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Identification of the chromosomes of the rye translocation tester set

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Summary. Intercrossing the Wageningen translocation tester set of rye and the series of 'Imperial' rye additions to 'Chinese Spring' wheat of Sears yielded 29 chromosome disomic translocation hybrids. Observation of trivalents led to the identification of the chromosomes of the tester set in terms of the terminology system used in the Triticinae. The analysis was complicated by very low chiasma frequencies in some short chromosome segments in the hybrids. Nevertheless, it could be safely concluded that 1R = VII; 2R = III; 3R = II; 4R = IV; 5R = VI; 6R = V; 7R = I, which deviates slightly from previous classifications based on other methods.

Key words: Chromosome identification – Rye-wheat addition lines

Introduction

The identification of the chromosomes of rye in terms of the nomenclature used for the Triticinae is of interest both for evolutionary studies and for plant breeding. Since it became clear that homoeologous substitution of rye chromosomes (or parts thereof) for wheat chromosomes in wheat can have practical application, the study of homoeology relations has especially become of increasing importance. There are several ways of identifying rye chromosomes and this has led to several different classifications that can not easily be compared. Some are based on chromosome length and arm index, some on Giemsa C-banding pattern, some on translocations, some on trisomics and others on individual additions to wheat. Only in the latter case have detailed homoeology studies by substitution been possible (Gupta 1971; Koller and Zeller 1976). Since both wheat and rye chromosomes have evolved since their separation from a common ancestor, the analysis of homoeology is complicated. In addition to substitution compensation, the use of homoeologous genes and other unique DNA sequences, and of pairing relations, are necessary to complete the picture. A rather satisfactory system has been constructed primarily based on substitution compensation which permits the numbering of the 'Imperial' to 'Chinese Spring' addition series in as good a correspondence with the wheat system as presently practically possible. This has been accepted by the Wageningen Workshop on rye chromosome Nomenclature and Homoeology Relationships (Sybenga 1983) as the (preliminary) rye/wheat homoeology standard. Still, however, the chromosomes of diploid rye and their numerous classifications cannot readily be correlated with this standard. The most complete review of rye chromosome classifications and their possible relations is given by Schlegel and Mettin (1982). The most successful in correlating the nomenclature of the 'Imperial' addition series to that of diploid rye have been Zeller et al. (1977). Their study involves a comparison of published and new C-banding patterns of the 'Imperial'/'Chinese Spring' addition series and Zeller's series of trisomics. In addition, the trisomics were combined with the translocation tester set (Sybenga and Wolters 1972; de Vries and Sybenga 1976; Sybenga 1983) in order to correlate the nomenclature used for this set to that used for the trisomics and the additions. This is of considerable importance for the cytogenetics of rye and for the study of rye-wheat relations, as the translocation tester set has been widely distributed, is easily maintained, and is used in many cytogenetic studies (Sybenga 1983, de Vries and Sybenga 1984). Since a few uncertainties were left in

the classification of Zeller et al. (1977), it was decided (Sybenga 1983) to test the translocation tester set directly on the addition series.

The hybrid between a (disomic) addition of a rye chromosome to wheat and a rye translocation homozygote has 29 chromosomes: 21 of wheat, 5 normal chromosomes, 2 translocation chromosomes and 1 extra chromosome of rye. When the extra chromosome corresponds with one of the five normal chromosomes of rye, one rye (ring) bivalent will be formed at MI of meiosis when the level of chiasma formation is high enough. In addition, bivalents and some trivalents may be formed (infrequently) by homoeologous wheat chromosomes, less frequently between homoeologous rye and wheat chromosomes. When the extra chromosome corresponds with one of the translocation chromosomes, it will associate with only a segment of that chromosome and also with a segment of the second translocation chromosome. The result is a trivalent in meiosis at MI, provided the level of chiasma formation is high and the translocated segments large enough to accommodate a chiasma in a sufficient number of cases. The difference between a trivalent and a (ring) bivalent thus permits the identification of the chromosomes involved in the translocation in terms of the system of classification of the additions. For full identification of a translocation, of course, trivalent formation with two additions is required.

Previous studies had revealed that this approach to identification of the chromosomes of the translocation tester set is complicated by two factors: 1. Rye chromosomes in a wheat background show considerably reduced chiasma-frequencies, especially in the small segments that occur in some translocations of the tester set. This reduces the probability of a trivalent being formed when one would be expected, and it also reduces the probability of formation of a ring bivalent when the addition chromosome is not homologous with the translocation chromosomes. Some rye chromosome arms appear to have especially low chiasma frequencies in a wheat background. 2. Wheat chromosomes without homologues, especially in combination with a rye genome, show considerable bivalent and even trivalent formation. Without C-banding, these are not always readily distinguished from rye bivalents. For a reliable identification based on the distinction between bivalent and trivalent formation a quantitative approach, involving a relatively large material and at least some C-banding, is required.

