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***Thinopyrum Distichum* Addition Lines: Production, Morphological and Cytological Characterisation of 11 Disomic Addition Lines and Stable Addition-Substitution Line**

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Abstract Plants of the partial amphiploid Inia 66/*Thinopyrum distichum* ($2n = 70$)/Inia 66 ($2n = 56$) were used as male parents in crosses with the monosomic series in the common wheat cultivar Inia 66. The genome and homoeologous group of the monosomic used in the cross affected the distribution of chromosome number of the progeny plants in the F_2 and F_4 . Meiosis in the pollen mother cells of the B_1F_7 partial amphiploids was not stable, and not different from that of the B_1F_1 in which univalents and multivalents were observed. Disomic addition lines were selected on the basis of morphology and meiotic stability in the F_2 , F_4 and F_5 . Eleven of the fourteen possible wheat-*Th. distichum* disomic addition lines were identified using chromosome C-band pattern, as well as size and arm ratio, as genetic markers. Addition of *T. distichum* chromosome J^{all} produced a phenotype indicating homoeology with wheat group-2 chromosomes. Clear indications of homoeology based on morphological characteristics were not obtained in any of the other addition lines, probably due to the mixed homoeology of the *Th. distichum* chromosomes relative to wheat. The addition lines were all susceptible to leaf rust, unlike the germplasm-line Indis which carries a leaf rust resistance gene on a translocation segment derived from *Th. distichum*. Instability of meiotic pairing was observed in all addition lines. The stability, or not, of progeny chromosome counts did not reflect the level of chromosome pairing instability in the parental plants. SDS-PAGE for gliadin-type seed proteins revealed two addition lines which expressed seed storage proteins uncommon to Inia 66 but typical of *Th. distichum*.

Key words C-Banding · Partial amphiploid · Coastal wheatgrass · Leaf rust · SDS-PAGE

Introduction

Addition lines of species related to common wheat can be produced if it is possible to obtain hybrids with wheat. These lines may be used to determine the genetic basis for characteristics of interest in the related species and thereby determine the procedure required to transfer the characteristics to wheat (Gale and Miller 1987). Characteristics which are largely determined by genes carried on a single alien chromosome can be identified and it is often possible to determine the homoeology of the alien chromosomes to those of wheat (Miller and Reader 1987). Disomic addition lines of *Thinopyrum* species in common wheat have been produced using *Th. elongatum* (Dvorak and Knott 1974; Hart and Tuleen 1983), *Th. bessarabicum* (Forster et al 1988), *Th. ponticum* (Knott 1961) and *Th. intermedium* (Cauderon 1966; Wienhues 1973). Wheat curl mite resistance (Larson and Atkinson 1973), stem rust resistance (Knott 1961; Wienhues 1973), leaf rust resistance (Sharma and Knott 1966; Wienhues 1973; Cauderon 1979), yellow rust resistance (Wienhues 1979) and wheat streak mosaic virus resistance (Cauderon 1979), as well as salt tolerance (Forster et al. 1988), are associated with specific chromosome addition lines, and in many cases the disease or insect resistance has been transferred to common wheat.

A variety of methods have been developed to produce addition lines (Lukaszewski 1988), the most common entailing backcrossing the interspecific hybrids, or amphiploids thereof, to the recurrent wheat parent and selecting monosomic addition lines (O'Mara 1940). These monosomic additions are selfed to produce disomic addition lines. Various methods can be used to identify the alien chromosome pair including morphological characteristics (Knott 1958), N-band patterns (Islam 1980), C-band patterns (Jewell and Driscoll 1983), and isozyme analysis (Forster et al. 1987).

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Thinopyrum distichum ($2n = 28$, genomic formula $J_1^d J_2^d J_2^d$) was hybridized with common and durum wheat in the late 1970s and amphiploids were produced (Pienaar 1981, 1983). The amphiploids were backcrossed to the wheat parents to produce partial amphiploids and other derivatives (Pienaar 1988).

A leaf rust-resistant, hexaploid line was identified from the B_2F_3 derivatives of the backcrosses of the common wheat cultivar, Inia 66, to the Inia 66-*Th. distichum* partial amphiploid (Pienaar et al. 1985). The leaf rust resistance in the germplasm line, named Indis, is associated with a translocation on chromosome 7D of Inia 66 derived from *Th. distichum* (Marais and Marais 1990). To study the genic content of *Th. distichum* it was felt necessary to produce disomic addition lines of *Th. distichum* in common wheat.

Sears (1954) showed that tetrasomy produced consistent changes in the plant phenotype. Subsequent production of many series of addition lines in common wheat have shown that the addition of pairs of chromosomes to the wheat complement produces changes in the plant and seed morphology, which are ascribable to the condition of tetrasomy rather than the origin of the added chromosome pair (Miller and Reader 1987). Thus, analysis of the morphological characteristics of disomic addition lines can indicate the homoeology of the alien chromosome. This method was used to determine the homoeology of *Thinopyrum bessarabicum* chromosomes 2J and 5J (Forster et al. 1988).

Riley (1960) c' served differences in the transmission rates of individual rye chromosomes through male gametes in wheat-rye disomic addition lines, indicating that each chromosome affects the degree to which it is transmitted through the male gametes.

The present paper describes the production and morphology of the disomic wheat - *Th. distichum* addition lines, their cytology, reaction to leaf rust, and gliadin-type protein separation by SDS-PAGE.

