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The assignment of a *Thinopyrum distichum* (Thunb.) Löve-derived translocation to the long arm of wheat chromosome *7D* using endopeptidase polymorphisms

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Summary. Endopeptidase zymograms of the translocation line 'Indis' revealed the presence of several major and minor bands that had differential expression in coleoptile and seed tissues. While 'Indis' lacks *Ep-D1a*, which is present in the parental cultivar 'Inia 66', it also may not express any of the *Th. distichum* bands. The 'Indis' zymogram was found to be identical to that of an isogenic line of 'Inia 66' possessing *Lr19*. Since the absence of an *Ep-D1a* product appears to be linked to the *7DL* translocation, it is possible to use the null condition as a marker for both the *Lr19* or 'Indis' translocations. The 'Indis' translocation also did not show recombination with the *cn-D1* chlorophyl mutant on *7DL*, confirming that a part of *7D* was involved. The results of a telocentric mapping experiment involving the *7D* telosomes indicated that in 'Indis' a chromosome segment from *Th. distichum* replaced a large section of *7DL* of 'Inia 66'.

Key words: Endopeptidase zymogram – Leaf and stem rust resistance – Telocentric mapping

Introduction

Marais et al. (1988) reported resistance to leaf and stem rust of wheat in 'Indis', a selection from the BC_2F_3 progeny following backcrossing of a primary 'Inia 66/*Th. distichum*' amphiploid to 'Inia 66'. This provisional study indicated that a *Th. distichum* chromosome segment apparently spontaneously translocated to chromosome *7D* of 'Inia 66'. The translocation chromosome behaved fairly normally during meiosis in heterozygotes. The foreign segment may be homoeologous to the *Th. ponticum*-derived translocation *Lr19/Sr25* on chromosome *7DL* of 'Agatha', described by Dvořák and Knott (1977). In 'Agatha' a gene(s) for yellow endosperm colour is probably located distal to the closely linked *Lr19* locus (Knott 1980). 'Indis' likewise has yellow-pigmented endosperm (Marais et al. 1988) and, compared to 'Inia 66', yielded more grain, yet had reduced 1000 kernel weight, flour yield, flour protein content and mixograph mixing time. At least some of these defects may relate to the late maturity and high fertility of 'Indis' that result in it being ill-adapted to the short and relatively dry growing seasons encountered locally.

The isozyme locus *Ep-D1* and the chlorina mutant *cn-D1* are useful markers on chromosome *7DL* (McIntosh 1988). Hart and Langston (1977) described four endopeptidase bands in the zymogram of 'Chinese Spring' following starch gel electrophoresis of extracts of etiolated 7-day-old seedlings. The genes coding for the isozymes in order of increasing mobility were assigned to chromosomes *7DL, 7AL, 7BL* and *7BL*, respectively. The fastest migrating isozyme did not occur in scutellar extracts and probably belonged to a different homoeoallelic group or is coded by a diverged duplicate of the scutellar gene on *7BL*. McMillin and Tuleen (1977) reported three structural endopeptidase genes on chromosome *7AL* and two on chromosome *7BL* of the wheat 'PI 357307'. Koebner et al. (1988) separated endopeptidases extracted from embryo halves of mature seeds and found two bands (EP-D1a and EP-B1a) in 'Chinese Spring'. A third band, corresponding in position to the *7AL* band of Hart and Langston (1977), occurred only in some 'Chinese Spring' plants. A study of other hexaploid wheats revealed four more *Ep-B1* alleles and two more *Ep-A1* alleles, one of which was a null allele. The only variation found at the *Ep-D1* locus was contributed by the *Aegilops ventricosa*-derived translocation in 'Rendezvous' and a null allele proposed to exist in 'Synthetic'.

The purpose of the present study was to determine the chromosome arm location of the *Th. distichum* segment in 'Indis' by studying its recombination with the centromere, as suggested by Sears (1962), as well as its position relative to the *Cn-D1* and *Ep-D1* loci. The endopeptidase variation observed in 'Indis' was also investigated with the objective of finding a biochemical marker to distinguish it from the *Lr19/Sr25* translocation and eventually to identify the donor chromosome in *Th. distichum*.