Materials and methods

The translocation tester set was the set available at this Laboratory (Sybenga and Wolters 1972; Sybenga 1983). The series of additions of 'Imperial' rye to 'Chinese Spring' was obtained from E. R. Sears, Columbia, Missouri and C. J. Driscoll, Adelaide, Australia. A parallel set was obtained from T. E. Miller, Cambridge, England, originally also from E. R. Sears. Crosses were made in the greenhouse in 1981, 1982, 1983 with the additions as the female parent. Fixations of meiosis of F_1 plants were made in acetic alcohol 1:3, and stored at −10 °C. Preparations were permanent aceto-carmine preparations, and (Giemsa) C-banded preparations were made according to Giraldez et al. (1979) with slight modifications. The chromosome number in the roottips was determined in temporary Feulgen squashes after bromonaphthalene pretreatment.

In addition to the translocation tester set, Robertsonian (telocentric) substitutions for chromosomes II and VII (nomenclature as used for the tester set) from this Laboratory were used.

Not all combinations were made.

Results and discussion

All pertinent quantitative results are given in Table 1.

Addition 1. Since the additional rye chromosome is the nucleolar chromosome, it is expected that translocations involving this chromosome and the Robertsonian substitution will all show a trivalent in meiosis. The main reason for analysing these combinations is to test the effect of the wheat background on rye chromosome behaviour and vice versa, and the effect of the size of the translocated segments. Translocation 273 (chromosomes VI and VII) has rather large interchanged segments, which in a disomic heterozygote leads to predominant ring quadrivalent formation. The translocated arm is the non-satellited arm of VII. In 44 cells of the hybrid, only three trivalents were found. There were no ring bivalents, but almost every cell carried an open bivalent.

Translocation 248 (chromosomes V and VII) gave only one cell out of 92 with a trivalent. The translocation has a break in the satellite of the nucleolar chromosome, and in the diploid tends to have a minority of ring quadrivalents. Only about two-thirds of the remaining cells in the hybrid had an open bivalent, indicating that not only the translocated segment but also the long arm of the nucleolar chromosome tended to have strongly reduced chiasma frequencies. A very limited number of bivalents could be shown to be wheat-wheat bivalents (C-banding).

The Robertsonian substitution gave a similar picture. There were no trivalents in 100 cells, but there were 44 heteromorphic bivalents, only three presumably involving the short (nucleolar) arm. The heteromorphic bivalents in 34 additional C-banded cells all showed the associated telo to be the one without the NOR. The fact that in most cells a heteromorphic bivalent was found shows that chromosome VII is addition chromo-

Table 1. Chromosome associations at first meiotic metaphase of hybrids between 'Imperial' rye additions to 'Chinese Spring' wheat and translocations of the Wageningen tester set. Chromosomes according to system of Sybenga and Wolters 1972. Chiasmata: arms associated by chiasmata

Addition	Translocation chromosomes	Cells	Chiasmata		Open bivalents		Ring bivalent		Trivalents	
			Total	Per cell	Total	Per cell	Total	Per cell	Total	Per cell
									S: 3	0.03[a]
1	2 telos VII	100	78	0.78	38	0.38	0	0	L: 37	0.39[a]
1	248 V–VII	95	65	0.68	63	0.66	0	0	1	0.01
1	273 VI–VIII	44	48	1.09	42	0.96	0	0	3	0.07
2	305 III–VI	141	257	1.82	201	1.43	3	0.02	25	0.18
3	2 telos II	9	5	0.56	1	0.11	0	0	4	0.44[a]
3	240 II–VI	30	42	1.40	18	0.60	0	0	12	0.40
3	242 III–V	53	121	2.28	37	0.70	42	0.79	0	0
3	305 III–VI	6	16	2.67	12	2.0	2	0.33	0	0
3	501 IV–VI	71	152	2.14	88	1.24	32	0.45	0	0
4	282 I–VI	56	103	1.84	43	0.77	30	0.54	0	0
4	305 III–VI	79	100	1.27	34	0.43	33	0.42	0	0
4	501 IV–VI	50	66	1.32	34	0.68	0	0	16	0.32
5	240 II–VI	45	74	1.64	62	1.38	1	0.02	5	0.11
5	242 III–V	138	210	1.52	182	1.32	14	0.10	0	0
5	273 VI–VII	170	337	1.98	255	1.50	4	0.02	37	0.22
6	2 telos VII	25	28	1.12	24	0.96	2	0.05	0	0
6	242 III–V	116	120	1.03	104	0.90	3	0.03	5	0.04
6	248 V–VII	72	33	0.46	31	0.43	0	0	1	0.01
6	273 VI–VII	50	49	0.98	29	0.58	9	0.18	1	0.02
6	501 IV–VI	50	36	0.72	28	0.56	4	0.08	0	0
7	242 III–V	64	43	0.67	25	0.39	9	0.14	0	0
7	282 I–VI	72	172	2.39	78	1.08	11	0.15	36	0.59
7	501 IV–VI	81	80	0.99	48	0.59	16	0.20	0	0

[a] Heteromorphic bivalents. S: short arm; L: long arm

some 1, as was expected. It is clear that in both arms the chiasma frequency is reduced, but that particularly the short, nucleolar arm was affected. In the addition line used, the banding pattern in this arm was very light with hardly a terminal band visible, in contrast to the observations of Darvey and Gustafson (1975). The arm may be structurally abnormal, preventing normal association, but more probably it is especially sensitive to the chiasma inhibiting effect of the wheat genome, as earlier suggested by Naranjo and Lacadena (1980). We conclude that chromosome VII is 1R.

