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Thinopyrum distichum chromosome morphology and C-band distribution

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Abstract *Thinopyrum distichum* is indigenous to the southern and south western coastal shores of South Africa. Like many of the *Thinopyrum* species it can be hybridized with wheat. The resulting progeny treated with colchicine produce fertile amphiploids. The need to distinguish the *Th. distichum* chromosomes from one another and from those of wheat prompted the investigation of the C-band distribution. The chromosome pairs of *Th. distichum* were distiguishable from each other and from those of wheat using C-band patterns, morphology and size as identification criteria. The chromosomes ranged from heterobrachial to metacentric with interstitial and telomeric C-bands. The C-band patterns of *Th. distichum* were similar, but not identical, to those of other *Thinopyrum* species.

Key words Coastal wheatgrass · Genome relatedness Heterochromatin distribution · C-band polymorphism

Introduction

Thinopyrum distichum (Thunb.) Löve, or coastal wheatgrass, is indigenous to the Cape Province, South Africa. It grows on the sand dunes of the south-western, southern and south-eastern sea shores, often in the zone of constant sea spray. It can be used as a stock grass, and is also used as a sand binder in reclamation work. It is a littoral perennial and appears to be largely self-pollinating, although it also reproduces vegetatively by rhizomatous off-shoots (Pienaar 1981). Pienaar (1981, 1983) found *Th. distichum* to be a segmental allotetraploid with genomes unlike those of wheat. Meiotic investigations concluded that the genomes of *Th. distichum* ($J^d_1J^d_1J^d_2J^d_2$) were distinct from, but closely related to, those of *Thinopyrum elongatum* (J^eJ^e) and *Thinopyrum junceiforme* ($J_1J_1J_2J_2$) (Pienaar et al. 1988).

A conventional karyotype analysis of *Th. distichum* (Pienaar 1981) placed the 28 chromosomes in seven groups of four. In each group of four chromosomes the pair-wise correlation was good, but there were small differences in length, arm ratio and centromeric index between the pairs.

Th. distichum was hybridized with *Triticum turgidum L.* cv group durum and *Triticum aestivum* L. em Thell cv group aestivum to produce sterile hybrids (Pienaar 1981, 1983). Colchicine treatment resulted in fertile amphiploids, which when backcrossed to *Triticum* produced partial amphiploids and many other aneuploid derivatives.

Dewey (1960) tested the salt tolerance of *Th. distichum* under field conditions, and found this species to be fairly salt tolerant. Under greenhouse conditions *Th. distichum* was found to be as salt tolerant as *Th. elongatum* (Littlejohn 1988). However, none of the amphiploids and backcross derivatives with partial *Th. distichum* genomes were more salt tolerant than the control wheat cultivars. One explanation was the possible difference in chromosome composition of the derivatives. The need to determine the chromosome constitution of the derivatives led to the investigation of the morphology and the C-band patterns of *Th. distichum* chromosomes reported here.

Materials and methods

Roots from shoot cuttings of *Th. distichum* collected near East London and roots from seedlings of *Th. distichum* collected from Strand were used for C-band pattern and karyotype analysis. The two samples differ morphologically in their natural habitat and the differences were expressed under greenhouse conditions. The wheat cultivar Inia 66 was used for a comparative wheat C-band karyotype. The C-band staining technique used was as follows:

Pre-fixation:	2 °C in H_2O for 24–26 h.
Fixation:	45% acetic acid for 2–20 h, or 3:1 ethanol-acetic acid for 1–5 days.

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Hydrolysis: 1NHCl at 60 °C for 5 min.
Maceration: 5% pectinase (Spark L pc) for 30–50 min at 40 °C.

Slide preparation by squashing.
Dehydration: absolute ethanol 1 h followed by dessication overnight.
$Ba(OH)_2$: saturated $Ba(OH)_2$ for 5 min at 20°–30 °C.
2×SSC: 15 min 2×SSC at room temperature, 80–90 min 2× SSC at 52 °C, starting at room temperature.
Staining: Leishman's Stain in phosphate buffer, 0.01 M, in a ratio of 1:5 respectively for 30–240 min.
Mounting: the dry slides were immersed in xylene and mounted in D.P.X.

