The choromosomal assesment of salt tolerant substituted tritipyrum using genomic fluorescent *in situ* hybridization (FISH)

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Abstract

Wheat, although moderately tolerant to salt, can not be cultivated in many areas. However, in the triticeae tribe, some of the wild wheat relatives are highly tolerant, e.g. Thinopyrum bessarabicum, which grows on the sea shore. Eight primary hexaploid tritipyrum lines, amphiploids between Triticum durum and Thinopyrum bessarabicum have been produced which can set seed in at least 250 mM NaCl. These tritipyrums (2n=6x=42, AABBE^{b}E^b) due to reasons such as brittle rachis, continuous production of tillers, late maturity, tall stature and meiotic instability will not fulfill the requirements of a successful commercial salt tolerant crop. To overcome such problems the substituted tritipyrum, in which selected E^b chromosomes are replaced by D genome chromosomes of 6x wheat, was produced from 6x tritipyrum x 6x wheat hybrids (F1: 2n=6x=42, AABBDE^b) followed by selfing and backcrossing with 6x tritipyrum. The fertile plants among the above progenies were screened by the genomic fluorescent in situ hybridization technique to identify their E^b and D chromosome constitution. This study showed that producing tritiprum with variable numbers of E^b and D genome chromosomes is feasible and that FISH is a useful technique for determining the number of E^b chromosomes present.

Keywords: *Tritipyrum, substituted tritipyrum, Fluorescent In situ Hybridization, salinity.*

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INTRODUCTION

The relatives of wheat represent an enormous pool of genetic variation which has important potential for use in wheat improvement. One way of exploiting this variation is the production of hexaploid amphiploid combinations for use as cereal crops. Triticale (2n=6x=42, AABBRR) the amphiploid between tetraploid wheat (AABB) and cultivated rye (2n=14, RR) is a successful example. Other examples are tritordeum (2n=6x=42, AABBH^{ch}H^{ch}), the amphiploid between tetraploid wheat and *Hordeum chilense* (2n=14, H^{ch}H^c) and tritipyrum (2n=6x=42, AABBE^{b}E^b) another amphiploid between *Triticum* Spp. and *Thinopyrum bessarabicum* (2n=2x=14, E^{b}E^b), the most notable salt tolereant wild species of wheat which is a littoral diploid grass native to the Crimea, Ukraine (Gorham *et al*., 1985). This wild relative of wheat is able to withstand prolonged exposure to 350 mM sodium chloride (NaCl). It has been identified as a useful source of salt tolerance genes for transfer to wheat for the following reasons: a) It is highly tolerant to prolonged exposure to salt (Gorham *et al*., 1985). b) It is a diploid species (2n=2x=14, E^{b}E^b). c) It has been hybridized with wheat (Alonso and Kimber, 1980; Forster and Miller, 1985). d) The genes conferring tolerance to salt are expressed in a wheat genetic background (Gorham *et al*., 1986; Forster *et al*., 1987). e) Its chromosomes can pair and presumably recombine with wheat homologous in the absence of chromosome 5B (Forster and Miller, 1985; King *et al*.,

1993b).

The Chinese Spring 5E^b addition line had a 10.4% frequency of survival greater than that of euploid Chinese Spring (CS) at 250 mM NaCl (King *et al.*, 1996). This observation demonstrates that a gene(s) on chromosome 5E^b confers a degree of salt tolerance, supporting previous observation made by Forster *et al.* (1988a). Surprisingly, not only did the CS5E^b(5A) and the CS5E^b(5D) substitution lines show a higher frequency of survival than CS, they also showed a significantly higher frequency of survival than the CS5E^b addition line itself. These results demonstrate that the gene(s) conferring salt tolerance located on chromosome 5E^b has a greater effect when substituted for homologous wheat group 5 chromosomes than when present as an additional chromosome, as in the CS5E^b addition line. Increased survival of the substitution lines, up to 29% over CS demonstrates the potential of the salt-tolerance gene(s) located on chromosome 5E^b from *Thinopyrum bessarabicum* , for the production of new salt-tolerant wheat varieties (King *et al.*, 1996).

The incorporation of wild species in amphiploids of this type results in hexaploid combinations with undesirable as well as useful characters from the wild parent. In the case of tritipyrum the undesirable characters include brittle rachis, late maturity, low fertility and meiotic instability, but a major desirable character is salt tolerance (Shahsevand Hassani *et al.*, 1997; Shahsevand Hassani *et al.*, 2000).

