

## A universal reference karyotype in rye, *Secale cereale* L.

R. Schlegel<sup>1</sup>, G. Melz<sup>2</sup> and R. Nestrowicz<sup>2</sup>

<sup>1</sup> Zentralinstitut für Genetik und Kulturpflanzenforschung, Akademie der Wissenschaften der DDR, DDR-4325 Gatersleben, German Democratic Republic

<sup>2</sup> Institut für Pflanzenzüchtung, Akademie der Landwirtschaftswissenschaften der DDR, DDR-2601 Gülzow-Güstrow, German Democratic Republic

<sup>3</sup> Institut für Züchtungsforschung, Akademie der Landwirtschaftswissenschaften der DDR, DDR-4300 Quedlinburg, German Democratic Republic

Received March 24, 1987; Accepted April 21, 1987

Communicated by P. L. Pfahler

**Summary.** Haploid, dihaploid and tetrahaploid rye plants of the variety 'Petka' have been produced to establish a homozygous genotype and karyotype. The morphology, karyotypic characteristics and the metaphase I chromosome behaviour are described demonstrating the utility of the material as a universal standard tester in rye cytogenetics. Clones of several ploidy levels are maintained by long-term in vitro sprout culture. They are available for international testing. Sexual propagation of the clones is possible using a heat treatment procedure to break the self-incompatibility of the homozygotes.

**Key words:** Haploids – Dihaploids – Standard karyotype – Meiotic configuration – Rye

### Introduction

In the past, several attempts have been made to establish a common chromosome designation, a general karyological characterization and a standard karyotype in diploid rye (Heneen 1962; Gupta 1971; Schlegel and Mettin 1982). All efforts, however, did not completely account for the natural variation of chromosome morphology and structural heterozygosity in allogamous rye populations. To overcome the difficulties at least partly, the participants of the first and second Workshops on Rye Chromosome Nomenclature and Homoeology Relationships (see Sybenga 1983) decided to consider the 'Imperial' rye additions to the hexaploid wheat variety 'Chinese Spring' as the standard rye chromosome set, although these chromosomes are not completely identical with those of the population of 'Imperial' variety (cf. Singh and Röbbelen 1975). Based on the agreements, a

preliminary karyotype and its C-banding pattern, a homoeologous designation of added rye chromosomes and a generalized C-banding pattern of rye were proposed. The participants anticipated producing a standard diploid rye homozygous for genetical, biochemical and/or molecular studies and which should, be in addition, available for cytogenetical test crosses. Thus, in the present paper the development and various morphological and cytogenetical features of a dihaploid rye as an international tester line are described.

### Materials and methods

Since there was an urgent need to develop a homozygous line of and advanced rye material, the variety 'Petka' was chosen. It has spring growth habit, green grains and traits of modern cultivated varieties. 'Petka' was bred in Petkus and was released in 1961 (DDR); it is related to 'Petkus' rye which has been used world-wide in cytogenetical experiments. Moreover, from 'Petka' rye a complete series of primary trisomics, originated by several backcrosses to the trisomics of 'Esto' winter rye which have widely been used as excellent testers in genetical analysis (Melz et al. 1984; Schlegel et al. 1986).

In one of the spring type trisomics (4R), twin seedlings were observed quite frequently. In 1984, a large sample of twins was screened in BC<sub>7</sub> for the occurrence of haploids. From a number of 7-chromosome offspring, the most vigorous plant (rosta-7) was selected, propagated in vitro and some clones were included in chromosome doubling by colchicine treatment. This resulted a dihaploid and a tetrahaploid which are now, along with a clone of the original haploid, available for genetic and cytological work. The stocks are maintained and multiplied by in vitro plant propagation. For morphological and cytological comparisons the haploid, diploid and tetrahaploid clones were grown in phytotrons under controlled environmental conditions. Sexual propagation has been achieved despite the self-incompatibility of the homozygotes by keeping the plants in growth chambers at 30°C during flowering to suppress the incompatibility system.

## Results

### *Plant morphology*

The haploid (*rosta-7*) is about half the size of the corresponding dihaploid, but is otherwise comparably developed (see Fig. 1). The adult plant shows not only a reduced height but also shorter and more narrow leaves, thinner culms and retarded development. The spikes are more dense, smaller and awnless. There seems to be a dosage effect for awn length and this becomes apparent after re-diploidization and at the tetraploid level. Generally, the homozygous dihaploid and tetraploid clones are characterized by somewhat weaker growth than in the diploid or tetraploid plants of the population which show erect semi-long straw, fishshaped and slightly noded spikes and a normal thousand kernel weight.