Materials and methods

The wheat material used for the endopeptidase zymogram studies included 'Indis', its progenitors ('Inia 66', *Th. distichum* and their amphiploid) and, for the purpose of reference, 'Chinese Spring' and some of the wheats studied by Koebner et al. (1988). Also included were a number of segregating populations and 'A2558', an isogenic line of 'Inia 66' and 'Indis' that carries *Lr19/Sr25*. 'A2558', produced by and obtained from Dr. B. Lombard (Sensako, Welgevallen Experimental Farm, Stellenbosch), has the pedigree 'Inia 66 * 6/Agatha' and differs morphologically from 'Indis'. Compared to 'Indis', the spikes of 'A2558' had long internodes, fewer spikelets per spike and less florets per spikelet. The 'A2558' plants also matured earlier than the 'Indis' plants.

Extracts for endopeptidase zymogram comparisons were based on kernels or etiolated seedlings. The extracts from immature kernels were prepared by macerating the total kernel in water or buffer. Either embryo or endosperm sections were cut from mature kernels. One to three embryo sections were incubated at room temperature for 2–14 h in 120 µl of distilled water. Endosperm sections were easier to macerate if cut from kernels germinated for about 4 days in the dark at 9 °C. To obtain seedling extracts, samples were planted in pots in a greenhouse (18 °/12 °C). An empty pot was placed upside down over the planted pot in a shaded area. After 13–14 days, samples were cut from the etiolated seedlings. Seedling extracts were obtained by macerating 50–70 mg of leaf pieces in 50 µl of the extraction buffer described by Tang and Hart (1975). Following centrifugation at 13,000 *g* for 20 min, 30 µl of the supernatant was loaded directly onto the gel surface (cathodal end). Gels (150 mm × 125 mm × 0.25 mm) were prepared as described by Koebner et al. (1988), except that the ampholyte mixture was Pharmalyte 4.2–4.9 (2 parts) and Pharmalyte 4–6.5 (1 part). Electrophoresis was carried out at 4 °C using a Hoefer Isobox unit. Gels were prefocused for 30 min at 13 W and run for 3 more h at the same setting. Staining was done as outlined by Tang and Hart (1975). Stained gels were washed from the support plates with water, spread onto white paper sheets and air dried.

The chromosome arm location and recombination between the translocation point and the centromere were studied as suggested by Sears (1962). A 'Chinese Spring' ditelocentric *7DS* line (obtained from E. R. Sears, Department of Agronomy, University of Missouri, Columbia) was pollinated with 'Indis'. Monotelodisomic F_1 plants were selected from the progeny and used to pollinate 'Chinese Spring'. F_1 seeds were germinated to count the chromosomes in their root tips and for leaf rust seedling tests. To obtain the *7DL* stock, 'Indis' was used to pollinate a F_1 plant that was suspected to be double monotelosomic, derived from the cross 'Inia 66 mono *7D*/Chinese Spring ditelosomic *7DL* monotelosomic *7DS*'. Four monotelodisomic plants were identified and crossed as male parents onto 'Chinese Spring'. Spikes on each male parent were bagged to provide kernels for progeny testing. Since 'Indis' does not produce EP-D1a, plants carrying *7DS* do not produce progeny having this band; two such plants were found among the four monotelodisomic plants. The remaining two plants each produced three progeny having the EP-D1a band and five without this band. The testcross-derived kernels involving the latter two plants were subsequently used.

Results and discussion

Figure 1 is a diagrammatic representation of the bands encountered in 'Indis', 'Chinese Spring' and the segregating populations derived from their hybrid, and shows their relationship to the bands named by Koebner et al. (1988). Bands 1–9 and 11 were present in the leaf extracts, but only bands 4–9 and 11 appeared at the same intensities in kernel extracts. The differential expression of the kernel and seedling genes is also reflected in Figs. 2 and 3. Band 10 was only seen in kernel extracts. The banding patterns of developing kernels (milk stage) and embryos or endosperms of either mature dry kernels or germinating kernels were virtually identical. The only exception was the faint band in position 10, occasionally