The permanent slides were viewed at 1 000× magnification and photographed with a Zeiss Axioscope. Kodak Technical Pan film was used to photograph the spreads at 500× magnification. The short and long arm lengths of the chromosomes were measured on photomicrographs using a digital Vernier rule. The measurements from 12 whole cells were used to calculate the relative chromosome lengths. The relative length of the chromosomes was determined as chromosome length expressed as a percentage of the total haploid autosomal length. Measurements of 20 chromosomes, not necessarily in the same cells, were used to determine the arm ratio and centromeric index. The arm ratio was calculated as long arm length divided by short arm length, expressed as a percentage. The centromeric index was calculated as short arm length divided by whole chromosome length also expressed as a percentage.

Results

It was possible to group the 28 chromosomes of *Th. distichum* into 14 pairs on the basis of their C-band patterns. In all 14 pairs of chromosomes at least one C-band could be distiguished in each chromosome arm (Fig. 1). The karyotypes of the two accessions of *Th. distichum* were almost identical, any C-band polymorphism detected being a product of the staining technique and not of intrinsic differences between the genotypes. The chromosomes in the karyotype are numbered in Roman numerals from I to XIV, in order of descending relative length (Fig. 2). Chromosomes IV and VII are satellited. In some C-banded cells secondary constrictions or satellites were observed on chromosomes I and VI (Fig. 2).

The chromosomes ranged in length from approximately 10 μm–13 μm. The individual chromosome arm ratios ranged from 1.03–1.99 and the centromeric indices from 34–49. The mean of each criterion calculated for each of the 14 chromosomes is given in Table 1. The coefficient of variation for each relative length is also given. The low coefficients of variation for repeated measures of relative length in this study indicate that the measuring technique was accurate. An analysis of variance was performed on the relative lengths, arm ratios, and centromeric indices. The F-values for the between-chromosome measurements and the within-chromosome measurements in all three analyses indicate significant difference between chromosomes, but no significance between measurements of the same chromosome in different cells (Table 2). An idiogram, based on the relative length and arm ratio of each chromosome and relative positions of major C-bands, was drawn (Fig. 3). The 95% confidence intervals for relative

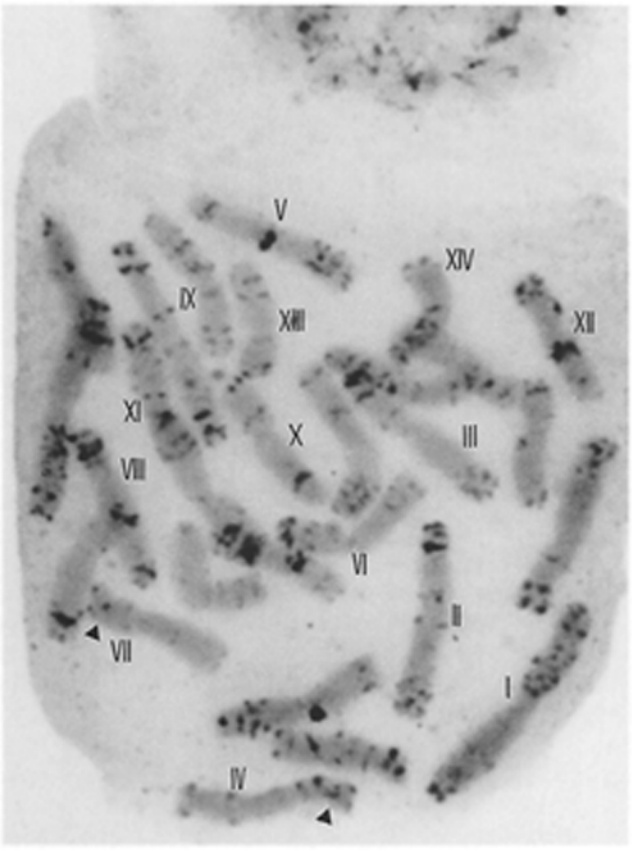

Fig. 1 Photomicrograph of *Thinopyrum distichum*, single cell with C-banded chromosomes numbered in Roman numerals from largest to smallest; ▶ = satellite

Table 1 The mean relative chromosome length, arm ratio, and centromeric index of *Th. distichum* chromosomes measured with a digital Vernier rule on photomicrographs. The coefficient of variation (CV) of the relative length of each of the 14 chromosomes is given. The number in brackets is the number of observations per chromosome