The D genome is what made bread wheat the most important cereal in the world. The difference between triticale and bread wheat is that, in triticale, the D genome has been replaced by the R genome. The benefits of the R genome over the D genome in triticale, 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 *et al.*, 1996). Similarly in tritipyrum the E^b genome is superior to the D genome in terms of salt tolerance potential. In the light of the studies of the substitution of D genome chromosomes into triticale, it seems that similar substitution into tritipyrum could prove valuable. There are a number of detrimental characters in tritipyrum and beneficial characters 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 detrimented 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 (Shahsevand Hassani, 2000).

The work presented here describes a preliminary programme to remove undesirable characters of tritipyrum by modification of primary tritipyrum lines via the introduction of D genome chromosomes to replace E^b chromosomes. It is theoretically possible for specific substitutions of chromosomes from the D genome for those of the E^b genome to play a very significant role in the development and improvement of secondary tritipyrum lines. It is expected that specific chromosomes of the D genome can substitute only for their homoeologues in the tritipyrum constitution, therefore, 128 combinations are possible as follows which can be calculated from the following equation:

$$(D+E^b)^7=\mathbf{1}D^7+\mathbf{7}D^6(E^b)^1+\mathbf{21}D^5(E^b)^2+\mathbf{35}D^4(E^b)^3+\mathbf{35}D^3(E^b)^4+\mathbf{21}D^2(E^b)^5+\mathbf{7}D^1(E^b)^6+\mathbf{1}(E^b)^7$$

Of these theoretically possible combinations (Table 1),

Table1. Theoretical combination of the D genome chromosomes substituted for E^b genome chromosomes in 6x tritipyrum.

Genomes (chromosome pairs)	Tritipyrum	Substituted tritipyrum						Wheat
Number of E^b chromosome pairs	7	6	5	4	3	2	1	0
Number of D chromosome pairs	0	1	2	3	4	5	6	7
Number of possible combinations	1	7	21	35	35	21	7	1

Table 2. Summary of results of the 6x tritipyrum / 6x wheat crosses.

Crosses (Female x male)	Female plants cross	Spikes pollinated (No.)	Florets pollinated (No.)	Hybrid Seed (No.)	Seed set %
Aziziah/*Th. bess*[+] x Wheats[++]	18	19	470	110	24
Creso/*Th. bess* x Wheats	15	21	416	58	14
Langdon/*Th. bess* x Wheats	11	20	430	80	23
Langdon(4B/4D)/*Th. bess* x Wheats	17	25	512	54	9
Karim/*Th. bess* x Wheats	14	18	291	31	8
Macoun/*Th. bess* x Wheats	17	22	478	51	11
Neodure/*Th. bess* x Wheat	15	27	638	65	9
Stewart/*Th. bess* x Wheats	12	19	438	83	20
Total	119	171	3673	532	118

[+]*Thinopyrum bessarabicum*, [++]*Triticum aestivum*, cvs: Wembley, Axona, S_{2-4} and S_{6-4}

126 represent substituted tritipyrum; seven pairs each from the A and B genomes will be present and the E^b chromosome pairs would vary from 6 to 1, the remaining chromosomes of the genome coming from the D genome. These 126 combinations do not include any possible substitutions where the chromosomes of the D genome substitute for E^b chromosomes regardless of their homology. If non-homologous substitutions, from hexaploid tritipyrum x hexaploid wheat crosses are also considered, the number of possible combinations will be much greater. The possibility of a very high degree of reorganization of the chromosomal and genomic composition could be obtained although they are not effective.

We also know that very few of the possible combinations have occurred so far in triticale populations and that all of these combinations may never be obtained. There are several reasons which could limit the possibilities of chromosome substitution and may apply to tritipyrum (Gupta and Priyadarshan, 1982).

Fluorescent *in situ* hybridization (FISH) is proving valuable for identifying the origin of chromosomes in first generation and backcrossed hybrids between wheat and its relatives, particularly, for single alien chromosomes and small alien segments that may carry important characters but are difficult to detect by other methods (Schwarzacher *et al.*, 1992, Miller *et al.*, 1995). FISH has been shown to be effective for identifying whole and partial *Thinopyrum bessarabicum* chromosomes in a wheat background and for studying chromosome pairing between wheat and *Thinopyrum bessarabicum* chromosomes (King *et al.*, 1993a, b; Miller *et al*., 1996). FISH using total genomic DNA of *Thinopyrum bessarabicum* as a probe (GISH) has been used in this study to determine the number of E^b chromosomes present in the potential substituted tritipyrums.