Materials and Methods

B_1F_1 Inia 66/*Th. distichum* ($2n = 70$)/Inia 66 (78S125, $2n = 56$) partial amphiploids (Pienaar 1981, 1983) were selfed for seven generations. Plants from each selfing were selected in the greenhouse for well-filled, large, kernels and high fertility. Inia 66 and some partial amphiploid plants with 56 chromosomes in the B_1F_7 generation were meiotically analysed as controls. A random sample of pollen from a number of 56 chromosome B_1F_7 plants was used to pollinate the complete set of Inia 66 monosomic lines. The resulting F_1 hybrids were selfed for a few generations as shown in Fig. 1.

Plants with $2n = 44$ chromosomes in the B_2F_2 and B_2F_4 generations were selected as possible *Th. distichum* addition lines. The 44 chromosome B_2F_2 plants were meiotically analysed. The B_2F_3 seed was sown and single spikes differing in morphology from Inia 66 were selected when ripe. The spikes were threshed individually and five seeds per spike were germinated to give the B_2F_4 . The chromosomes of these were counted and meiotic analysis was done on the $2n = 44$ B_2F_4 plants. The $2n = 43$ B_2F_4 plants were selfed and the chromosomes of the seedlings from the selfed seed were counted. These B_2F_5 , $2n = 44$ progeny plants were meiotically analysed. Of the 60 plants from the B_2F_4 and B_2F_5 in which more than 8 out of 10 PMCs had 22" at metaphase-1, the root tip chromosomes of 39 were stained to

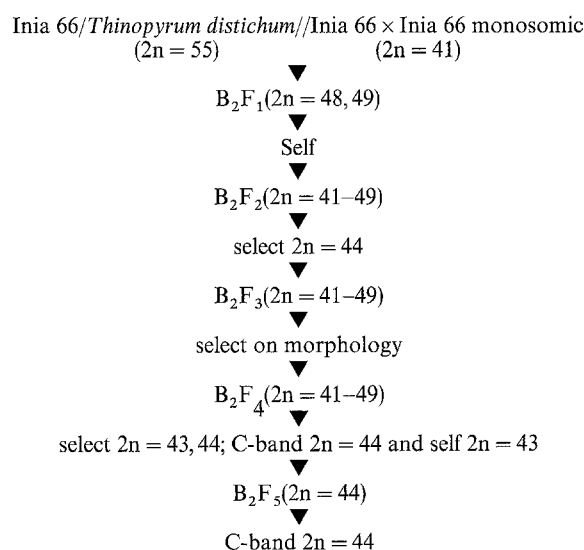


Fig. 1 The flow diagram of the crosses made to produce lines which were possible disomic addition lines of *Th. distichum* in the wheat cultivar Inia 66

determine C-band patterns. Most of these 39 plants were selected on the basis of meiotic stability and differences in whole plant, spike, or kernel morphology compared to the Inia 66 control plants and the other addition lines. The chromosomes of some 44 chromosome lines which did not differ visually from Inia 66 were also C-banded.

Chromosome counts were made using the Feulgen technique. Spikes were fixed in Carnoy's (6:3:1, ethanol-chloroform-acetic acid) and stained in aceto-carmin for the meiotic analyses. The C-band identification of the *Th. distichum* and Inia 66 chromosomes was done according to Littlejohn (1994). Eleven disomic wheat - *Th. distichum* addition lines, and an addition-substitution line which has no 5D but carries J^{dXI} and J^{dXIV} in the disomic condition, were grown in the greenhouse. The leaf rust-resistant line, Indis, the cultivar, Inia 66, and the Inia 66/*Th. distichum* ($2n = 70$)/Inia 66 partial amphiploid with $2n = 56$, were grown as controls. All the seedlings were inoculated with leaf-rust UV8 at the two-leaf stage. The leaf-rust race was obtained from Dr. B. Lombard (Sensako, Stellenbosch). Leaf-rust readings were taken 2 weeks after inoculation. At maturity the plant height was measured. The primary spikes per plant, the spikelets per spike, florets per spikelet, and kernels per spike were counted using all the spikes of both plants. The spike length and the culm diameter of two primary spikes per plant was measured.

A spike was cut from each of the disomic addition plants, Inia 66, and the F_1 hybrid Inia 66/Chinese Spring, for meiotic analysis and fixed in Carnoy's (6 ethanol: 3 chloroform: 1 acetic acid) for up to 4 weeks. Pollen mother cells (PMCs) were stained and squashed in aceto-carmin, and the meiotic configurations analysed at 1000 X magnification. The selfed progeny seed was germinated and the root-tip chromosomes of 20 seedlings stained using the Feulgen technique to obtain a chromosome count.

Selfed progeny seed was cut in half, the embryo side used to check chromosome number, and the endosperm half used to separate the gliadin-type proteins electrophoretically using the SDS-PAGE method of De Villiers and Laubscher (1989).

Results and Discussion

The fertility of the three B_1F_7 plants was not high since on average they produced 43 kernels from 154 florets per spike. This represents a fertility of 27.9% compared to 81% for Inia 66. The chromosome counts of 20 B_1F_7

plants ranged from $2n = 54$ to $2n = 59$, peaking at 56 (51.7%). In Table 1 the mean meiotic configurations of *Inia 66* and three B_1F_7 partial amphiploids with $2n = 56$ chromosomes are compared to the meiotic configurations observed in the B_1F_1 (Pienaar 1981). Chromosome pairing in *Inia 66* was stable but in the partial amphiploids univalents and multivalents were frequently observed (Fig. 2). The meiotic analysis of the B_1F_7 plants did not differ significantly from that of the B_1F_1 . C-banding indicated a full complement of wheat chromosomes (Fig. 3) and no translocations were found in the three control B_1F_7 plants.