Chromosome number	Relative) length (12)	CV (%)	Arm ratio (20)	Centromeric index (20)
I	9.60	8.61	1.07	48
II	8.90	6.20	1.13	47
III	8.21	8.91	1.55	39
IV	7.96	7.55	1.65	38
V	7.66	8.16	1.03	49
VI	7.46	7.15	1.38	42
VII	7.01	10.89	1.57	39
VIII	6.75	7.03	1.33	43
IX	6.66	7.05	1.06	49
X	6.51	6.78	1.55	39
XI	6.15	6.15	1.99	34
XII	5.99	10.19	1.56	39
XIII	5.70	8.91	1.77	36
XIV	5.54	5.39	1.29	44

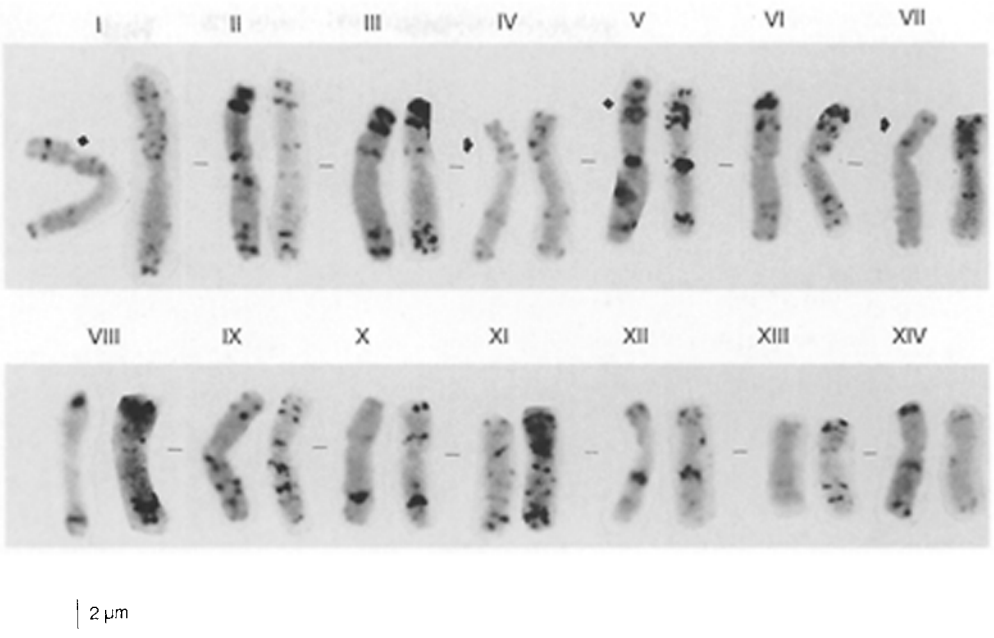

Fig. 2 Karyotype of *Th. distichum* arranged and numbered in descending order using Roman numerals. Each karyotype was cut out from the photomicrograph of a single cell, thus the size of the chromosomes is relative. The left chromosome is representative of the Strand population and the right of the East London population

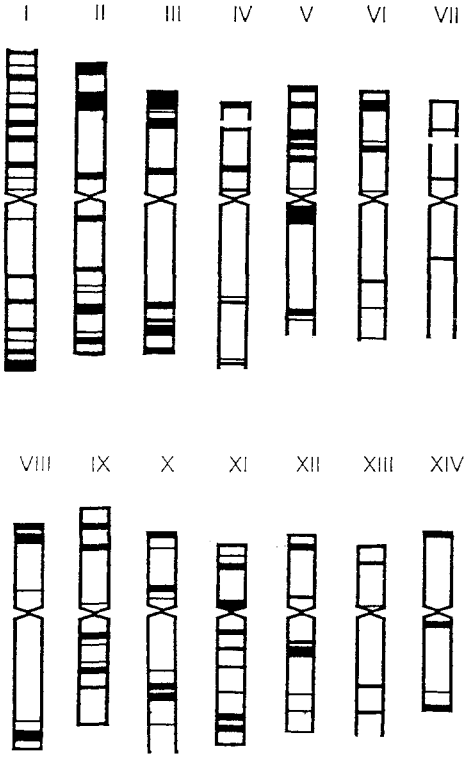

Fig. 3 Idiogram of *Th. distichum* C-banded chromosomes

length, arm ratio and centromeric index of each chromosome are indicated in Figs. 4, 5 and 6.