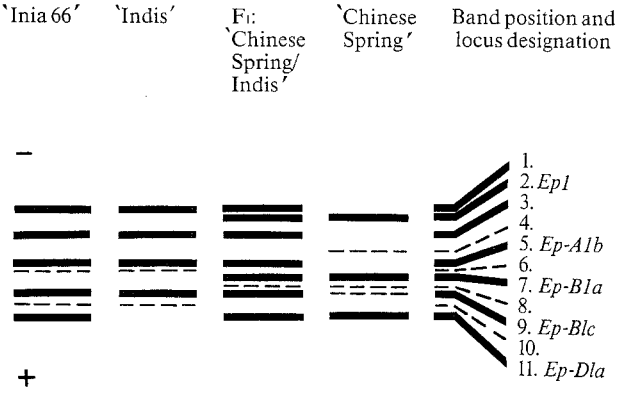

Fig. 1. Relative positions of endopeptidases encountered in the segregation studies

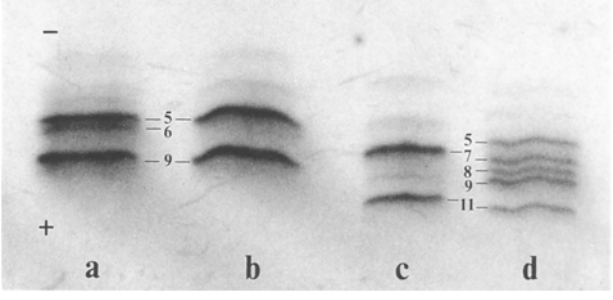

Fig. 2 a–d. Expression of endopeptidases in embryos of **a** 'Indis'; **b** 'A2558'; **c** 'Chinese Spring'; **d** the F_1 'Chinese Spring/Indis'

visible in embryo extracts of 'Inia 66', 'Indis' and 'A2558', and more regularly in endosperm extracts. Whether this is a member of a further group of tissue-specific isozymes remains to be determined.

Depending on the quality of the extracts, very faint bands could at times be seen in positions 1–3 of kernel extracts of genotypes displaying the corresponding bands in seedling extracts. These bands were slightly more pronounced in endosperm than in embryo-derived extracts. This would hint at a low level of production of the corresponding seedling isozymes in kernel tissues. The intensity of the bands did not increase in embryo or endosperm tissues obtained from whole kernels germinated for up to 10 days at 9 °C. Occasionally very faint bands were also discernible at positions 4 and 9 in leaf or kernel extracts of 'Chinese Spring'. Whether these relate to any of the null alleles suggested to exist in 'Chinese Spring' by Koebner et al. (1988) or to the results of the present study remains to be determined. A 'Chinese Spring' seedling was encountered in which bands 4 and 9 were expressed at the same intensities as the regularly occurring bands. Also, in some extracts of 'Chinese Spring ditelosomic *7DS*' seedlings, band 4 was expressed at a high level. This observation suggests that the null condition may in some instances be brought about by regulatory genes rather than being due to defective structural genes. Position 2 corresponds to endopeptidase 4 (EP-4) of Hart and Langston (1977). The isozymes occupying positions 7 and 11 were also described by the latter authors, and were renamed EP-B1a and EP-D1a, respectively, by Koebner et al. (1988). Zymograms of F_2 embryos from the cross 'Chinese Spring/Inia 66' confirmed that the 'Inia 66' band in position 11 was EP-D1a. Comparison of embryo extracts of 'Inia 66', 'Capelle-Desprez' and 'Ciano 67' suggested that the bands in positions 5 and 9 are EP-A1b and EP-B1c, also described by the latter authors. Two minor bands appeared at positions 6 and 8 of some plants of 'Inia 66' and 'Chinese Spring', respectively. The band at position 8 was also described by Hart and Langston (1977) and associated with chromosome *7A*. Koebner et al. (1988) referred to the band as EP-A1d? since they could not unambiguously associate it with chromosome *7A* of 'Chinese Spring'. The 'Inia 66' band at position 6 could occasionally be seen in extracts of 'Indis'. Band 6 appeared irregularly in extracts of the progeny of a single isolated 'Inia 66' plant as well as the relatively pure breeding 'Indis'. Thus, it is possible that this band is not always expressed. Due to their erratic incidence and the difficulty in consistently detecting the low levels of expression of the minor bands (6 and 8), no further attempts were made to describe them.