MATERIALS AND METHODS

Plant and genetic materials

These consisted of eight hexaploid tritipyrum genotypes (2n=6x=42, $AABBE^bE^b$), four hexaploid wheat cultivars (*Triticum aestivum*, 2n=6x=42, AABBDD) and total genomic DNA of *Thinopyrum bessarabicum, Triticum durum* and *Triticum aestivum* cv Chinese Spring.

Crossing and self pollination programme

42-chromosome 6x tritipyrum seedlings were selected by counting their root-tip chromosomes using the Feulgen squash method (Huchinson *et al*., 1980). To produce substituted tritipyrum, spikes of each line were emasculated and then pollinated with pollens from the hexaploid wheat cultivars (Table 2).

Mitotic preparations

The chromosome numbers of the resulting F_1 hybrids by mitotic chromosome counting were determined (Table 3) and the plants were grown in two successional sowings in a greenhouse with 16 hour photoperiod at 20°C and 8 h dark period at 15°C. The mitotic chromosome preparation technique for the resulting F_2 (Table 4) from the F_1 hybrids was used. The 40-44-chromosome plants were then sown in two successions in a greenhouse at ambient temperature.

In situ hybridization on F_1 hybrids

The meiotic and mitotic chromosome preparation and *in situ* hybridization techniques for the F_1 hybrids were carried out. The labelling, the hybridization and prehybridization mixtures per slide were prepared (Table 5).

Identification of E^b chromosomes of F_2 plants by *in situ* hybridization

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 in which wheat blocking DNA was hybridized to the preparation for one hour prior to normal hybridization (Shahsevand Hassani, 1998 & Shahsevand Hassani *et al.*, 2000). The chromosome constitution of possible substituted tritipyrum plants of the F_2 progeny was assessed by scoring the number of E^b chromosomes present in meiotic cells at Metaphase I of meiosis for individual fertile plants of the progeny. The F_3 seeds of F_2 fertile plants screened by *in situ* hybridisation (Table 6), with a range of 3-12 E^b chro-

Table 3. Aneuploidy of the F_1 hybrids.

Hybrids (6x tritipyrum x 6x wheats)	Seeds germinated (No.)	Chromosome counts	
		42	Non-42
Azizia/*Thinopyrum bessarabicum* x Wheats	28	23	2
Creso/*Thinopyrum bessarabicum* x wheats	24	11	8
Langdon/*Thinopyrum bessarabicum* x Wheats	27	20	5
Langdon (4B)/4D/*Thinopyrum bessarabicum* x Wheats	22	14	3
Karim/*Thinopyrum bessarabicum* x Wheats	21	17	2
Macoun/*Thinopyrum bessarabicum* x Wheats	22	17	1
Neodure/*Thinopyrum bessarabicum* x Wheats	20	16	2
Stewart/*Thinopyrum bessarabicum* x Wheats	26	19	5
Total	190	137	28

Table 4. Aneuploidy of tritipyrum / 6x wheat F_2 progenies involving cultivars Wembley and S_{6-4}.

F_2 progeny	Seeds germinated (No.)	Chromosome counts	
		42	Non-42
Aziziah/*Thinopyrum bessarabicum* x Wembley	2	0	1
Langdon/*Thinopyrum bessarabicum* x Wembley	4	1	1
Langdon(4B)4D/*Thinopyrum bessarabicum* x Wembley	97	15	52
Macoun/*Thinopyrum bessarabicum* x Wembley	12	3	2
Néodure/*Thinopyrum bessarabicum* x Wembley	31	3	15
Langdon (4B)4D/*Thinopyrum bessarabicum* x S6-4	8	3	5
Total	156	25	76

Table 5. The requirement mixtures of labelling, hybridisation and prehybridization *for in situ* hybridisation procedure.