The distribution of chromosome counts from a random sample of seed from the progeny of the B_2F_1 ranged from $2n = 41$ to $2n = 50$ (Fig. 4), peaking at 44 chromosomes. The chromosome counts were grouped according to the homoeologous group of the monosomic female parent and according to the genome of the monosomic female parent. In the analysis of the means of the groups, the B_2F_2 plants derived from the crosses in which monosomic group 1 was used as female parent were found to have significantly-higher chromosome counts than the crosses involving groups 3, 6 and 7, but not groups 2, 4 and 5. No significant differences were found in the grouping according to genome. Meiotic analysis of 19 of the B_2F_2 plants with 44 chromosomes revealed that only one plant had pollen mother cells at metaphase-I which gave a configuration of 22".

In plants of the B_2F_4 the chromosome counts ranged from $2n = 41$ to $2n = 50$ (Fig. 4), peaking at 42 chromosomes, with the second largest group having 44 chromosomes. The B_2F_4 chromosome counts were grouped according to the homoeologous group of the monosomic female parent, and according to the genome of the monosomic female parent. The analysis of the means of the groups showed significantly-higher chromosome counts in the B_2F_4 plants derived from the crosses in which monosomic group 5 was used as female parent. In the grouping according to genome, the derivatives of crosses in which A-genome monosomics were used as female parents had significantly-higher chromosome

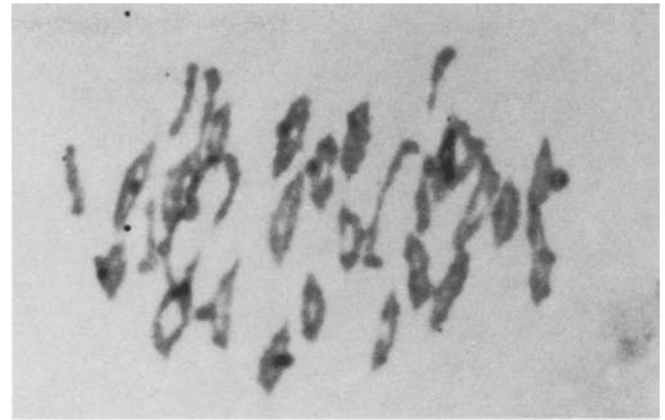


Fig. 2 Metaphase-I in a pollen mother cell of *Inia 66/Th. distichum* ($2n = 70$)//*Inia 66* partial amphiploid ($2n = 56$) showing a quadrivalent, two univalents and 25 bivalents

counts than those derived from crosses in which the B-genome monosomics or the D-genome monosomics were used. There were no significant differences observed when B- or D-genome monosomics were used as female parents in the second backcross.

The B_2F_4 lines with 44 chromosomes were meiotically analysed and out of these, 29 showed bivalent pairing in at least 8 out of 10 PMCs while 11 were not stable, resulting in univalents and multivalents. The B_2F_4 plants with $2n = 43$ chromosomes were selfed. Of the 74 lines, 25 gave progeny with 44 chromosomes. Thirty-three of these B_2F_5 lines were meiotically analysed of which 30 showed stable chromosome pairing and three were unstable.

The C-band karyotype of *Th. distichum* (Fig. 5) was used to identify the *Th. distichum* chromosomes in the addition lines. Of the 14 possible addition lines, 11 disomic addition lines were identified by C-banding the 39 meiotically-stable lines with 44 chromosomes (Fig. 6). Generally, between one and five plants of each

Table 1 The meiotic configurations observed in pollen mother cells (PMCs) of the three B_1F_7 partial amphiploid plants compared to *Inia 66* and the B_1F_1 partial amphiploid

Line	Chromosome count	Number of PMCs	Meiotic configurations						
			I	II rod	II ring	II total	III	IV	XMT
<i>Inia 66</i>	42	80	0.22	2.00	19.36	20.84			40.72
B_1F_7			0-2	0-7	14-21	20-21			33-42
78S125-1	56	25	3.96	2.28	22.28	24.56	0.16	0.16	49.24
B_1F_7			0-6	0-8	18-27	25-27	0-2	0-1	43-54
78S125-2	56	25	3.24	4.56	21.36	25.92	0.20	0.08	48.00
B_1F_7			0-6	2-8	19-24	24-27	0-2	0-1	44-54
78S125-3	56	25	5.56	4.44	20.20	24.64	0.16	0.16	47.24
B_1F_7			2-10	1-8	17-23	23-27	0-1	0-1	42-50
78S125 ^a	56	100	5.34	6.77	17.88	24.76	0.18	0.18	45.32
B_1F_1			2-10	1-13	11-22	18-27	0-2	0-2	37-51

^a Pienaar 1981

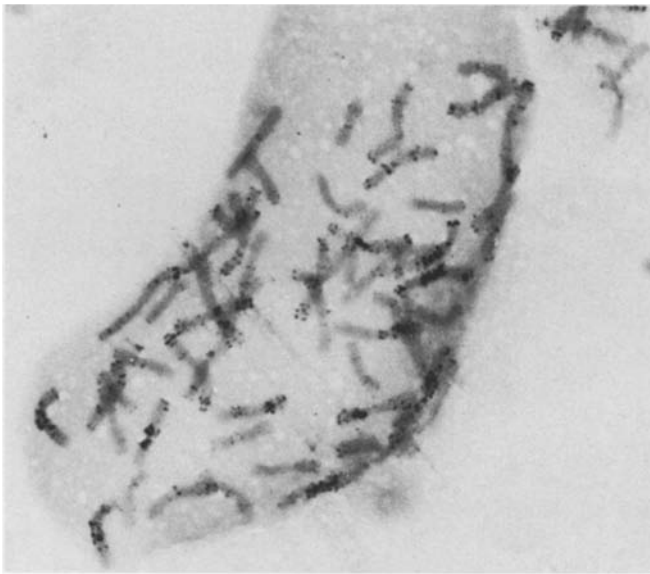


Fig. 3 The C-banded chromosomes of the Inia 66/*Th. distichum* ($2n = 70$)//Inia 66 partial amphiploid with $2n = 56$