The standard Chinese Spring karyotype (Gill 1987; Gill et al. 1991) was used as a reference karyotype to identify the 'Inia 66' chromosomes (Fig. 7). All the chromosomes of 'Inia 66' were identifiable. Each of the chromosomes of *Th. distichum* could be differentiated from those of 'Inia 66'.

Table 2 The F-ratios for sources of variation for (a) relative chromosome length, (b) arm ratio and (c) centromeric index

Source of variation	F-ratio	Significance level
(a) Between cells	0.0310	1.0000
Between chromosomes	49.137	0.0000
(b) Between cells	0.6560	0.8598
Between chromosomes	41.778	0.0000
(c) Between cells	0.570	0.9250
Between chromosomes	49.137	0.0000

Discussion

Karyotype analyses of various *Thinopyrum* species reveal that the chromosomes vary in length from 7 μm–10 μm in conventionally (Feulgen or aceto-carmine)-stained preparations (Cauderon and Saigne 1961; Heneen and Runemark 1972; Dvorak and Knott 1974; Pienaar 1981; Moustakas and Coucoli 1982; Dvorak et al. 1984; Wang 1985; Hsaio et al. 1986). In the diploids, three chromosomes are metacentric or near metacentric (arm ratio 1.0–1.3), three pairs are sub-metacentric (arm ratio 1.3–1.6), and one pair is heterobrachial (arm ratio 1.7–2.0). Generally, two chromosome pairs have satellites, those on one pair being much shorter. Although the karyotypes of the *Thinopyrum* species appear very similar the significant differences in up-to-four out of seven chromosomes indicate structural rearrangements, especially segmental interchanges (Pienaar 1990).

Pienaar (1981) arranged the chromosomes of *Th. distichum* into seven groups of four on the basis of chromosome size and morphology. Of the 14 pairs, only two showed satellites. One pair of satellited chromosomes showed a much shorter satellite than the other. This was

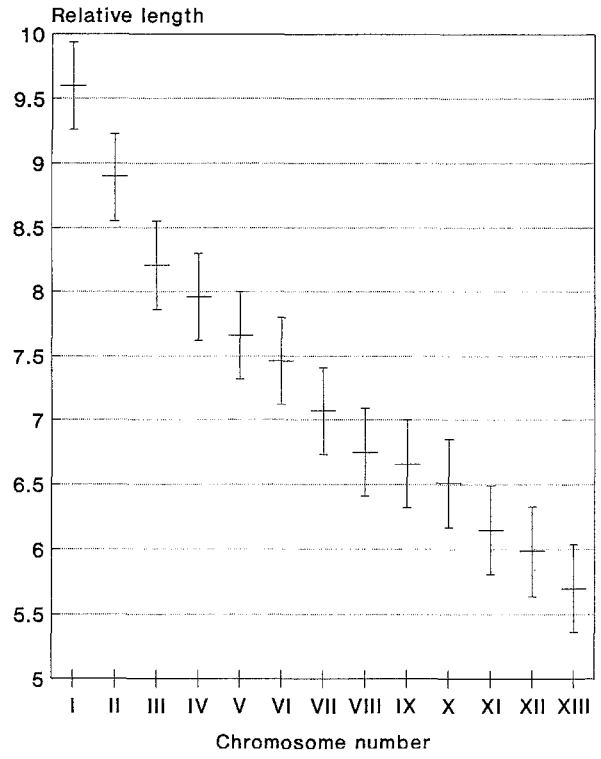

Fig. 4 The 95% confidence intervals for the measurements of relative chromosome length of each of the 14 chromosomes of *Th. distichum*

