 plant stained with DAPI showing 42 chromosomes, 13 ring bivalents, four rod bivalents and eight univalents. g) A pollen mother cell at Metaphase I of a Tritipyrum x Wembley F_2 plant showing eight E^b chromosomes, one ring bivalent, one rod bivalent and four univalents. h) A pollen mother cell at metaphase I of a BC_1 plant showing five E^b chromosomes, two ring bivalents, one univalent and one rod bivalent consisting of a wheat-*Th*. *bessarabicum* Robertsonian translocation chromosome (arrowed).

Figure. *5.* a) Multiple pistils in a single floret of the Creso/*Th*. *bessarabicum* line. b) Multiple seeds of individual florets (photo, King *et al.* 1997). c) A PMC at Metaphase I of (Creso/*Th besarabicum) x* Creso F_2 plant shows one univalent E^b chromosome. d) Seven morphological distinct 29-chromosome potential tetraploid-*Th*. *bessarabicum* addition lines from (Cr/b) x Cr F_4 progeny. e) A meiotic preparation at Metaphase I of Cr/b x Cr F_1 hybrid with 35 chromosomes, 14 pairs of A and B and seven univalents of E^b genomes stained with DAPI. f) *In situ* hybridization on a meiotic preparation at Metapbase I Cr/b x Cr F_1 hybrid with 35 chromosomes, 14 of A and B and 7 of E^b genomes under bright red filter. g) Potential addition line groupings of Cr/b x Cr F_3 progenies. h) A PMC at Metaphase I of (Creso/*Th*. *bessarabicum)* x Creso F_1 plant showing one univalent E^b chromosome.

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آیا تریتیپیرم (TRITIPYRUM) ، آمفی پلوئیدی جدید با پتانسیل تحمل به شوری، می تواند غله‌ای موفق مانند تریتیکاله باشد؟

چکیده

خاکهای متاثر از شوری مشکل عمده و فراگیر جهانی است که سطح اراضی قابل توجهی را در بسیاری از کشورها از تولید محصولات زراعی خارج نموده است. اصلاح واریته های ژنتیکی متحمل به شوری مستلزم دانش و آگاهی

ما در درک بهتر ماهیت و کنترل ژنتیکی پیچیده این صفت خواهد بود. تریتیپیرم آمفی پلوئید جدید متحمل به شوری حاصل از تلاقی بین گونه های دو جنس گندم نان (پایه مادری) و تینوپیرم وحشی (پایه پدری) می تواند نوید و امیدی تازه در این زمینه باشد. اگر چه در این غله جدید بویژه ارقام هگزاپلوئید، تلاقی بین ارقام گندم دورم و یک گونه علف شورپسند دیپلوئید، شواهدی محکم دال بر دارا بودن پتانسیلی بالقوه مبنی بر ظهو به عنوان یک گیاه زراعی جدید و مقاوم به تنش شوری دیده می شود ولیکن این غله مصنوعی نوظهور دارای معایب ناپایداری جزئی کروموزومی و باروری کم همانند تریتیکاله در اوان پیدایش خود به عنوان یک غله مصنوعی می باشد. همانگونه که صفات نامطلوب در آن گیاه به تدریج با کارهای اصلاحی مرتفع گردید به نظر می رسد که غلبه بر این صفات در این گیاه نیز با جایگزین نمودن کروموزومهای ژنوم D گندم نان با کروموزمهای ژنوم وحشی پایه پدری آن (E^b) میسر گردد. در راستای تحقق عملی این فرضیه در آزمایشاتی نتایج حاصل از تلاقی آن با ارقام مختلف گندم هگزاپلوئید با تکنیک هیبریداسیون DNA فلورسنت در محل طبیعی خود (FISH) مطالعه گردید. علاوه بر آن خصوصیات مرفولوژیکی، سیتولوژیکی، و رفتار زراعی این گیاه برای اولین بار در مزرعه بررسی گردید. همچنین صفت جدید چند بذری در یک لاین بررسی و اساس ژنتیکی – کروموزومی آن تا حدود زیادی پایه ریزی شد. نتایج اولیه و امیدوار کننده حاصل از تحقیقات فوق الذکر نشان داد اولا" تولید لاین های ثانویه تریتیپیرم حاوی کروموزومهای جایگزین شده از ژنوم D به جای ژنوم E^b جهت حذف برخی صفات نامطلوب و دستیابی به لاینهای با ثبات کروموزومی توام با عملکرد و باروری بیش از لاینهای موجود از طریق روشهای اصلاحی امکان پذیر می باشد. ثانیا" وجود خواص پنجه زنی مستمر و چند ساله بودن این گیاه امکان اصلاح یک نبات زراعی دو منظوره (تولید علوفه و دانه) امری دور از انتظار و دسترس نیست. ثالثا" به احتمال بسیار قوی صفت چند بذری تحت کنترل دو ژن مغلوب یکی در رقم وحشی و دیگری در ارقام گونه زراعی گندم دوروم بوده و شواهد موجود در بخش مطالعه اساس کروموزومی پدید ه نادر چند بذری در خانواده گندمیان به احتمال زیاد دلالت بر ایجاد یک مجموعه هفت تایی از یک رقم گندم تتراپلوئید (Creso)، هر یک حاوی یک کروموزوم اضافه متفاوت از گونه وحشی والد پدری، برای اولین بار در سطح دنیا دارد.