Labelling mixture	Hybridisation mixture	Prehybridization mixture
5 µl of 10 x nick translation (NT) buffer (0.5 M Tris. Hcl, pH 7.8, 0.05 M Mgcl2, 0.5 mg/ml bovine serum albumin (Sigma A7638).	20 µl of dextran sulphate (25%)	20 µl of dextran sulphate (25%)
5 µl of Unlabelled numleotide mixture (0.5 ml solutions of dCTP, dGTP and dATP in 100 mM TrisHcl, pH 7.5 were prepared and mixed in the ratio 1:1:1)	5 µl of 20 x SSC	5 µl of 20 x SSC
3.5 µl of Fluorochrome-labelled nucleotide mixture [0.05 mM solution of dTTP was prepared in 100 mM TrisHcl, pH 7.5. one mM Fluorogreen (dUTP) was mixed at the ratio of 2.5:1 with dTTP. One mM Fluorored (dUTP) was also mixed in the tatio of 1:1 with the dTTP].	1.25 µl of SDS (10%) 2.65 µl of probe containing 50 ng of labelled probe DNA (total genomic DNA of *Thinopyrum bessarabicum* labelled with fluorescent Texas red dCTP nucleotide)	1.25 µl of SDS (10%) 1.5 µl of blocking DNA containing 50 ng of unlabelled wheat cv Chinese Spring DNA
1 µl of 100 mM dithiothreitol (DTT) and 1 µg of the target and sonicated DNA	21.1 µl of Sigma water	22.25 µl of Sigma water
Total: 45 µl	Total: 50 µl	Total: 50 µl

Table 6. 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	4	4	12	1	7	3	13
2	6	3	12	2	4	4	12
3	5	6	11	3	6	3	12
4	4	3	10	4	5	3	11
5	1	4	9	5	3	4	11
6	3	3	9	6	3	4	11
7	6	1	8	7	2	4	10
8	4	2	8	8	3	3	9
9	2	3	8	9	3	3	9
10	2	3	8	10	4	2	8
11	3	2	7	11	2	3	8
12	1	3	7	12	6	1	8
13	3	2	7	13	4	2	8
14	4	1	6	14	5	1	7
15	2	2	6	15	3	2	7
16	3	1	5	16	4	1	6
17	5	0	5	17	3	1	5
18	2	1	4	18	5	0	5
19	1	1	3	19	3	1	5
Mean	3	2	8	Mean	4	2	9

Univ.= Univellent chromosomes, Biv.= Bivallent chromosomes

Table 7. Frequency of aneuploidy in the backcross progenies (BC_1).

Backcross Progeny	Seeds Germinated (No.)	Chromosome counts	
		Euploid	Aneuploid
[Langdon(4B)/4D/Th. bess$^+$]/Wembley x Langdon/*Thinopyrum bessarabicum*	8	2	3
[Langdon(4B)4D/Th. bess$^+$]/Wembley x Macoun/*Thinopyrum bessarabicum*	7	___	3
(Karim/Th. bess$^+$)/Wembley x Karim/*Thinopyrum bessarabicum*	6	1	1
(Macoun/Th. bess$^+$)/Wembley x Macoun/*Thinopyrum bessarabicum*	18	3	6
(Néodure/Th. bess$^+$)/Wembley x Macoun/*Thinopyrum bessarabicum*	3	1	2
(Néodure/Th. bess$^+$)/Wembley x Karim/*Thinopyrum bessarabicum*	5	___	2
[Langdon(4B)4D/Th. bess$^+$]/S_{6-4} x Langdon(4B)4D/Th. bess$^+$	6	1	1
(Stewart/Th. bess$^+$)/S_{6-4} x Stewart/Th. bess$^+$	4	___	1
Total	69	8	19

$^+$, *Thinopyrum bessarabicum*

mosomes, germinated and grown in a glass-house.

Producing (tritipyrum x 6x wheat) x tritipyrum progeny (BC_1)

The spikes of each F_1 hybrid were emasculated and then pollinated with pollen from the hexaploid tritipyrum genotypes. The chromosome numbers of the resulting BC_1 progeny were counted (Table 7) and grown in two successional sowings over spring and summer in a greenhouse at ambient temperature.