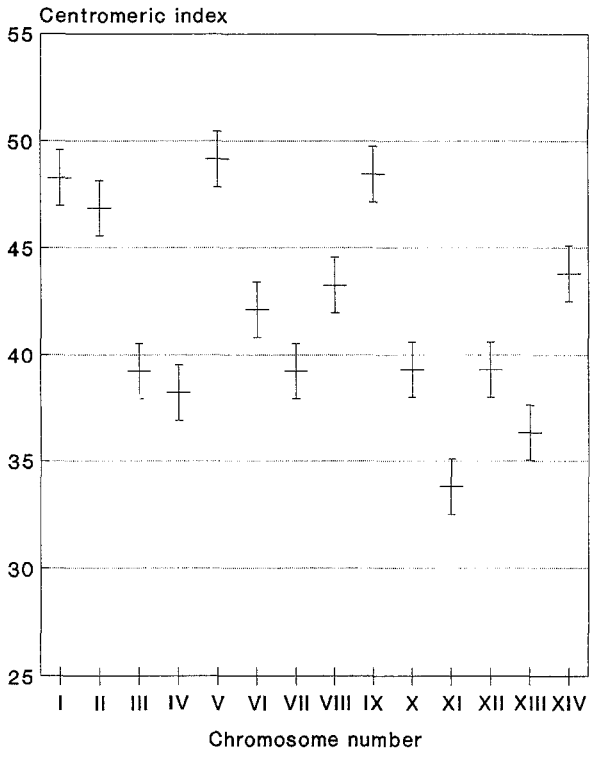

Fig. 6 The 95% confidence intervals for the measurements of centromeric index of each of the 14 chromosomes of *Th. distichum*

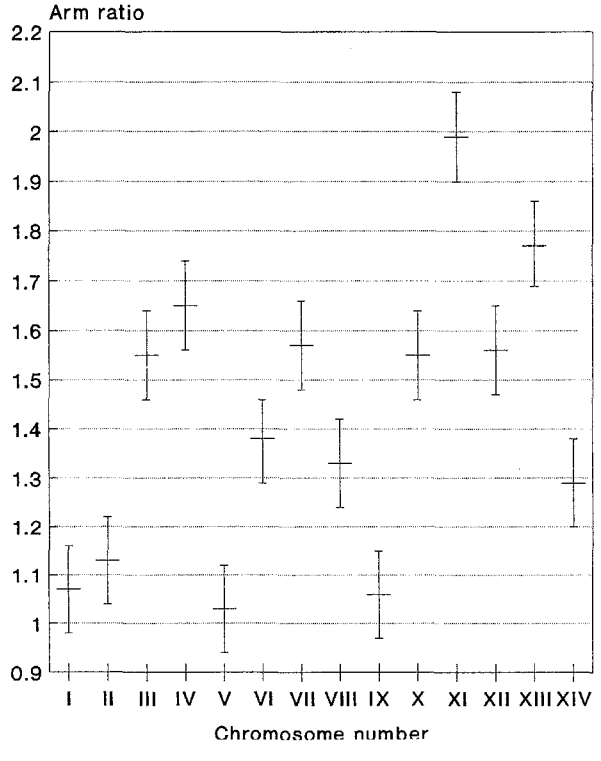

Fig. 5 The 95% confidence intervals for the measurements of arm ratio of each of the 14 chromosomes of *Th. distichum*

confirmed in the C-banded chromosomes. Grouping of the C-banded chromosomes according to arm ratio into the three groups, metacentric, sub-metacentric and heterobrachial, and then pairing according to most similar arm ratio, did not show clear patterns of C-band relatedness between chromosomes. Pairing according to similar C-band patterns resulted in pairs which differed in arm ratio. Clearly *Th. distichum* is not autotetraploid. The karyotype can be grouped into two putative 'genomes', one which consists of chromosomes with many interstitial less-prominent bands (I, IV, VI, VII, IX, XIII XIV) and the other with more-prominent telomeric and interstitial C-bands (II, III, V, VIII, X, XI, XII). In any of the above groupings the satellited chromosomes do not appear to be homoeologous (Pienaar 1981), but rather form two non-homologous chromosomes (Armstrong et al. 1991).

Armstrong et al. (1991) identified two satellited chromosomes of *Th. distichum* by C-banding, silver staining and in-situ hybridisation with the rRNA vector pTa 71. Pienaar (1990) postulated that two nucleolar organizer regions (NORs) could be suppressed in tetraploid *Th. distichum*, as two satellites are observed in closely-related diploid *Thinopyrum* species. In-situ hybridization should reveal all NORs, irrespective of activity (Armstrong et al. 1991). Therefore, tetraploid *Th. distichum* has only two identifiable NORs. The secondary constrictions observed on chromosomes I and IV then do not have NORs. Grouping of