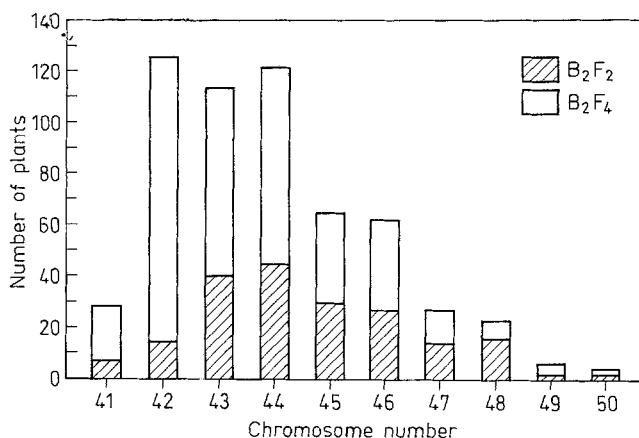


Fig. 4 Distribution of the chromosome counts of the *Th. distichum*/3* Inia 66 B_2F_2 and B_2F_4 plants

addition were identified, excepting chromosome J^{dX} which was identified in 15 of the 39 lines. There was no correlation between the number of addition lines identified and the original monosomy in Inia 66.

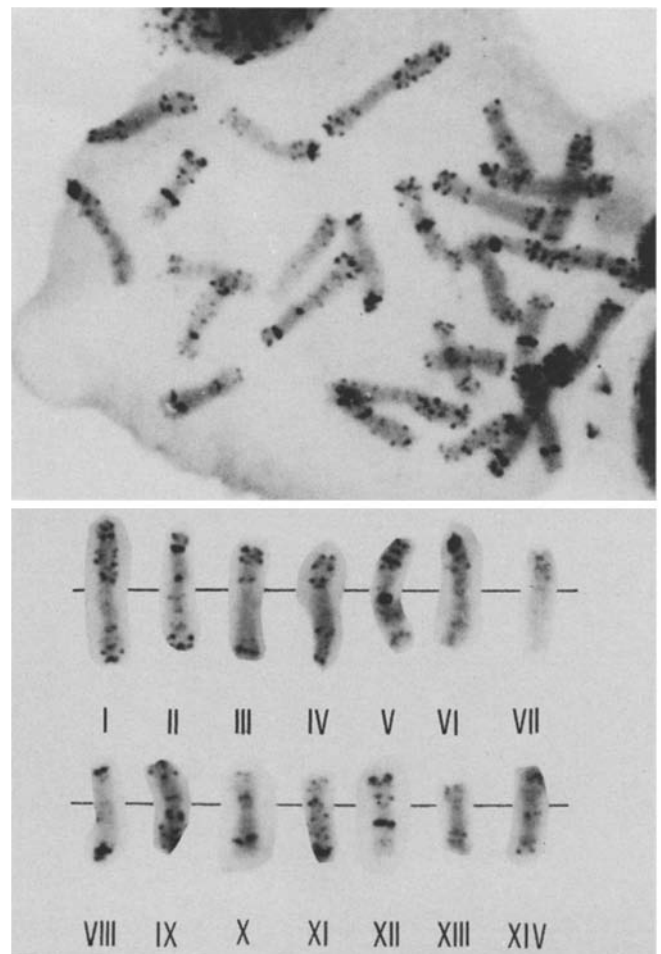
The purpose of using the Inia 66 monosomics as female parents in the second backcross in this study was to obtain substitution lines. Although substitutions must have been present in the B_2F_2 plants with 44 chromosomes which gave $20'' + 4'$, by the B_2F_4 few such plants remained. The likelihood of obtaining a compensating substitution by this random method is small (Gale and Miller 1987), and as non-compensating substitutions are generally non-viable they are naturally selected against.

In the B_2F_4 the plants derived from the A-genome monosomics had higher chromosome numbers than those derived from the B- and D-genome monosomics. Thus there is interaction between the wheat and *Th.*

distichum chromosomes which is linked to genome relatedness. Dvorak (1980) postulated, on the basis of good pairing (up to five bivalents) between J^e and D-genome chromosomes in *Th. elongatum*/*Ae. squarrosa* hybrids, that the J^e chromosomes are more-closely related to the D genome than the A or B genome. If J^d chromosomes are thus also less related to A-genome chromosomes then compensation of A-genome chromosomes by J^d chromosomes would occur less readily. Thus in the B_2F_2 few substitutions would be viable without other partially-compensating *Th. distichum* chromosomes being present. It is not expected that this would persist into the B_2F_4 .

The use of group-1 and group-5 monosomics as maternal parents resulted in significantly higher chromosome numbers in the B_2F_2 and B_2F_4 respectively but the reason for the difference between the B_2F_2 and B_2F_4 is not clear. The meiotic configurations observed in the B_2F_4 and B_2F_5 are as expected. A higher number of selfed $2n = 43$ plants gave configurations with $22''$ than the B_2F_4 plants which were randomly selected. Similarly, a higher proportion of homologous addition chromosome pairs were observed in the B_2F_4 than in

Fig. 5 The C-banded chromosomes of *Th. distichum*, arranged and numbered in descending order of size



the B_2F_2 . No correlations between the genomes or homologous groups of the parental monosomic were observed in the meiotic analyses of the selected $2n = 44$ plants in any generation. One addition-substitution line was found amongst the 44-chromosome plants. In this line, two pairs of *Th. distichum* chromosomes, J^{dXI} and J^{dXIV} , were present and no 5D chromosomes were observed. The maternal parent in the original cross was Inia 66 mono-5D. In this line either one J^d chromosome, or a single arm of both the J^d chromosomes present, must compensate for the loss of the 5D chromosome pair. Therefore, either J^{dXI} , J^{dXIV} , or an arm of each chromosome, must be homologous to group 5.

