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

H. S. Hassani¹, I. P. King², S. M. Reader³, P. D. S. Caligari⁴ and T.E. Miller³

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, AABBE⁶B⁶) derived from Triticum durum (2n=4x=28, AABB) and Thinopyrum bessarabicum (2n=2x=14, E²E²) 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 E² 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 km² 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-
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. Triticaceae 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 F1 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 Triticaceae appear to be responsible for tolerance to several abiotic stresses (Forster, 1990). Chromosome 5E<sup>b</sup> was found to carry a dominant gene for salt tolerance (Forster et al., 1988).

The gene(s) conferring salt tolerance located on chromosome 5E<sup>b</sup> 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<sup>b</sup> addition line. This characteristic of 5E<sup>b</sup> 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 from a related species are added to wheat, were first produced by O’Mara (1948), who succeeded in adding rye (Secale cereale) 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, $DDDE^bE^b$ 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 (Cresco/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 octoploid tritipyrum lines ($2n=8x=56$, AABBDDE$^bE^b$ and AABBE$^bE_1E_2E_3$). 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 *T. 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. Azizia; Creso, Karim, Langdon (4B)4D, Macoun, Neodure, Stewart and Th. Bessarabicum. b) From left to right 6x Tritiprum Az/b, Cr/b, La/b, La (4B)4D, Ka/b, Ma/b, Neb/b, St/b and 8x Tritiprum, CS/b, St/Th. junceiforme. c) From right to left T. aestivum cvs. Chinese Spring, Wembly, Axona and two breeding lines S_{54} and S_{64}. d) The Tritiprum section of the field trail, note the lateness of the lines as compared with the surrounding wheat crop. e) Brittle rachis of Tritiprum. f) Perennial habit of Tritiprum.
Six 42-chromosome seedlings of each line were selected from among 20 seedlings and transplanted into plastic pots. Three were

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

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<th>Tritipyrum lines</th>
<th>PMC Univ.</th>
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**Analysis of variance**

- Ms between 6x lines: 7df 1.30<sup>ns</sup> 0.52<sup>ns</sup> 0.87<sup>ns</sup> 0.36<sup>ns</sup> --- --- 2.61<sup>**</sup> 0.0016<sup>**</sup>
- Ms within 6x lines: 7df 0.64 0.55 1.17 0.15 --- --- 1.15<sup>**</sup> 0.0006
- Ms between all lines: 9df 1.73<sup>ns</sup> 2.06 4.11 10.15<sup>**</sup> --- --- 27.61<sup>**</sup> 0.0014<sup>**</sup>
- Ms within all lines: 9df 1.02 0.47 1.21 0.28 --- --- 1.69 0.0007

<sup>ns,**,***</sup> Non-significant, significant at P=0.05 and P=0.01, respectively.
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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°C 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.1b). For selecting, the 42-chromosome F2 seedlings from the F1 hybrids, the mitotic chromosome preparation technique was made. To identify the number of E\(^b\) chromosomes of F2 and BC1 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 F2 and the BC1 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

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**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         |                 |             |

\(x^2\) 0.058** .006**  

Expected values in parenthesis  
**Significant P=0.01**
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 F1’s (2n=5x=35; AAB-BE2) 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 F3 (Fig. 5g) and F4 (Fig. 5d) progenies were classified into seven morphologically distinct groups.

**RESULTS AND DISCUSSION**

Tritipyrum is a new amphiploid combination between wheat (*Triticum* ssp.) and *Thiioopyrum* ssp., and to date, there have only been three scientific publications, two of which are the work of the author of this paper. In the first paper, all produced Triti-
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Table 4. Mean of morphological characters of tritipyrum and wheat cultivars.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Survival %</th>
<th>Height (cm)</th>
<th>Tiller number</th>
<th>Spikelet number %</th>
<th>Fertility %</th>
<th>Fmgerance (day)</th>
<th>1000-Grain weight (g)</th>
<th>Protein %</th>
<th>Grain Hardness</th>
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<td>58.8±5.5</td>
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Grand Mean  52.6  78.3  10.5  15.44  72.78  239  45.72  16.41  50.67
CV%        21.0  59.5  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 NaCl, whereas both Neodur 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 Tritipryum exhibited a considerable amount of instability in the form of aneuploidy (45.5%) 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 suggesting that the Eb 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 Eb chromosomes in tritipyrum genotypes (Table 2, Fig. 4a-b). Although the random univalent and rod bivalent ratio was expected to be 1 (E<sup>b</sup>): 2 (A+B) and 1 (E<sup>E</sup>)<sup>b</sup>): 2 (AA+BB) respectively, but ratios of 2 (E<sup>b</sup>): 1 (A+B) and 1 (E<sup>E</sup>)<sup>b</sup>): 1.5 (AA+BB), respectively, were found and confirmed by Chi-Square analysis. These results show that although the Eb chromosomes play a major part in the pairing failure and meiotic instability of tritipryum, the A and B genome chromosomes, must not be ignored, as univalent and rod bivalent chromosomes occur in both Eb and AB genomes.
(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<sup>b</sup> 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. <i>bessarabicum</i> 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 <i>Th. bessarabicum</i>, 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. <i>bessarabicum</i> [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. <i>bessarabicum</i> 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 Tritipyrum including the normal Langdon/Th. <i>bessarabicum</i> (La/b) line (Ta-
Can Tritipyrum... Be a Successful Cereal Like Triticale?  

The highest protein amount result, that of Cr/b x Ks/b, showed the greatest delay in ear emergence, presumably due to the presence of the two Thinopyrum genomes (Hassani et al., 1998).