None of the hemizygous haploid, homozygous dihaploid and tetraploid clones produced any seeds, due to either meiotic irregularities of the haploids or to incompatibility. Seed set in the dihaploids and tetraploids can be induced up to 30%, however, by heat treatment. Egg cells and pollen cells do function if the incompatibility system is inactivated. The kernels are plump which is within the range of shape of the 'Petka' population. The testa shows a green colour genetically linked with anthocyanin expression in the coleoptile. Germination of the seeds is normal.

### *Karyotype structure*

Since only a few karyograms in rye are based on chromosome measurements, information on chromosome morphology in a structurally homomorphic rye is needed for better identification of individual chromosomes. In the present study, therefore, special emphasis was

placed on the factors involved in sampling and characterization of C-banded chromosomes which might contribute to variation in chromosome length. Thus, ten somatic chromosome spreads of ten different root tips were used for statistical analysis. The results are given in Table 1. The data demonstrate remarkable differences in mean total length as well as relative arm length which range from 124.24 units in chromosome 1R to 162.46 units in 2R and from 45.03 units in chromosome arm 5Rp to 93.25 units in 5Rq, respectively. The arm ratios vary from 1.02 in chromosome 3R to 2.07 in 5R. The small sample standard deviations mean that most of the length differences or arm index variations are statistically significant (Table 1). This was due to measurements of identified C-banded chromosomes which reduce mistakes in chromosome determination (Fig. 2). The karyogram drawn from the data has been used for a more detailed description using the C-banding pattern. Prominent blocks of telomeric heterochromatin are stained, which is a common pattern of diploid rye. The band positions and band sizes are related to relative arm length, so there are sufficient references for identifying each of the seven chromosomes individually. Applying the standard chromosome band nomenclature taken from Schlegel et al. (1986), a specific reference karyogram is established (Fig. 3).

### *Chromosome pairing in meiosis*

*Meiosis of haploid rye 'rosta-7'*. Meiotic studies of rye haploids have been reported by several workers. Associations of two or more apparently non-homologous chromosomes at metaphase I are often observed (Puertas and Giraldez 1974). Levan (1942) was the first to demonstrate statistically that chiasma formation between the seven chromosomes was not random. He suggested

**Table 1.** The variation in length of chromosomes units in haploid rye '*rosta-7*'. Confidence limits at 1% level of probability for total chromosome length = 6.30, for arm length = 3.64, for the arm ratio = 0.06

Characteristics	Chromosome						
	1R	2R	3R	4R	5R	6R	7R
Satellite	15.24						
SD	1.40						
Short arm	39.32	75.44	63.73	65.25	45.03	50.51	58.78
SD	0.40	0.63	0.65	1.26	1.30	0.99	0.76
Long arm	69.68	87.02	65.63	88.21	93.25	89.67	71.54
SD	1.26	1.15	1.26	4.70	1.18	0.82	4.10
Total length	124.24	162.46	129.36	153.47	138.29	140.19	130.33
SD	1.35	1.60	1.82	5.99	1.47	1.48	5.51
Arm ratio	1.27 <sup>a</sup>	1.15	1.03	1.35	2.07	1.78	1.22
SD	0.06	0.02	0.02	0.03	0.08	0.04	0.01