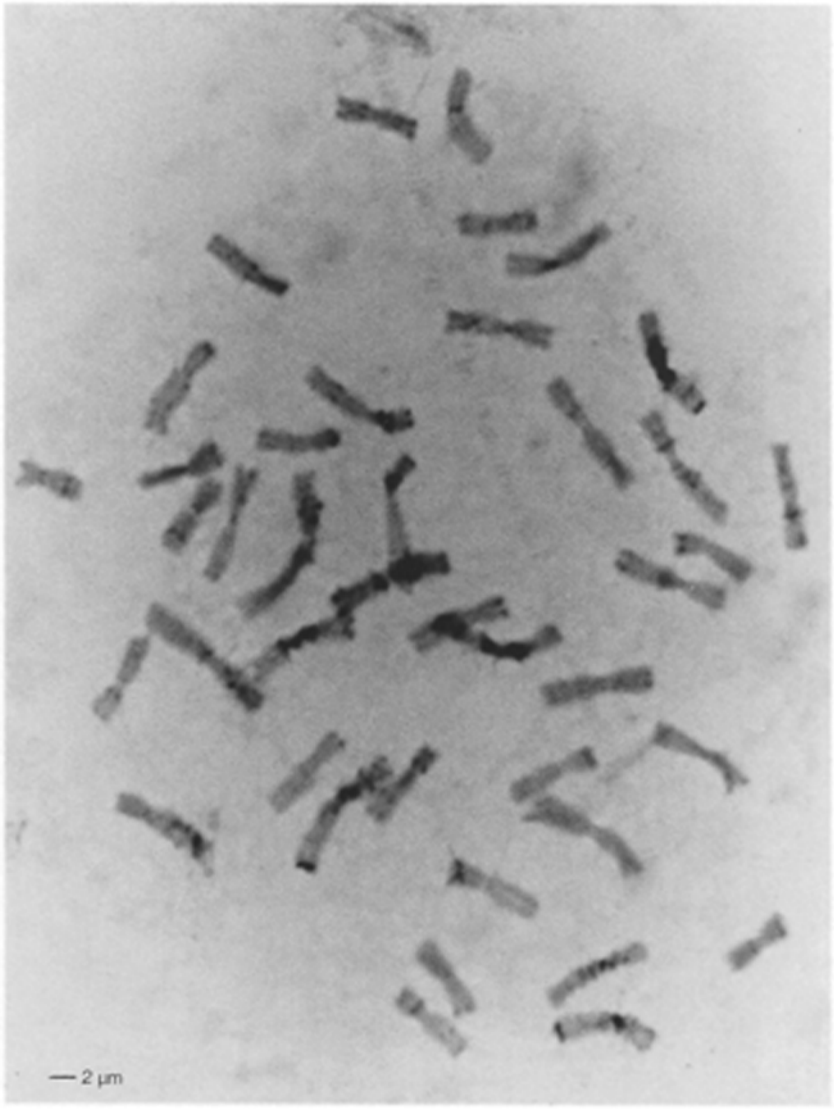

Fig. 7 The C-band patterns of Inia 66 chromosomes

the chromosomes according to similar type of C-banding pattern into two 'genomes' results in one genome not contributing any NOR. Suppression of the NORs is common in interspecific hybrids in the Triticeae (Appels et al. 1986; Friebe et al. 1987; Armstrong et al. 1991). It is not uncommon in polyploid Triticeae that one genome lacks an NOR (Flavell and O'Dell 1976; Dvorak and Appels 1982). Dominance of NORs is associated with a change in the gene structure (Flavell et al. 1988); therefore, it is concievable that, during the evolution of the tetraploid, *Th. distichum*, suppressed NORs became totally redundant and changed in structure, so that only those NORs contributed by one genome are now present. Armstrong et al. (1991) called the two satellited chromosomes 5E and 6E because of C-band similarity with the corresponding chromosomes of *Th. elongatum*. This is contrary to the nomenclature for *Th. distichum* chromosomes proposed by Pienaar et al. (1988), namely J_1^d and J_2^d for the two similar, but not identical, genomes of *Th. distichum*.

The morphological measurements on C-banded *Th. distichum* chromosomes could not always be correlated with those made on Feulgen-stained chromosomes (Pienaar 1981). This is probably due to the greater accuracy of identification of C-banded chromosomes, which distinguishes more easily between chromosomes with similar length and arm ratio. C-banded chromosomes are, however, more difficult to measure and the outline of the chromosome and the centromere is often unclear. Endo and Gill (1984) found that chromosomes can change length during the C-banding process. Possibly the C-banding technique differentially changes the length of the chromosome arms, resulting in altered arm ratios. The change in chromosome length could also explain the longer absolute chromosome lengths observed compared to other *Thinopyrum* species measured after conventional staining (Cauderon and Saigne 1961; Heneen and Runemark 1972; Dvorak and Knott 1974; Pienaar 1981; Moustakas and Coucoli 1982; Dvorak et al. 1984; Wang 1985; Hsaio et al. 1986).