The Production of Substituted Tritipyrum

In spite of considerable variation between the Tritipyrum lines, the morphology of F1 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 genomes by FISH on meiotic preparations in F1 progeny (Fig. 4c-d). The in situ results on mitotic preparations also confirmed the presence of seven E genomes (Fig. 4e). Due to the large number of univalents in F1, a high range of aneuploidy and cytological segregation was, as expected, observed in the F2 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 F2 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 S6-4 combinations. This suggests that the choice of wheat parent, both primary

| Table 6. Segregation ratio for multiple pistil in F2 progeny. a) Small population of Cr/b x Ks/b. b) Large population of Cr/b x S/b, Ma/b and Sub. | 
|-----------------|-----------------|---------------|-----------------|---------------|
| Progeny         | Observed plants | Expected plants | Expected plants | Expected plants |
|                 | with pistil     | on 3:1         | with pistil     | on 15:1        |
|                 | Single Multiple |               | Single Multiple |               |
| a               | 13 2           | 11.25 3.75     | 14.063 0.938    | 0.036** 0.01   |
| b               | 92 5           | 72.75 24.25    | 90.938 6.063    | 0.056** 0.01   |
| a+b             | 105 7          | 84 28         | 105 7           | 0.038** 0.01   |

ns, ** Non-significant, significant p=0.01 respectively.
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 S6-4. 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 F3 (1.5:1) and the BC1-F2 (1.83: 1) progenies in comparison with F2 (0.41:1) and BC1 (0.55:1) generations were very much higher, respectively. This is presumably the result of higher chromosomal stability in F3 and BC1-F2 in comparison with F2 and BC1 generations, respectively (Hassani, 1998). The GISH on meiotic slides from fertile F2 and BC1 plants ranging (2-11) and (5-11) of Eb chromosomes show that it is possible to produce Tritipyrum with variable numbers of Eb and D genome chromosomes (Table 5, Fig. 4f-g), and that FISH is a useful technique for determining the number of Eb chromosomes present. To recognize Eb 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 Eb 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 Eb 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 chromosomal 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.

**ACKNOWLEDGEMENTS**

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Figure 3. Morphological variation of Tritipyrum x wheat F2 plants a) Vegetative stage. b) At maturity. c and d) A range of (Tritipyrum x wheat) x Tritipyrum BC1-F1 and BC1-F2 plants at maturity. e) Mature plants of Tritipyrum x wheat F1 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 *Cresol/Th. bessarabicum* stained with DAPI, showing two rod bivalent and nineteen ring bivalent, undifferentiated chromosomes. b) Meiotic preparation of *Cresol/Th. bessarabicum* showing one E⁶ rod bivalent and six E⁵ ring bivalents. c) GISH on a meiotic preparation of a Tritipyrum x wheat F₁ hybrid. A metaphase I cell stained with DAPI, showing 14 undifferentiated univalent chromosomes of the E⁶ and D genomes. d) The same cell, the seven E⁵ univalent fluorescent (bright red). e) GISH on mitotic cells of Tritipyrum x wheat F₁ hybrids. A mitotic preparation of the St/b x S₆₄ hybrid showing seven univalent chromosomes of the E⁵ genome (Single filter). f) A pollen mother cell at Metaphase I of a Tritipyrum x Wembley F₂ 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 F₂ plant showing eight E⁵ chromosomes, one ring bivalent, one rod bivalent and four univalents. h) A pollen mother cell at metaphase I of a BC₁ plant showing five E⁵ 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 bessarabicum) x Creso F2 plant shows one univalent E3 chromosome. d) Seven morphological distinct 29-chromosome potential tetraploid-Th. bessarabicum addition lines from (Cr/b) x Cr F4 progeny. e) A meiotic preparation at Metaphase I of Cr/b x Cr F1 hybrid with 35 chromosomes, 14 pairs of A and B and seven univalents of E3 genomes stained with DAPI. f) In situ hybridization on a meiotic preparation at Metaphase I Cr/b x Cr F1 hybrid with 35 chromosomes, 14 of A and B and 7 of E3 genomes under bright red filter. g) Potential addition line groupings of Cr/b x Cr F1 progenies. h) A PMC at Metaphase I of (Creso/Th. bessarabicum) x Creso F1 plant showing one univalent E3 chromosome.
REFERENCES


ما در درک بهتر ماهیت و کنترل زنیکی پیچیده این صفت خواهد بود. تریتیپ آمی فلورسنس گذشته و متحمل به شوری حاصل از ثلافی بین گونه های دو جنس گندم نان (پایه مادری) و تینوزیر و وحشی (پایه پدری) می تواند نوبه و امیدی تازه در این زمینه باشد. گرچه در این علائم جدید بیشتر ارقام هگزالپولید، ثلافی بین ارقام گندم دوم و یک گونه میلیونی، گروه شویندگی، شوهر و مهکونی دل را دارا بودن پتانسیل بالقوه مزیت بر الگوی به عنوان یک گیاه زراعی جدید و ماقبل به تنش شوری دیده می شود و لیکن این علائم وضعیت نظیر مجازات و معاصر ناباید جزئی کروموزوم و باروری کم هم اند تریتیکال در اواک پیدا یک خود به عنوان یک علائم مثبت که احتمالاً که صفات مانندی در آن گیاه به دنبال بازاره اصلاحی مرتفع گردیده به نظر می رسد که این صفات در این گیاه نیز با جایگیر نمونه کروموزومهای زنوم D گندم نان با کروموزومهای زنوم و حیثی پایه پدری آن (E(2)) میسر گردند. در راستای تحقیق عملی این فرضیه در آزمایشات نتایج حاصل از ثلافی آن خ ای ارقام مختلف گندم هگزالپولید با تکنیک های جدیدی DNA فلورسنس در محل طبیعی خود خصوصیات مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحلهای مرحله