<sup>a</sup> Satellite included in the short arm

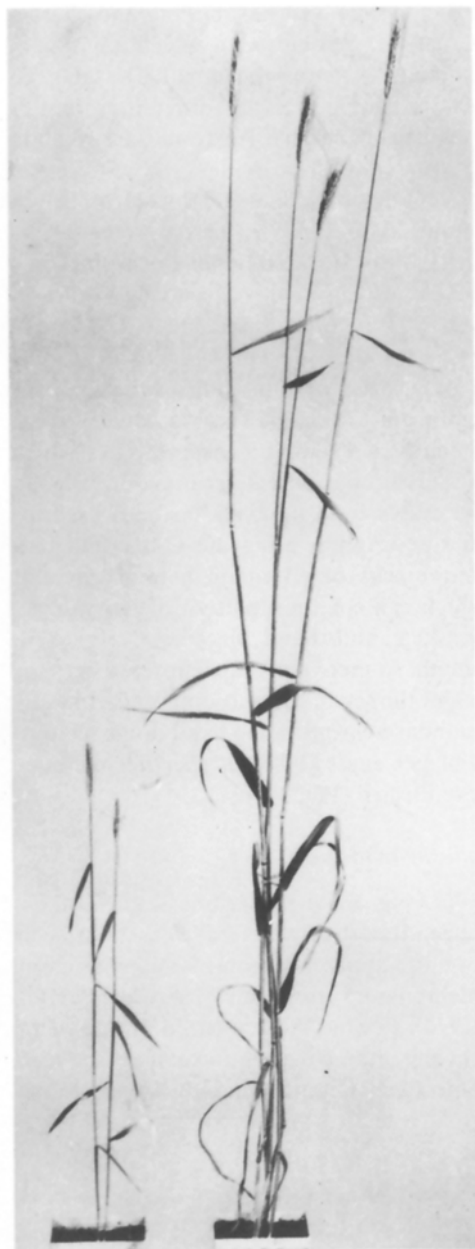


Fig. 1. Plant morphology of haploid (*left*) and dihaploid (*right*) clones of the 'rosta-7' genotype

that one particular chiasma is formed at quite a high frequency while the remaining arise at random. The mean chiasma frequency per pollen mother cell (PMC) as given by different authors ranged from 0.03 (Nordenskiöld 1939), over 0.26 (Heneen 1965) and 0.34 (Neijzing 1982) to 0.44 (Levan 1942). The present investigation on 'rosta-7' (haploid) revealed 6.38 univalents, 0.29 rod bivalents and 0.01 chain trivalents per PMC (=0.32 chiasmata/PMC) considering 200 cells, which corresponds well to previous results.

Using Giemsa C-banding, it was possible to discriminate between the meiotic chromosomes to some degree. The chromosomes can be divided into two groups according to telomeric band position: a group of four with a Giemsa band on both telomeres (chromosomes 1R, 2R, 3R, 7R) and a group of three chromosomes with one telomeric band (chromosomes 4R, 5R, 6R). On the latter, the single telomeric band is always very pronounced, while in the double-banded chromosomes one of the two telomeres is less marked. The difference of the two bands is too variable to allow more precise characterization of the chromosomes.

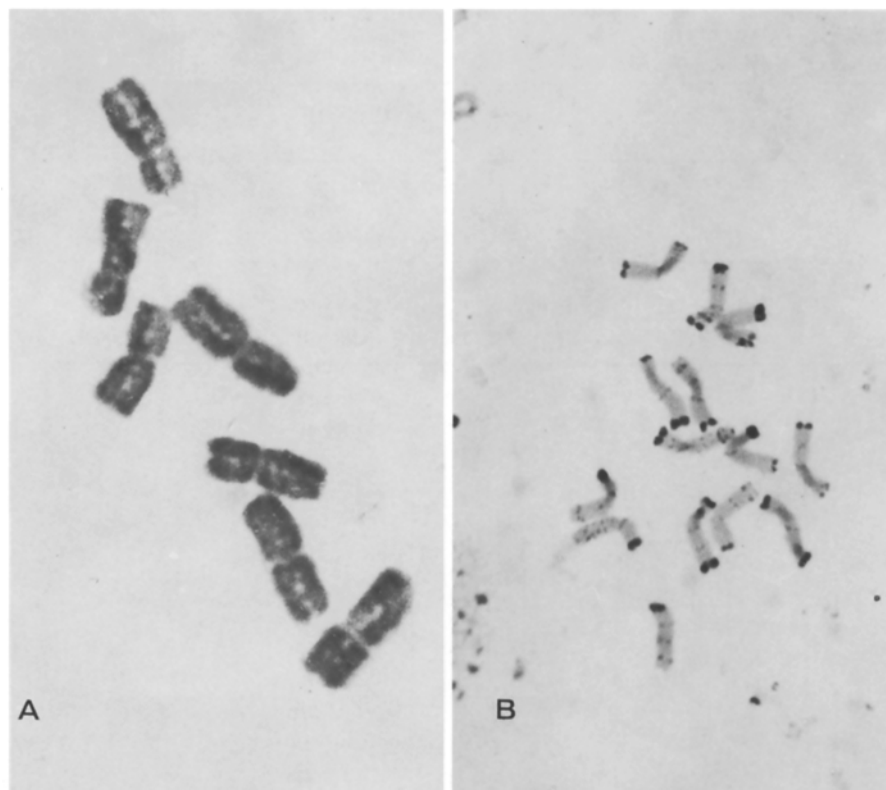
Associations of two and more single-banded and/or double-banded rye chromosomes were scored at metaphase I. Differentiation between true chiasma formation and secondary end-to-end attachments can be attempted either by chromosome co-orientation or by proving chromatid exchange in anaphase I. Aberrations other than chromatid bridges and acentric fragments were also observed at anaphase I.