Not one of the addition lines, or the Inia 66 used as control, were resistant to the leaf-rust race UV8. Indis gave a characteristic fleck resistance reaction with this leaf-rust race. The partial amphiploid, Inia 66/*Th. distichum* ($2n = 70$)/Inia 66, was susceptible to the leaf-rust race used. Either the chromosome on which the

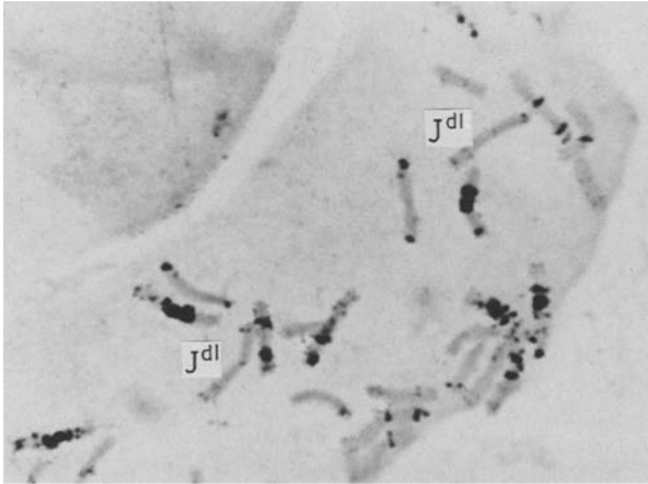


Fig. 6 The C-banded chromosomes of the wheat - *Th. distichum* addition line J^{dl}

gene for leaf-rust resistance is found was not amongst the addition lines, or the resistance was not expressed in the addition chromosome.

The 11 addition lines and the substitution-addition line differed morphologically from one another and from Inia 66 (Table 2, Fig. 7) as follows:

the J^{dII} disomic addition has thin culms, narrow spikes, leaves, glumes and kernels. The flag leaves are twisted during growth and the kernels are vitreous. These characteristics are indications of homoeology with group 2 of wheat (Miller and Reader 1987).

the J^{dIII} disomic addition is shorter than Inia 66, with smaller, denser spikes, thicker culms and wrinkled kernels.

the J^{dV} disomic addition produces fewer tillers than Inia 66 and is taller.

the J^{dVI} disomic addition looks somewhat similar to the J^{dIII} disomic addition line, with small, narrow spikes and narrow kernels. The culm diameter differs little from Inia 66, but the spikes are denser than those of Inia 66. the J^{dVII} disomic addition produces many tillers but is shorter than Inia 66, with thicker culms and denser spikes. the J^{dVIII} disomic addition differs little from Inia 66.

the J^{dIX} disomic addition has a tapered spike, is taller than Inia 66 with thinner culms. The kernels are redder in colour.

the J^{dX} disomic addition has more florets per spikelet giving a broad appearance to the spike, and thick culms. The kernels are coarse. The kernels are easily threshed, being seated very loosely in the florets when ripe.

the J^{dXI} disomic addition has a long lax spike, thick culms, broad leaves and large coarse kernels. This could be a group-5 addition.

the J^{dXIII} disomic addition is shorter than Inia 66 with small spikes, thin culms and the top two to three florets infertile.

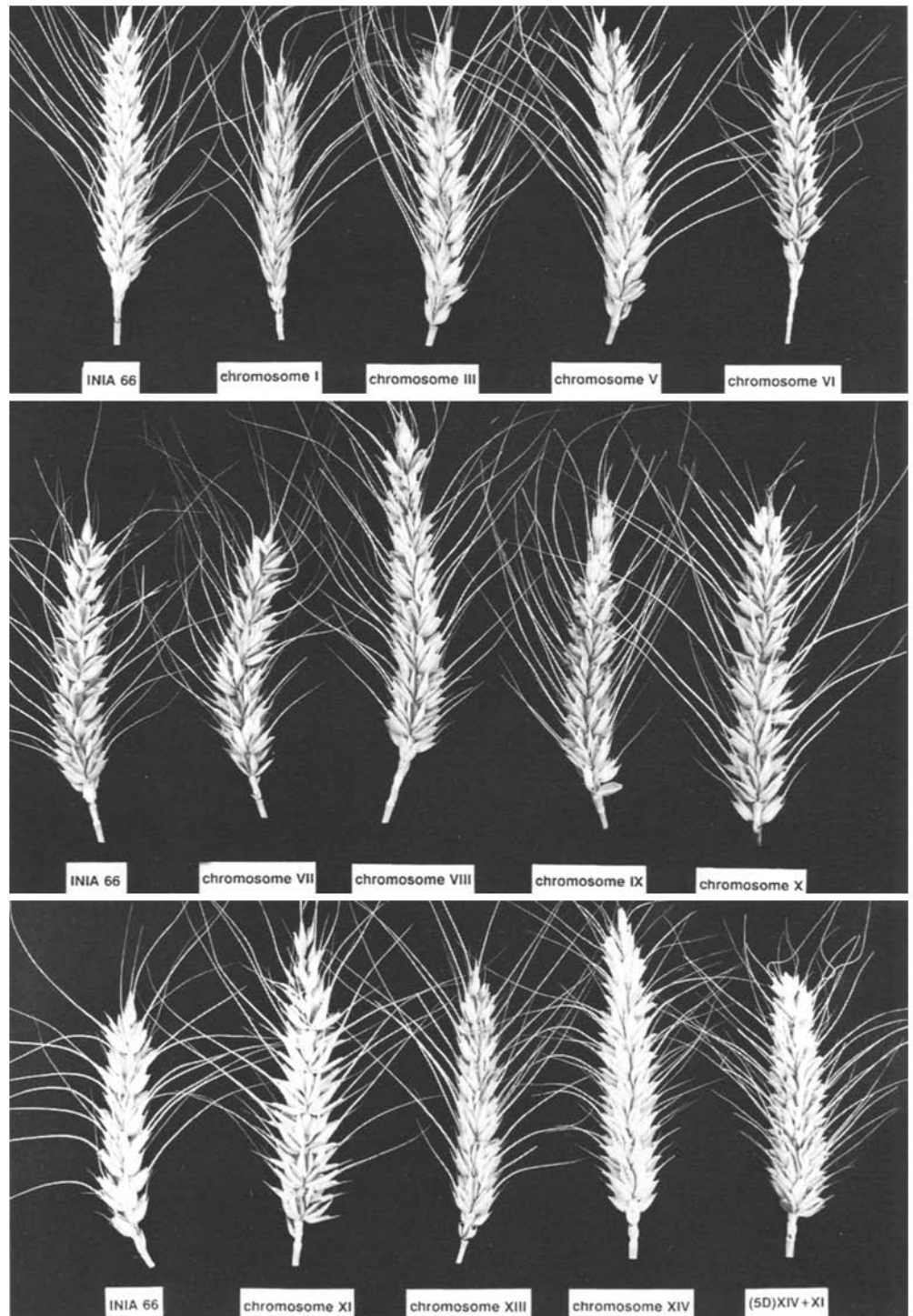
the J^{dXIV} disomic addition is taller than Inia 66, with dense clavate spikes and large kernels.