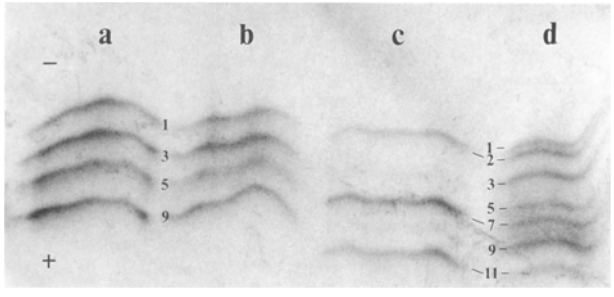

Fig. 3a–d. Seedling-expressed endopeptidases of **a** 'Indis'; **b** 'A2558'; **c** 'Chinese Spring'; **d** the F_1 'Chinese Spring/Indis'

In order to determine whether any of the major bands of 'Indis' are coded for by genes on chromosome *7D*, three F_2 and one testcross population were analyzed for their endopeptidase variation. The F_2 results are listed in Table 1. Bands 1 and 5 ('Indis') appeared to have corresponding 'null' bands in 'Chinese Spring' and were associated with chromosome *7A*. Segregation for the presence : absence of bands 1 and 5 (in populations disomic

Table 1. F_2 segregation for endopeptidase isozymes in crosses of 'Indis' (bands 1,3,5 and 9) and 'Chinese Spring' (bands 2 and 7)

F_2 population	A genome bands No. of individuals having endopeptidases		Chi-square[a]	Probability[a]	B genome bands No. of individuals having endopeptidases				Chi-square[a]	Probability[a]
	1,5	–,–			2,7	2,3,7,9	3,9	–,–,–,–		
'Chinese Spring/Indis' (seedling extracts)	101	8	17.2	0.00	25	58	26	–	0.5	0.79
'Chinese Spring/Indis' (kernel extracts)[b]	52	18	0.0	≈1.00	17	38	15	–	1.6	0.20
'Chinese Spring mono *7A*/Indis'[c]	75	0	23.7	0.00	16	40	19	–	0.6	0.75
'Chinese Spring mono *7B*/Indis'[c]	37	13	0.0	≈1.00	–	–	48	2	10.7	0.00

[a] For conformation to single factor segregation
[b] Not scored for the seedling bands 1, 2 and 3
[c] The progeny of monosomic F_1 plants

for chromosome *7A*) conformed to a 3:1 ratio, except among F_2 seedlings from the cross 'Chinese Spring/ Indis'. The reason for the latter inconsistency is not clear. It is unlikely to have resulted from a sampling error due to chance, however, it is possible that seedlings with the 'null' alleles germinated slower or had weaker growth, resulting in such plants not being sampled. The latter possibility was not tested. No recombinant types involving bands 1 and 5 and the null bands were observed.

Bands 3 and 9 of 'Indis' appeared to be allelic to bands 2 and 7 of 'Chinese Spring'. These bands were coded for by chromosome *7B* (Table 1) and were inherited as closely associated traits. No recombinant forms were observed and segregation in progenies disomic for chromosome *7B* conformed to a 1:2:1 ratio. The F_2 results were confirmed with a study of endopeptidase zymograms of 138 individuals from the testcross population 'Chinese Spring//Chinese Spring/Indis'. The male parent produced 71 gametes with the alleles for bands 2 and 7 ('Chinese Spring') and 67 gametes with the alleles for bands 3 and 9 ('Indis'). No recombinant-type gametes were formed. Sixty-one gametes possessing the alleles for bands 1 and 5, and 77 gametes with the corresponding 'null' alleles were found. Similar to the F_2 result, no recombinant-type gametes were evident. The results indicate either a close linkage of the genes coding for kernel- and seedling-expressed isozymes, or may imply that the seedling-expressed isozymes 1, 2 and 3 are respectively modified products of the genes coding for isozymes 5, 7 and 9. Since the status of the genes coding for bands 1, 2 and 3 is not clear, no gene symbols are proposed. Thus, the major bands of the 'Indis' zymogram (seedlings) include EP-A1b and the closely associated band 1, as well as EP-B1c and its closely associated band 3.