To calculate the types of chromosomes involved in the inhomologous associations, each of the rod bivalents was analysed. Assuming random chiasma formation in cells with five univalents and one bivalent, six different types of rods can be expected if there are four double-banded and three single-banded chromosomes. If each chromosome is equally likely to be bound, the probability of a bivalent of two double-banded chromosomes is 6/21, that of bivalent of two single-banded chromosomes is 3/21 and that of a bivalent including single-banded and double-banded chromosomes is 12/21. Using the 14 chromosome arms which can be

Table 2. Possible rod bivalent formation by one chiasma of two types of C-banded chromosomes in haploid rye (rosta-7)

	Types and frequencies of different rod bivalents						Total	$\chi^2$
	—/—	—/—	—/—	—/—	—/—	—/—		
Observed	43 (86)	1 (2)	2 (4)	3 (6)	0 (0)	1 (2)	50 (100)	
Expected	24/84 (28.57)	24/84 (28.57)	24/84 (28.57)	3/84 (3.57)	6/84 (7.14)	3/84 (3.57)	84/84 (100.00)	85.3**

\*\*  $P < 0.001$



**Fig. 2.** Somatic chromosomes of **A** the haploid after aceto carmin staining and **B** of a dihaploid after C-banding

**Table 3.** The mean chromosome pairing of 10 heterozygous tetraploid 'Petka' plants compared to the pairing behaviour of the homozygous tetraploid line 'rosta-7'

Material	No. of PMC's scored	Uni-valents	Bivalents			Trivalents			Quadrivalents			
			Ring	Rod	Total	Chain	Pan	Total	Ring	Chain	Others	Total
Petka-tetra	1,095	0.30	6.12	0.93	7.05	0.10	0.02	0.12	2.23	0.96	0.12	3.31
Tetrahaploid	100	0.32	6.15	1.95	8.10	0.02	0	0.02	1.45	1.22	0.17	2.87

paired, 91 different combining events are possible. These arms can be described by the quarterly units  $n_i$  for chromosome numbers 1 to 7,  $k_1$  for short and  $k_2$  for long arms,  $t_1$  for the presence and  $t_2$  for the absence of heterochromatic telomeres, and  $y_1$  for the double-banded and  $y_2$  for the single-banded chromosome types. Thus for each arm one obtains the formulas  $n_1 k_1 t_1 y_1 \dots n_7 k_2 t_1 y_1$ . Associations of two arms of a given chromosome after diallelic combination are impossible and cannot contribute to heteromorphic rod bivalents, as is shown by identical  $n_i$  in the formulas. Thus, just 84 reliable pairings are expected. The six types of chromosome arm associations are determined by the formulas and frequencies demonstrated below. There is a strong deviation between the expected and observed frequencies of rod bivalent configurations as tested with

$\chi^2$  (Table 2). Also, there is a clear indication that associations of double-banded chromosomes are much more frequent than those including either double-banded and single-banded chromosomes or single-banded chromosomes only. Apparently, chiasma formation in the haploid is non-random, as in Neijzing (1982). The occurrence of true chiasmata and the non-randomness of their position along chromosomes 1R, 2R, 3R and 7R demonstrate homologous segments distributed non-randomly within the genome. Their constitution, however, remains unknown. Partially in agreement with Jones (1978), Giraldez and Orellana (1979) and Neijzing (1982) on diploid rye, no chiasma formation was observed in the heterochromatic telomers. This indicates that this kind of repetitive DNA does not function as a homologous region contributing to crossing over.