the J^{dXI} - J^{dXIV} substitution-addition line differed from

Table 2 The mean measurements of morphological characteristics of wheat-*Th. distichum* disomic addition lines, an addition-substitution line and Inia 66

Addition line	Height as % of Inia	Spikes/plant (A)	Spikelets/spike (B)	Florets/spike (C)	Kernels/spike (D)	Length of spike (mm) (F)	Density of spike (F/B)	Culm diam.
II	113	6	15	41	31	79	5.27	1.48
III	88	4	15	53	47	86	5.73	2.00
V	112	3	16	51	46	92	5.75	1.86
VI	100	4	18	39	29	89	4.94	1.98
VII	87	7	19	53	30	79	4.16	2.07
VIII	107	4	16	57	48	94	5.88	1.89
IX	116	3	16	54	42	92	5.75	1.70
X	96	4	16	56	39	94	5.88	2.21
XI	103	5	17	37	33	107	6.29	2.07
XIII	84	4	14	37	31	79	5.64	1.42
XIV	112	4	22	59	40	93	4.23	1.89
XI + XIV	109	5	21	67	43	78	3.71	2.39
Inia	100	5	16	45	40	98	5.76	1.89

Fig. 7 The spike morphology of the wheat – *Th. distichum* addition lines and one addition-substitution line compared to the wheat cultivar Inia 66



Inia 66 in having dense, tapered spikes, thick culms and small, round kernels. The ligules are large and intensely purple. Flowering is 10–12 days later than in Inia 66. All the addition lines except J^{dVII} produce larger kernels than Inia 66.

Armstrong et al. (1991), on the basis of C-banding similarity, postulated, that the satellited J^{dIV} and J^{dVII}

chromosomes were homoeologous to $5 J_1^e$ and $6 J_1^e$ respectively. The homoeology of J^{dVII} was not verified by morphological characteristics, as group-6 additions usually have round glumes and kernels and the plants are taller than the parental wheat cultivar (Miller and Reader 1987). Addition chromosome J^{dVII} is satellited, but the $2n = 44$ chromosome plants were small of stature and had small dense spikes, more characteristic of a

group-7 addition. Chromosome J^{dIV} was not obtained as an addition line and its homoeology could not be verified. Either chromosome J^{dXI} or J^{dXIV} must be homoeologous to group 5 as one or the other is able to compensate for the loss of chromosome 5D of wheat. Disomic addition line J^{dXI} has thick culms, broad leaves, large coarse textured grains and the J^{dXI} chromosome is the most heterobrachial of the *Th. distichum* chromosomes and is therefore indicative of a group-5 addition (Miller and Reader 1987). Disomic addition J^{dXIV} is tall and has dense spikes and this chromosome is the second most heterobrachial in the *Th. distichum* complement. These characteristics are also associated with homoeology with group 5. It is possible that only one arm of each of these chromosomes is homoeologous to group 5, in which case both chromosomes must be present to adequately compensate for the loss of 5D.

The lack of consistency in the morphological characteristics with those described by Gale and Miller (1987) indicate mixed homoeology. Arm homoeology of rye chromosomes to wheat, as determined by analysis of C-banded metaphase-I chromosome pairing and the chromosomal location of homoeoloci in wheat and rye, indicates that only 1RS, 1RL, 2RL, 3RS and 5RS are fully homoeologous with their wheat counterparts (Naranjo and Fernandez-Rueda 1991). The remaining arms of rye chromosomes appear to be involved in chromosomal rearrangements. *Th. elongatum* chromosomes do not show complete homoeology for wheat chromosomes, although adequate compensation for wheat chromosomes takes place (Dvorak and Knott 1974; Dvorak 1980; Tuleen and Hart 1988). *Th. distichum*, which is a segmental allopolyploid in which pairing of homoeologous J^d chromosomes occurs in partial amphiploids in the presence of Ph_1 (Pienaar 1981, 1983), is more likely to have a rearranged genome than a diploid species. Therefore, mixed homoeology in the disomic addition lines is not unexpected.