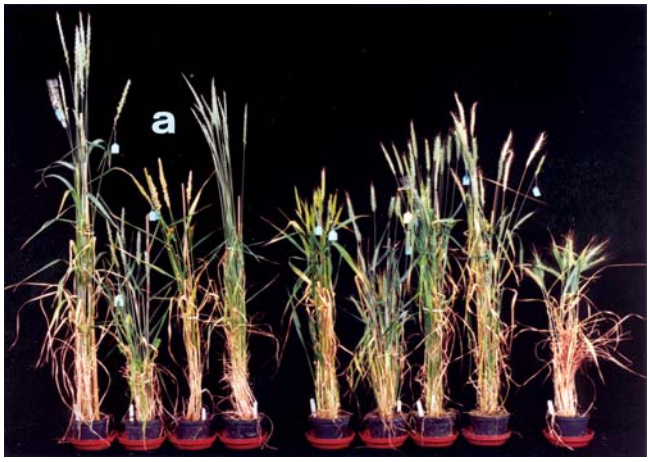
Identification of the number of E^b chromosomes in BC_1 plants by *in situ* hybridization

In situ hybridizations was carried out on BC_1 plants using more than 38 meiotic slide preparations. The seeds of fertile plants of the BC_1 generation (Table 6), with a range of 5-13 E^b chromosomes, were selected and grown over spring and summer in a greenhouse with normal ambient temperature.

RESULTS

Chromosome counts of the 6x tritipyrum lines and F_1 hybrid (tritipyrum x 6x wheats) plants showed a degree of instability in the form of aneuploidy (28 out of 190 plants) (Table 3), with a proportion of plants having lost a chromosome (41) and some having gained a chromosome (43). The chromosome counts of the F_2 (Table 4) and BC_1 progeny (Table 7) showed much higher levels of aneuploidy (75% and 70%, respectively) with plants having a wider range of chromosome number. All 6x tritipyrum lines showed a moderate crossabi-lity with 6x wheat cultivars with the range of 8 to 24 per cent seed set (Table 2). Hybrid necrosis, which leads to early death of F_1 hybrids was a severe barrier in crossing hexaploid tritipyrum with hexaploid wheat. F_1 hybrids plants involving wheat cultivars Axona and S_{2-4} (Fig. 1b) developed necrosis, i.e., their leaves showed discolouration and drying beginning at the tips of older leaves and progressing towards their leaf sheaths. The affected areas of leaves then turned darker green, greyish brown and finally the leaf tissues died, with the result that these hybrids failed to produce normal spikes. Consequently, only the F_1 hybrids involving cultivars Wembley and S_{6-4} were suitable for further study.

Hybrid necrosis in wheat is a physiological disor-



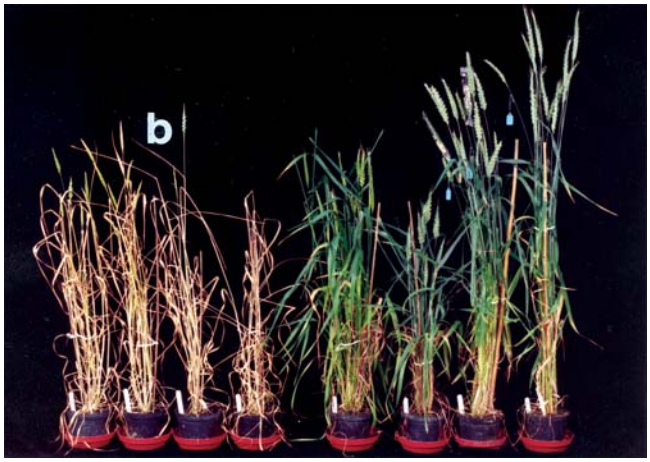


Figure 1. a) 6x tritipyrum, F_1 hybrids and 6x wheat cultivars. Left: tritipyrum genotypes involving Aziziah/*Thinopyrum bessarabicum*, Creso/*Thinopyrum bessarabicum*, Langdon/*Thinopyrum bessarabicum* and Langdon(4B)4D/*Thinopyrum bessarabicum*. Middle: F_1 hybrids involving the above tritipyrum x 6x Wembley. Right: hexaploid wheat cultivar Wembley. **b**) Mature plants of tritipyrum x wheat F_1 hybrids. Left: four necrotic plants of tritipyrum genotypes x 6x wheat Axona. Right: normal plants of tritipyrum genotypes x 6x wheat Wembley.

der caused by the presence of two complementary genes ($Ne_1$and Ne_2), which have been studied in some detail in several cultivars of common 6x wheat and hybrids (Hermsen, 1963a,b; Zeven, 1966, 1968, 1969, 1971; Gregory, 1980). The Ne_1 and Ne_2 genes are located on chromosome arms 5BL and 2BS, respectively. It is estimated that 18% of wheat cultivars posses Ne_1, while more than 42% of them have Ne_2 (Hermsen, 1963c).