Although C-banding has been used to determine genome relatedness between many Triticeae, the results have often been inconsistent (Gill and Kimber 1974; Gerlach 1977; Iordansky et al. 1978; Gill 1981, Teoh and Hutchin-

Fig. 8 The spike differences between the East London and the Strand accessions of *Th. distichum*

son 1983; Endo and Gill 1984). Some inconsistencies in banded karyotypes and evolutionary inferences drawn from the studies probably stem from intra- and inter-specific banding polymorphism, and to the non-standardized banding techniques used in different laboratories (Gill 1987; Gill et al. 1991). Gill (1981) reported *Thinopyrum bessarabicum* as having prominent terminal C-bands, and inferred that, at the generic level, *Th. bessarabicum* was more-closely related to rye than *Triticum*. DNA analysis indicated that *Thinopyrum* species are as distantly related to rye as to wheat (Dvorak and Chetgen 1973). Endo and Gill (1984) compared the C-band patterns of *Th. elongatum* (J^e-genome) and *Th. bessarabicum* (J-genome) chromosomes. They found that the C-band patterns were not completely equivalent. The longest chromosome of both karyotypes was very similar, showing a large satellite. The second largest chromosomes were also satellited, but differed in band pattern as well as in the size of the satellite. In general, the chromosomes of *Th. bessarabicum* showed prominent terminal C-bands, while *Th. elongatum* showed a greater number of interstitial bands. It was concluded that the C-band patterns were not similar enough to support the theory of the homology of the J and J^e genomes (Dvorak 1981; Dewey 1984; Wang 1985, 1989, Wang and Hsaio 1989), but rather that J^e and J are two distinct homoeologous genomes (Jauhar 1988 a,b), warranting classification in genera with different genome symbols (Jauhar 1990).

Intraspecific banding polymorphism between subspecies within a species (Teoh et al. 1983) and between different species of a genus sharing a common genome (Singh and Robbelen 1975; Zurabishvili et al. 1978; Endo and Gill 1984) have been described. Therefore, inferences about genome relatedness cannot be considered without cross-referencing with other studies determining genome relatedness. Hybrids between *Th. distichum* and *Th. elongatum* can be obtained fairly easily (Pienaar et al. 1988), substantiating their close relationship. Hybrids between *Th. distichum* and *Th. bessarabicum* have not yet been obtained, despite repeated attempts. If the chromosomes of *Th. distichum* are grouped according to the type of C-banding pattern, either as many interstitial less prominent bands (I, IV, VI, VII, IX, XIII, XIV) or as prominent terminal and interstitial C-bands (II, III, V, VIII, X, XI, XII), it can be postulated that *Th. distichum* is an allotetraploid between J and J^e genome predecessors. Segmental interchange can account for the presence of major interstitial bands which are not found in *Th. bessarabicum*. Although little C-band polymorphism was detected in *Th. distichum*, the extent of C-banding polymorphism within other *Thinopyrum* species is not known. The segmental allotetraploid nature determined from observations of meiosis of hybrids of *Th. distichum* with wheat indicate close homoeology between the J_1^d and J_2^d genomes (Pienaar 1981, 1983; Pienaar et al. 1988). The J and J^e genomes are known to be closely related (Dvorak 1981; Dewey 1984; Wang 1985, 1989; Wang and Hsaio 1989), substantiating the origin, as indicated by chromosome C-banding, of the *Th. distichum* genomes.

Although there was some polymorphism between the C-band patterns of the two *Th. distichum* accessions, it did not reduce the ability to identify the individual chromosome pairs within cells, or to distinguish homologues between the different plants and accessions. The polymorphism probably originates in differential staining during the preparation of the C-band slides, rather than in intrinsic differences between the chromosomes. The small amount of C-band polymorphism observed is consistent with the self-pollinating characteristic of the species (Endo and Gill 1984). The difference in morphology (Fig. 8) observed in the two accessions was not portayed in differences in C-band karyotype.

The karyotype can now be used to identify *Th. distichum* chromosomes in a wheat background, or to identify large chromosome exchanges between wheat and *Th. distichum* in the derivatives of hybrids between wheat and *Th. distichum*.

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