Quadrivalents in the meiotic configurations of the Chinese Spring/Inia 66 F_1 hybrid (Table 3, Fig. 8) indicated the presence of differences in chromosome-arm homoeology between the two cultivars. The lack of clear morphological indicators of homoeology of the *Th. distichum* addition lines may be influenced by the arms within a single *Th. distichum* chromosome being homoeologous to different Inia 66 chro-

mosomes than would be the case with Chinese Spring. This would also produce indications of mixed homoeology. The majority of sets of addition lines have been produced in Chinese Spring (Shepherd and Islam 1988). Alternatively, the morphological indicators may be clear when Chinese Spring is the common wheat cultivar which forms the genetic background of the addition lines, but less clear for other common wheat cultivars.

The meiotic analysis of the disomic addition lines and Inia 66 revealed some significant differences in the stability of chromosome pairing (Fig. 9). Univalent frequencies ranged from 0.07 in disomic addition J^{dIII} to a maximum of 0.57 in the substitution-addition line (Table 4). Trivalents and quadrivalents were not observed in the additions J^{dV} , J^{dX} and J^{dXIV} . The highest frequency of multivalents occurred in disomic addition J^{dXIII} . The multivalents could result from small translocations between the wheat chromosomes or between wheat and *Th. distichum*, but no translocations were detected by C-banding. Pairing regulatory genes on the *Th. distichum* chromosomes could affect the pairing of the wheat chromosomes. The chromosomes of the closely-related *Th. elongatum* have been shown to affect pairing between wheat chromosomes (Dvorak 1987; Charpentier et al. 1988). The effect on chromosome pairing may not be related to the presence of genes promoting pairing, as the presence of alien chromo-

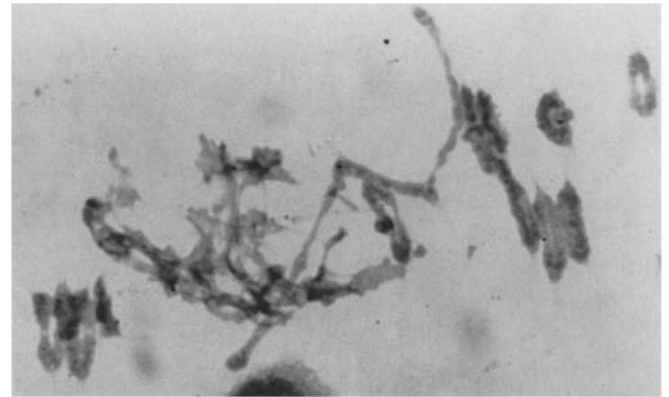


Fig. 8 Quadrivalent formation in a pollen mother cell of a Chinese Spring/Inia 66 F_1 hybrid

Table 3 The meiotic analysis at metaphase-I in pollen mother cells of the Inia 66 and the Chinese Spring/Inia 66 F_1 hybrid

Line	# PMCs	Meiotic configurations						
		I	II rod	III ring	II total	III	IV	XMT ^c
Inia 66	80	0.22 0-2	2.00 0-7	18.83 14-21	20.83 20-21			39.73 35-42
Ch. Spr. ^a / Inia 66	140	0.84 0-5	2.69 0-8	17.06 13-20	19.75 17-21	0.21 0-2	0.25 0-2	38.00 32-41

^a Ch. Spr., Chinese Spring

^b # PMCs, number of pollen mother cells

^c XMT, chiasmata

somes per se has an effect on the meiotic configurations observed in wheat-rye addition lines (Riley 1960; Orellana et al. 1983).

The stability of the progeny chromosome number was not highly correlated ($r = 0.40$) with the stability of meiotic pairing in the parent plants. Among the addition lines, J^{dVI} , J^{dVII} and J^{dXIV} gave fewer progeny plants with 44 chromosomes when selfed (Table 5). The instability of chromosome number indicates a lower transmission rate of chromosomes J^{dVI} , J^{dVII} and J^{dXIV} , probably through the $n + 1$ male gametes which are at a competitive disadvantage (Khush 1973). The stability of the wheat-*Th. distichum* disomic addition lines shows a greater range than that reported for the seven wheat-*Th. elongatum* addition lines. The percentage of progeny plants with 44 chromosomes from selfing wheat-*Th. elongatum* disomic addition lines ranged from 85.5% to 96.6% (Dvorak and Knott 1974), whereas in wheat-*Th. distichum* disomic addition lines it ranged from 65% to 100%. In wheat-rye addition lines the stability of the addition line has been linked to the identity of an addition chromosome (Riley 1960; Miazga et al. 1988).

The PAGE analysis showed that in the additions J^{dV} and J^{dIX} there were altered gliadin band patterns indicating the presence of proteins coded for by *Th. distichum* genes (Fig. 10). Neither of these lines could be identified as group-1 or group-6 additions based on morphological features. In the J^{dIX} line a major Inia 66 band was lost. This is a common polymorphism within Inia 66 and

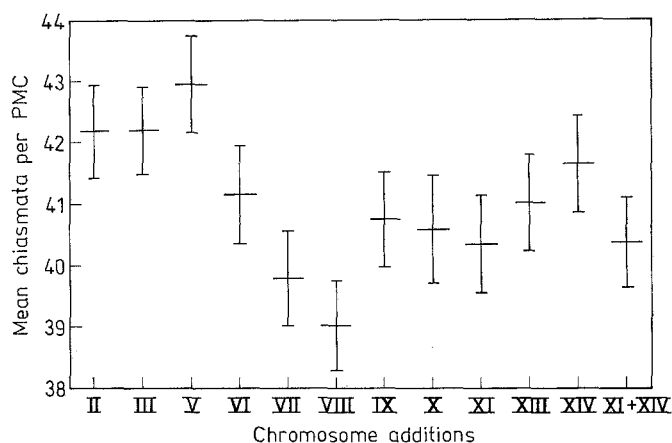


Fig. 9 The 95% confidence intervals for the mean chiasma frequencies observed in the Inia 66 - *Th. distichum* addition lines and the addition-substitution line

polymorphism was detected in *Th. distichum* as well, so definitive results could not be obtained.