Type of chromosome association	Frequency	Type of arm association	Formula	Frequency
	6/21		$n_1k_1t_1y_1 - n_1k_1t_1y_1$	24/84
	12/21		$n_1k_1t_1y_1 - n_1k_1t_1y_2$	24/84
			$n_1k_1t_1y_1 - n_1k_1t_2y_2$	24/84
	3/21		$n_1k_1t_2y_2 - n_1k_1t_2y_2$	3/84
			$n_1k_1t_1y_2 - n_1k_1t_2y_2$	6/84
			$n_1k_1t_1y_2 - n_1k_1t_1y_1$	3/84

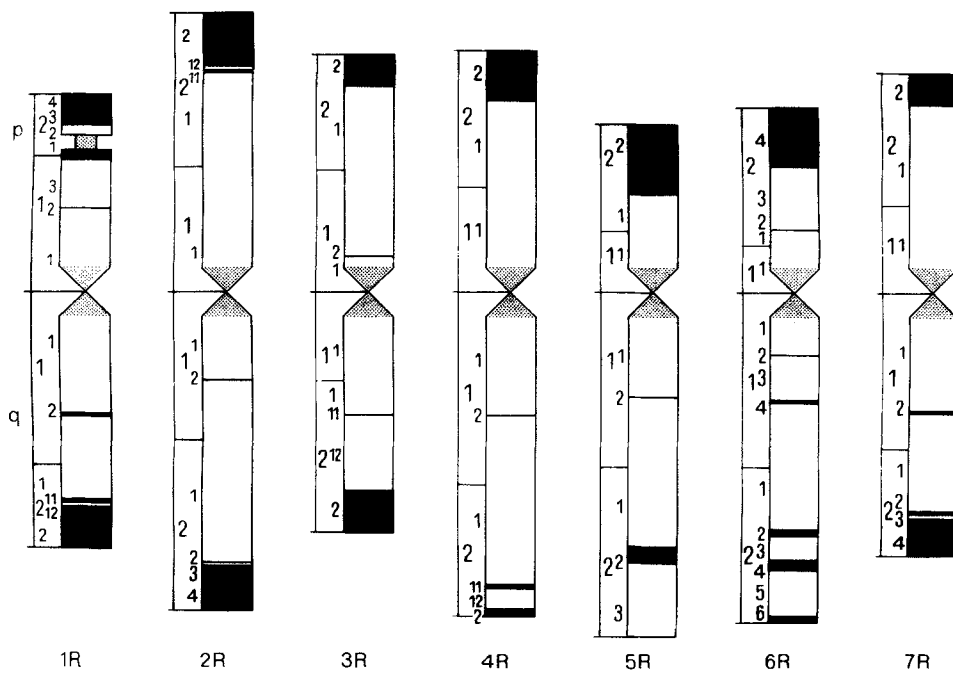


Fig. 3. Karyogram established according to the proposal of Schlegel et al. (1986)

*Meiosis of the experimental dihaploid of 'rosta-7'.* The recovered diploid genotype from the haploid plant 'rosta-7' should be completely homozygous and was thus expected to show some meiotic irregularities, a frequent attribute of inbred lines. The homozygous dihaploid line, however, exhibited a high meiotic stability. In metaphase I, an average of zero univalents, 5.50 ring bivalents and 1.10 rods are formed, as computed from 100 PMC's. Rod bivalents of double-banded chromosomes were more frequent (64.3%) than rods of single-banded chromosomes, according to the frequency of double-banded and single-banded chromosomes (4:3). This pattern of association conforms with results in disomic parental plants from which the haploid was derived (Schlegel 1973). It can be assumed that, in this particular genotype, homozygosity does not strongly af-

fect the meiotic pairing. This can be taken as an essential prerequisite for maintenance of the line and for crossing experiments.