As is shown in Figs. 2 and 3, 'Indis' does not produce Ep-D1a, and its zymogram is identical to that of 'A2558'. *Th. distichum* seed extracts produced a sharp band at position 12 (Figs. 4 and 5) and a minor band at position 11. A third faint band that co-focuses with band 6 of 'Inia 66' appeared in seedling extracts, and it was not clear which is expressed in the amphiploid and 'Indis'. Band 12 could be seen in the primary 'Inia 66/ *Th. distichum*' amphiploid, while the lowest pI band probably focuses in the same position as EP-D1a, and is therefore not visible. Extracts based on 50:50 mixtures of *Th. distichum* and 'Inia 66' kernels confirmed this conclusion. The results strongly suggest that the 'Indis' translocation involves at least part of wheat chromosome *7DL*. If a homoeologous transfer occurred, as is suggested by the generally improved phenotype and stable breeding behaviour of 'Indis', then the translocated segment may lack the endopeptidase locus (loci), it may possess a null allele(s) or the gene(s) may be suppressed. Since it is not known whether the endopeptidase locus (loci) on *7DL* of 'Indis' is intact yet inactive, structurally

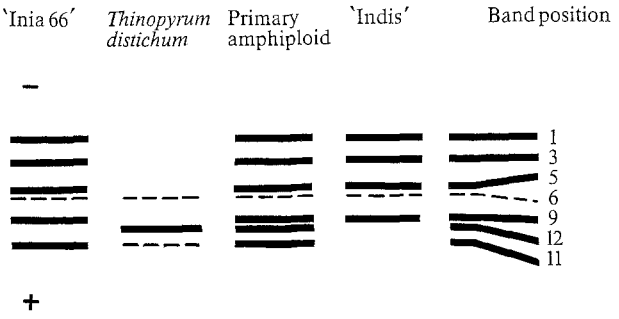

Fig. 4. Relative positions of the embryo and seedling-expressed endopeptidases of 'Indis' and its progenitors

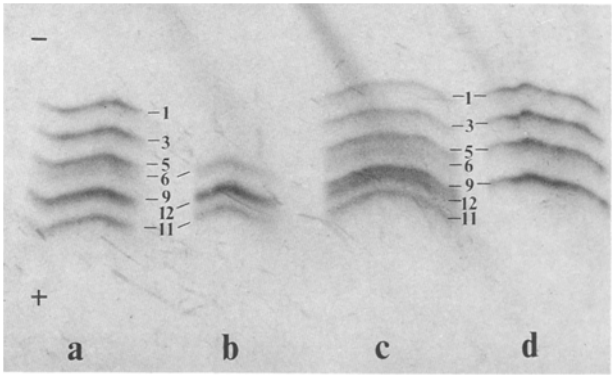

Fig. 5 a–d. Seedling-expressed endopeptidases of **a** 'Inia 66'; **b** *Th. distichum;* **c** the primary 'Inia 66/*Th. distichum*' amphiploid; **d** 'Indis'

altered or completely absent, it is not appropriate to assign the symbol *Ep-D1c,* as used to designate the null allele in 'Synthetic' (Koebner et al. 1988).

In order to confirm that the translocation is in fact associated with the absence of EP-D1a, the F_2 of the cross 'Indis/Chinese Spring' was analyzed for leaf rust resistance (race 3SA132) and endopeptidase phenotypes. Among 126 seedlings, 26 were susceptible and showed expression of *Ep-D1a,* 69 were resistant and expressed *Ep-D1a,* whereas the 31 plants showing absence of *Ep-D1a* products were all resistant. It seems possible, with a knowledge of the endopeptidase phenotype as well as the seedling leaf rust reaction, to classify single plants as homozygous or heterozygous for the translocation.