To fully determine the homoeology of the *Th. distichum* chromosomes use will have to be made of isozyme analysis such as that employed in determining the homoeology of *Th. intermedium* chromosomes (Forster et al. 1987), or else substitution lines will have to be produced as used in determining the homoeology of *Th. elongatum* chromosomes (Dvorak 1980; Tuleen and Hart 1988).

Table 4 The meiotic analysis at metaphase - I in pollen mother cells (PMCs) of the wheat-*Th. distichum* disomic addition lines and Inia 66 (XMT = chiasmata)

Line	# PMCs	Meiotic configurations						
		I	II rod	II ring	II total	III	IV	XMT
Inia 66	80	0.22 0-2	2.00 0-7	18.83 14-21	20.83 20-21			39.27 35-42
II	27	0.22 0-2	1.56 0-6	20.25 15-21	21.81 21-22		0.04 0-1	42.19 36-44
III	30	0.07 0-2	1.67 0-6	20.13 17-22	21.8 21-22		0.07 0-1	42.20 38-44
V	25	0.16 0-2	0.88 0-4	21.04 18-22	21.92 21-22			42.96 38-44
VI	25	0.24 0-2	2.56 0-5	19.20 17-22	21.76 20-22		0.04 0-1	41.16 38-44
VII	26	0.42 0-2	3.69 1-7	17.81 13-21	21.50 21-22	0.12 0-1	0.04 0-1	39.81 35-43
VIII	14	0.34 0-3	3.86 1-9	17.86 13-21	21.72 19-22	0.06 0-1		39.03 34-43
IX	25	0.19 0-2	2.96 0-6	18.77 16-22	21.73 19-22	0.12 0-1		40.76 38-44
X	20	0.1 0-2	3.30 1-6	18.60 17-21	21.90 21-22			40.60 38-43
XI	25	0.24 0-2	3.40 0-8	18.40 13-21	21.80 21-22		0.04 0-1	40.36 34-44
XIII	25	0.40 0-2	2.60 0-7	18.64 14-22	21.24 19-22		0.28 0-1	41.04 35-44
XIV	25	0.08 0-2	2.24 0-4	19.72 17-22	21.96 21-22			41.68 39-44
XI + XIV	28	0.57 0-2	2.96 0-6	18.68 16-22	21.64 21-22			40.39 37-44

Table 5 The distribution of the chromosome counts of the selfed seed from eleven disomic wheat – *Th. distichum* addition lines, and one substitution-addition line

Addition chromosome	Chromosome number of seedling progeny				Percentage progeny 2n = 44
	42	43	44	45	
II			20		100
III		1	18	1	90
V			20		100
VI	2	6	12		60
VII	3	4	13		65
VIII		4	16		80
IX	3	1	16		80
X		2	18		90
XI		2	18		90
XIII			20		100
XIV		5	15		75
XI + XIV		1	19		95

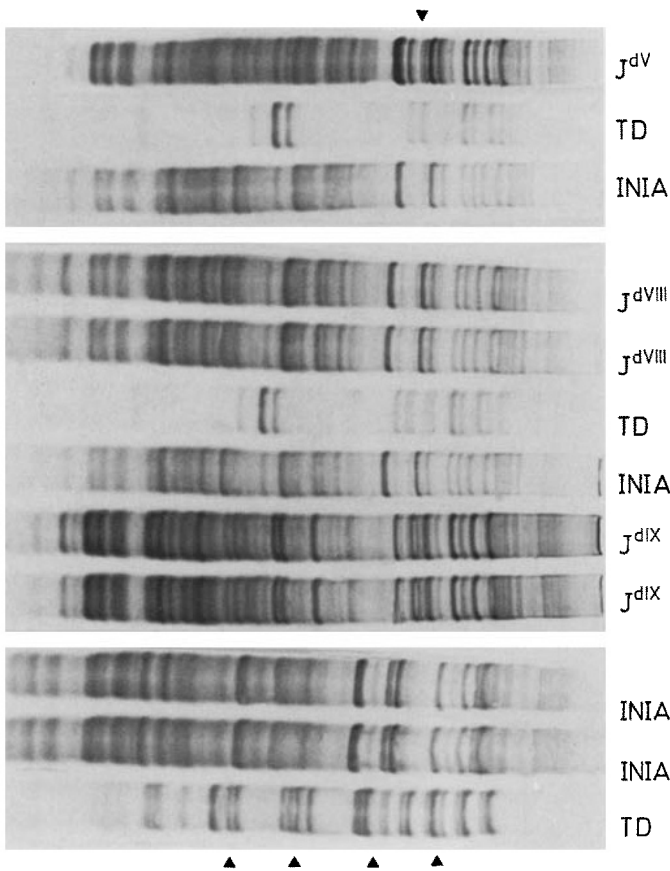


Fig. 10 PAGE of wheat *Th. distichum* addition lines J^{dV} and J^{dIX} with banding patterns differing from Inia 66, J^{dVIII} identical to Inia 66, and polymorphism in *Th. distichum* (TD).

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