*Meiosis of the tetrahaploid 'rosta-7'.* Even the MI pairing of the tetrahaploid plants is in accordance with that of heterozygous tetraploids (Table 3). The somewhat lower trivalent and quadrivalent frequencies are the only modifications which result in a slight reduction of chiasma frequency (25.59 compared to 24.26 chiasmata/PMC). The data are contradictory: previous experiments showed increased preferential bivalent formation by increased structural heterozygosity, while the present findings show increased bivalent pairing by a complete homozygous karyotype (cf. Schlegel and Mettin 1975). Summarizing the mean pairing configura-

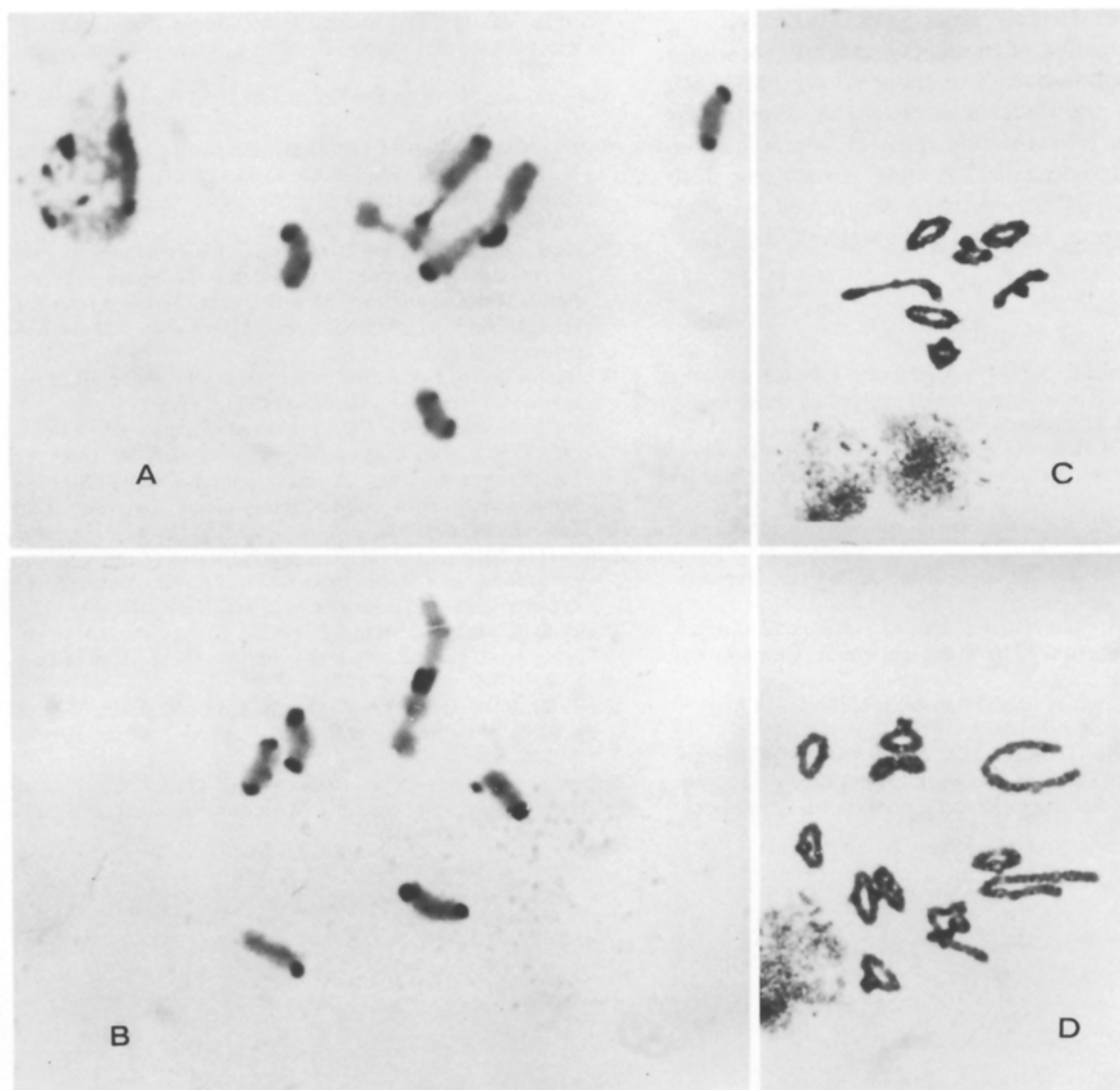


Fig. 4. Meiotic configurations of **A**, **B** haploid, **C** dihaploid and **D** tetraploid rye clones

tions, there is sufficient meiotic stability for sexual plant propagation with a high probability of obtaining euploid progenies.