Nishikawa (1967) found that Ne_1 is widely distributed among tetraploid varieties tested and its frequency amounted to about 75% , while Ne_2 has never been detected. Ne_1 may inhibit the gene flow from tetraploid to hexaploid or in the reverse direction, because Ne_2 considerably distributes among hexaploid wheat (Hermsen, 1963b, Tsunewaki and Nakai, 1967a). Therefore, occurrence of hybrid necrosis in a number of 6x tritipyrum (carrying the tetraploid background) x 6x wheat F_1's in this study is not an unusual phenomenon and supports the previous findings about hybrid necrosis.

In spite of considerable variation between the tritipyrum lines the morphology of the F_1 hybrids was predominantly wheat-like. Pollen sterility was observed in all hybrid combinations ranging from 2 percent (Creso/*Thinopyrum bessarabicum* x Wembley) to 63 per cent (Langdon /*Thinopyrum bessarabicum* x Wembley). Consequently a low level of seed set was also observed, however, the Langdon /*Thinopyrum bessarabicum*, Langdon (4B) 4D/*Thinopyrum bessarabicum*, Macoun /*Thinopyrum bessarabicum* and Néodure /*Thinopyrum bessarabicum* x Wembley and Langdon (4B)4D/*Thinopyrum bessarabicum* x S_{6-4} hybrids showed reasonable pollen fertility and seed setting (Shahsevand Hassani, 1998).

In spite of some difficulties with differentiation, seven of the unpaired chromosomes could be identified as E^b chromosomes by *in situ* hybridization in meiotic slides (Fig. 2a & b). The *in situ* results on mitotic preparations also confirmed the presence of seven E^b chromosomes (Fig. 2c & d). From F_2 plants of the F_1 hybrids involving wheat cultivars Wembley and S_{6-4}, plant seedlings with the chromosome range of 40-44 were selected. Due to the large number of univalents in the F_1s a high range of aneuploidy and cytological segregation was, as expected, observed in the F_2 generation (Table 4). A considerable range of segregation was also observed in plant morphology, level of sterility, production of back tillers and late maturity.

DISCUSSION

The introduction of the preblocking step to the *in situ* hybridization procedure improved the differentiation between E^b and D genome chromosomes (Shahsevand Hassani, 1998). GISH results from fertile F_2 self pollinated plants, mainly involving Langdon (4B)4D/*Thinopyrum bessarabicum*, Néodure

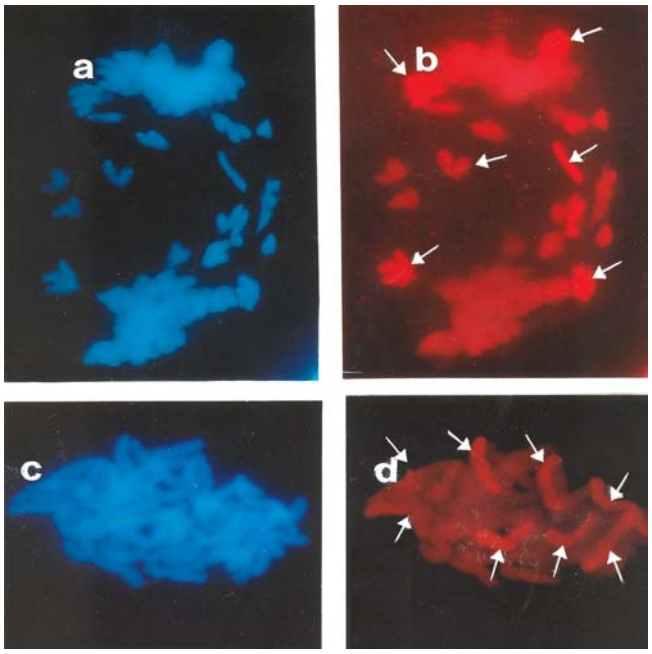


Figure 2. GISH on a meiotic (a&b) and mitotic (c&d) preparations of a tritipyrum x wheat F_1 hybrid. **a**) A Anaphase II cell stained with DAPI, showing many undifferentiated univalent chromosome of the E^b and D genomes. **b**) The same cell, the E^b univalents fluorescent bright red. **c**) A DAPI stained cell of Stewart/ *Thinopyrum bessarabicum* x Wembley showing 42 undifferentiated chromosomes. **d**) The same cell highlighting the seven bright red E^b chromosomes.