Recombination between the resistance gene and the chlorina mutant (*cn-D1*) was also studied. One-hundred-and-seventy-nine F_2 plants from the cross 'Indis/chlorina mutant' were inoculated with leaf rust race 3SA132. A ratio of 139 resistant plants with normal chlorophyll to 40 susceptible plants with the chlorina phenotype was observed. This would imply absolute linkage of the translocation with *cn-D1.*

Finally, use was made of telocentric mapping to confirm the translocation of the *Th. distichum* chromosome

Table 2. Chromosome numbers and seedling leaf rust resistances of the progeny obtained by pollinating 'Chinese Spring' with pollen from monotelodisomic plants. The pollen parents (2n = 40 + *t* + trans) possessed a single translocation chromosome in combination with either the normal *7DS* or *7DL* telosome

Telosome	No. of F_1 plants	Somatic chromosome number	Probable chromosome numbers of parental gametes		Seedling resistance (race 3SA132)
			Female	Male	
7DS	4	41	20	21	;
	124	42	21	21	;
	1	41 + *t*	20	21 + *t*	;
	1	42 + *t*	21	21 + *t*	;
	1	43	22	21	;
	7	41 + *t*	21	20 + *t*	4
	138				
7DL	111	42	21	21	;
	34	42 + *t*	21	21 + *t*	;
	2	43 + *t*	22	21 + *t*	;
	4	41	21	20	4
	11	41 + *t*	21	20 + *t*	4
	1	42 + *t*	22	20 + *t*	4
	163				

segment to *7DL* and to determine the distance of the translocation point from the centromere of *7DL*. Two F_1 populations were obtained by pollinating 'Chinese Spring' with pollen from monotelodisomic plants possessing one of the two *7D* telosomes, as well as the complete translocated chromosome. Root-tip chromosome counts and seedling leaf rust resistances were determined for the two groups. The relevant data are summarised in Table 2. Also given are the probable chromosome constitutions of the male and female gametes that gave rise to the respective F_1 groups. In deriving these, it was assumed that aneuploid male gametes were mostly inviable. From the implied genetic constitutions of the male gametes produced by the plants possessing *7DS*, it can be concluded that pairing of *7DS* and the translocation chromosome may have been fairly regular, resulting in only 1.4% of the pollen grains carrying both entities. This conclusion was confirmed by studying metaphase I in PMCs of the male parent. Pairing of all the chromosomes appeared to be normal. An unpaired telosome was seen in 4.4% of 68 cells. Two univalents occurred in 17.6% of the cells and are probably explained by the observation of R. de V. Pienaar (personal communication) that the 'Chinese Spring' and 'Inia 66' genomes may differ with regard to one or possibly two reciprocal translocations. When meiosis in the male parent having *7DL* was studied, 87% of the 54 PMCs contained the visibly unpaired telosome, while 25.9% of the cells contained more than one unpaired normal chromosome. The male parent possessing *7DL* produced a high proportion (22.1%) of pollen grains which carried both *7DL* and the translocation chromosome (Table 2). No recombination products of the translocated chromosome and the *7DL* telosome were evident. This is to be expected if the latter chromosomes often did not pair during meiosis, and would suggest that the translocation may have involved a large portion of *7DL*.

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References

Dvořák J, Knott DR (1977) Homoeologous chromatin exchange in a radiation-induced gene transfer. Can J Genet Cytol 19: 125–131

Hart GE, Langston PJ (1977) Chromosomal location and evolution of isozyme structural genes in hexaploid wheat. Heredity 39:263–277

Knott DR (1980) Mutation of a gene for yellow pigment linked to *Lr19* in wheat. Can J Genet Cytol 22:651–654

Koebner RMD, Miller TE, Snape JW, Law CN (1988) Wheat endopeptidase: genetic control, polymorphism, intrachromosomal gene location, and alien variation. Genome 30: 186–192

Marais GF, Roux HS, Pretorius ZA, Pienaar R de V (1988) Resistance to leaf rust of wheat derived from *Thinopyrum distichum* (Thunb.) Löve. In: Proc 7th Int Wheat Genet Symp, IPSR Cambridge, pp 369–373

McIntosh RA (1988) Catalogue of gene symbols for wheat. In: Proc 7th Int Wheat Genet Symp, IPSR Cambridge, pp 1225–1323

McMillin DE, Tuleen NA (1977) Determination of the linkage relationships and the gene-centromere genetic distances for endopeptidase structural genes in hexaploid wheat. Genetics 88 (Suppl):S44

Sears ER (1962) The use of telocentrics in linkage mapping. Genetics 47:983

Tang KS, Hart GE (1975) Use of isozymes as chromosome markers in wheat-rye addition lines and in triticale. Genet Res Camb 26:187–201