#### Discussion

The completely homozygous dihaploid rye presented seems useful as a karyological and genetic standard. Plants are vigorous and exhibit growth characteristics of advanced cultivated rye. Though self-incompatibility prevents seed production in a natural way this can be compensated for either by vegetative plant propagation or by artificial inhibition of the incompatibility reaction. It could even be advantageous for extended intercrosses without manual emasculation, since only female gametes are obtained. The material may then be more

useful than the proposed diploid inbred line '2D117-1' from T. Lelley (see Sybenga 1983). There are karyological similarities with that inbred line and also with the generalized karyotype based on the 'Imperial' rye chromosomes. The few deviations concern the relative chromosome arm length, the C-band size and band positions. The seven chromosomes of the haploid set can be unequivocally distinguished by these features.

The meiotic behaviour of the various ploidy clones does not restrict use of the material as a standard tester. Neither the metaphase I pairing of the dihaploid nor the meiotic behaviour of the tetraploid show remarkable disturbances, as are expected after homozygotization. It seems that the genotype selected does not carry alleles for desynaptic chromosome behaviour when homozygous. Also, the MI pairing of the haploid corresponds with related findings of other authors (Nor-

denskiöld 1939; Heneen 1962; Levan 1942; Neijzing 1982). The formation of bivalents and trivalents with a comparable high frequency of about 0.3 per PMC once more confirms the frequent intragenomic duplications in rye. Further efforts should focus on comprehensive molecular and biochemical studies, particularly since gene dosage effects can clearly be determined using the introduced haploid, dihaploid and tetrahaploid clones.

## References

- Giraldez R, Orellana J (1979) Metaphase I bonds, crossing over frequency, and genetic length of specific chromosome arms of rye. *Chromosoma* 72:377–385
- Gupta RK (1971) Homoeologous relationship between wheat and rye chromosomes. Present status. *Genetica* (the Hague) 43:199–213
- Heneen WK (1962) Chromosome morphology in inbred rye. *Hereditas* 48:182–200
- Heneen WK (1965) On the meiosis of haploid rye. *Hereditas* 52:421–424
- Jones GH (1978) Giemsa C-banding of rye meiotic chromosomes and the nature of 'terminal' chiasmata. *Chromosoma* 66:45–57
- Levan A (1942) Studies on the meiotic mechanism of haploid rye. *Hereditas* 28:177–211
- Melz G, Neumann H, Müller HW, Sturm W (1984) Genetical analysis of rye (*Secale cereale* L.). I. Results of gene localization on rye chromosomes using primary trisomics. *Genet Pol* 25:111–115
- Neijzing MG (1982) Chiasma formation in duplicated segments of the haploid rye genome. *Chromosoma* 85:287–298
- Nordenskiöld H (1939) Studies of a haploid rye plant. *Hereditas* 25:204–210
- Puertas MJ, Giraldez RL (1974) Meiotic pairing in haploid rye. In: Kasha KJ (ed) *Haploids in higher plants. Advances and potential. V. Utilization of haploids in research*. Guelph University, Canada (Abstr), p 390
- Schlegel R (1973) Experimentelle Untersuchungen zur chromosomalen Stabilität und deren Bedeutung für die Fertilität des autotetraploiden Roggens (*Secale cereale* L.). Dissertation A, Martin-Luther-Universität, Halle-Wittenberg, 172 pp
- Schlegel R (1986) Neocentric activity in chromosome 5R of rye revealed by haploidy. *Hereditas* 107 (in press)
- Schlegel R, Mettin D (1975) Untersuchungen an Valenzkreuzungen beim Roggen (*Secale cereale* L.). IV. Über die Beziehungen zwischen Meioseverhalten und Fertilität bei halbmeiotisch entstandenen tetraploiden Bastarden. *Biol Zentralbl* 94:295–315
- Schlegel R, Mettin D (1982) The present status of chromosome recognition and gene localization in rye, *S. cereale* L. Tagungsber Akad Landwirtschaftswiss DDR 198:131–152
- Schlegel R, Melz G, Mettin D (1986) Rye cytology, cytogenetics and genetics. Current status. *Theor Appl Genet* 72:721–734
- Singh RJ, Röbbelen G (1975) Comparison of somatic Giemsa banding pattern in several species of rye. *Z Pflanzenzücht* 75:270–285
- Sybenga J (1983) Rye chromosome nomenclature and Homoeology relationships. Workshop Report. *Z Pflanzenzücht* 90:297–304