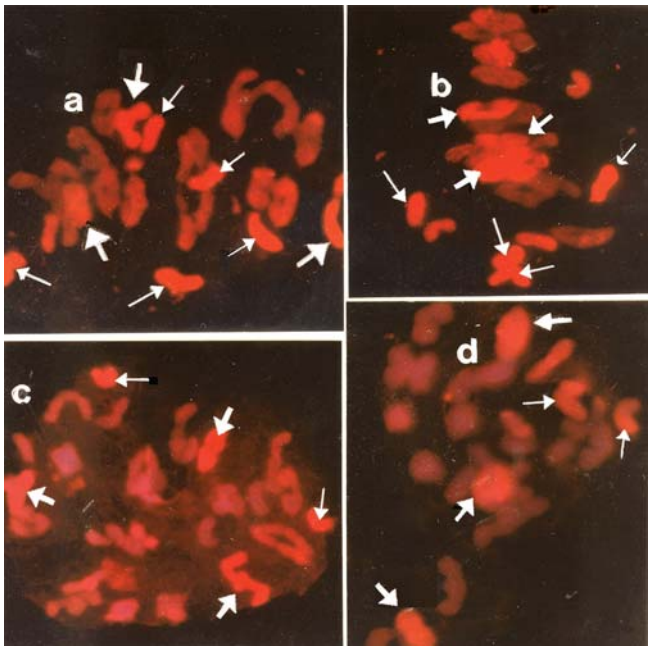
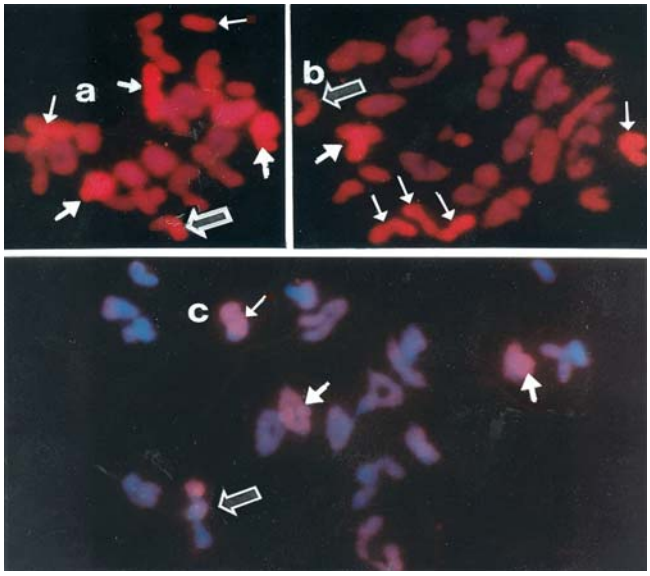


Figure 3. Examples of GISH on tritipyrum x wheat F_2 hybrid plants: meiotic Metaphase I cells showing different numbers of E^b bivalents (ring and rod) and univalents (bright red and arrowed, large arrows mark bivalents and small arrows univalents). **a**) A pollen mother cell at Metaphase I of a tritipyrum x Wembley F_2 plant showing 11 E^b chromosomes consisting of one ring bivalents, two rod bivalent and five univalents. **b)** A pollen mother cell at Metaphase I of a tritipyrum x Wembley F_2 plant showing ten E^b chromosomes, three ring bivalents and four univalents. **c)** A pollen mother cell at Metaphase I of a tritipyrum x Wembley F_2 plant showing eight E^b chromosomes, two ring bivalent, one rod bivalent and two univalents. **d)** A pollen mother cell at Metaphase I of a tritipyrum x Wembley F_2 plant showing eight E^b chromosomes, three ring bivalent and two univalents.

/*Thinopyrum bessarabicum* and Macoun/*Thinopyrum bessarabicum* tritipyrum (Table 6) highlighted the presence of a range of E^b chromosomes (3-12), in the form of univalents (1-6) and bivalents (0-4) (Fig. 3a-d). The GISH also showed the presence of *Triticum durum-Thinopyrum bessarabicum* Robertsonian translocated chromosomes (Fig. 4a-c). 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 disease. Although this type of chromosome may have a 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 *Thinopyrum bessarabicum* characters into wheat.

The results of *in situ* hybridization on meiotic slide preparations of fertile BC_1 plants showed variable numbers of E^b chromosomes (5-13) including up to four bivalents (Table 6 and Fig. 5a-e). The results showed that it is possible to produce tritipyrum with variable numbers of E^b and D genome chromosomes, and that fluorescent *in situ* hybridization is a useful technique for determining the number of E^b chromosomes present (Figures 3a-d, 4a-e and 5a-c). The study also highlighted a number of difficulties to differentiate the E^b chromosomes from the wheat chromosomes (A, B and D genomes chromosomes), hybridization and prehybridization mixtures with different fluorochrome labelled nucleotides. The best differentiation result was achieved (Figures 3a-d, 5a-e and 4a-c) when dCTP nucleotide, labelled with Texas Red flourochrome, was used following preblocking by total DNA of Chinese Spring wheat. The choice of wheat parent, both primary tetraploid and secondary hexaploid, is important if the problem of hybrid necrosis is to be avoided. The low level of fertility (the data not



Fifure 4. Examples of GISH on meiotic preparations of tritipyrum x wheat F_2 and (tritipyrum x wheat) x tritipyrum BC_1 plants showing different number of E^b bivalents (ring and rod), univalents and Robertsonian translocated chromosomes (bright red and arrowed, large arrows indicate bivalents, small arrows univalents and open arrows marks Robersonian translocations). **a)** A pollen mother cell at Metaphase I of an F_2 plant showing 8 E^b chromosomes, two ring bivalents, one rod bivalent, two univalents and one univalent Robertsonian translocation. **b)** A pollen mother cell at Metaphase I of a F_2 plant showing 7 E^b chromosomes, one ring bivalents, four univalents and one univalent consisting of a wheat chromosome and a wheat-*Thinopyrum bessarabicum* Robertsonian translocated chromosome (arrowed). **c)** 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-*Thinopyrum bessarabicum* Robertsonian translocation chromosome (arrowed).

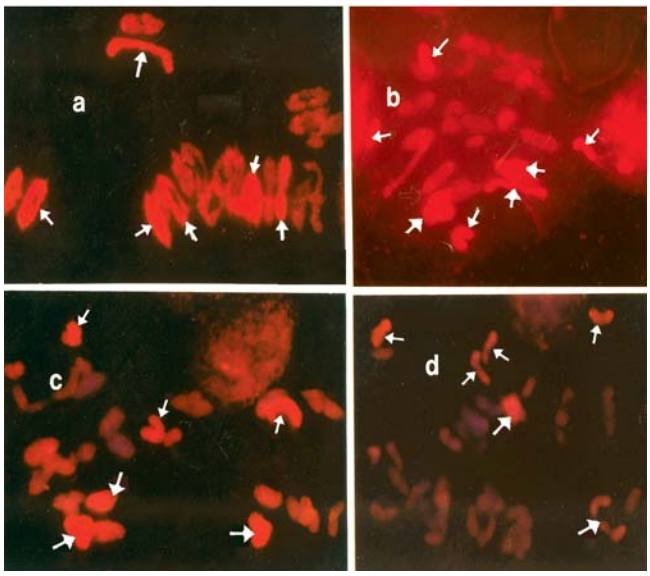


Figure 5. Examples of GISH on meiotic preparations of (tritipyrum x wheat) x tritipyrum BC_1 plants showing different numbers of E^b bivalents (ring and rod) and univalents (bright red and arrowed, large arrows mark bivalents and small arrows univalents). **a)** A pollen mother cell at Metaphase I of a BC_1 plant showing 11 E^b chromosomes, four ring bivalents, two rod bivalent. **b)** A pollen mother cell at Metaphase I of a BC_1 plant showing 10 E^b chromosomes, two ring bivalents, one rod bivalent and four univalents. **c)** A pollen mother cell at Metaphase I of a BC_1 plant showing nine E^b chromosomes, three ring bivalents and three univalents. **d)** A pollen mother cell at Metaphase I of a BC_1 plant showing eight E^b chromosomes, one ring bivalent, one rod bivalent and four univalents.

shown here) and crossability (Table 3) means that a considerable effort will be required to produce sufficient progeny in order to make specific combinations of E^b and D genome chromosomes. However, this study supports the feasibility of substituted tritipyrum.

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