Addition 2 appeared to be difficult to cross with the translocations. Success was obtained only with 305 (chromosomes IV and VI). The chiasma frequency was relatively high resulting in a number of wheat bivalents; even wheat rings were formed. The considerable number of large trivalents, typical of rye, indicated that the translocated chromosomes were (partly) homologous with the addition chromosome. The frequency of trivalents per cell was not very high, but sufficient, in view of the low ring bivalent frequency, to justify this conclusion. Since it appeared later that chromosome VI is not 2R, chromosome III is 2R.

Addition 3. Four translocation and a Robertsonian substitution (chromosome II) were combined with addition 3. Only eight cells of the Robertsonian hybrid could be analysed. The chiasma frequency was low. Three cells showed a heteromorphic bivalent. Therefore, although no trivalents were found, we conclude that chromosome II is 3R. This is confirmed by the observations on the translocation hybrids: Translocation 240 (II and VI) gave 13 trivalents in 31 cells and no ring bivalents.

Translocations 242 (III and V), 305 (III and VI) and 501 (IV and VI) gave no trivalents but high frequencies of ring bivalents. The chiasma frequencies were high, and there must have been several wheat (open) bivalents. Conclusion – chromosome II is 3R.

Addition 4 was combined with three translocations. The chiasma frequency was moderate. Translocation 282 (I and VI) had no trivalents but many ring bivalents, as did 305 (III and VI), but 501 (IV and VI) had a trivalent in one-third of the cells, no rings and an open bivalent in approximately two-thirds of the cells. Since it is clear that VI is not identical to 4R, the conclusion is that chromosome IV is 4R.

Addition 5 was combined with three translocations. With 240 (II and VI) there was a number of trivalents (11% of the cells), one ring in 45 cells, numerous open bivalents, and a reasonably high chiasma frequency. It may be assumed that the ring bivalent was a wheat bivalent and that the translocated segment, although not really very short, tended to lack a chiasma. In diploid translocation heterozygotes, chiasmata occur in over 80% of this segment (Sybenga 1970). Translocation 242 (III and V) did not give trivalents with this addition, but some ring bivalents and many open bivalents. It seems that this addition chromosome is also reluctant to associate with its full homologue from the tester set. In respect to the short arm, Drögemüller and Lelley (1984) reached the same conclusion. With 273 (VI and VII) there were trivalents again, in moderate frequency and a frequency of (wheat) rings comparable with that for 240. Again a high chiasma frequency lead to numerous open bivalents, many of which involving wheat chromosomes. There is no reason to question the conclusion that chromosome VI is 5R.

Addition 6. As expected, two telocentrics substituting for the nucleolar chromosome VII did not lead to the formation of trivalents or heteromorphic bivalents when combined with addition 6. The rather low chiasma frequencies resulted in low trivalent frequencies but ring bivalent frequencies were reasonably high. Although this could have disturbed the analysis, the conclusions are safe.

Translocation 242 (III and V) gave five trivalents in 116 cells, and three ring bivalents. As the two types of configurations occured in different cells, this result is ambiguous. Translocation 248 (again V but now with VII) had only one trivalent in 72 cells. Random searches in many more cells revealed six more trivalents and almost no rings, of which there were none in the well analysed sample of 72 cells. One exchanged segment was small and the chiasma frequency was low.

Table 2. A Nomenclature of rye chromosomes. *Top:* standard nomenclature of Triticinae. *Bottom:* nomenclature used originally for translocation tester set. **B** Translocation tester set and chromosomes involved according to standard nomenclature. Not all arm indications (S = short, L = long; Sybenga 1983) are certain

A	Standard:	1R	2R	3R	4R	5R	6R	7R
	Tester set:	VII	III	II	IV	VI	V	I
B	Translocation					Chromosomes		
	240					3R/5RL		
	242					2RL/6RL		
	248					1RS/6RS		
	273					1RL/5RS		
	282					5RL/7RS		
	305					2RL/5RS		
	501					4RL/5RL		

Translocation 273 (VI and VII) again produced one trivalent (50 cells) but in addition nine rings. Finally, 501 (IV and VI) had no trivalents but four rings in 50 cells with a low chiasma frequency. Drögemüller and Lelley (1984) concluded that the short arm of 6R tended to have very low chiasma frequencies when the overall chiasma frequency was low, as in these hybrids. It is quite reasonable, therefore, to conclude that chromosome V is 6R.

Addition 7. Here again, in one combination (282, I and VI) ring bivalents and trivalents were combined. The chiasma frequency was high and the trivalents were in excess. As translocation 242 (III and V) and 501 (IV and VI) had rings and no trivalents, the conclusion is that chromosome I is 7R.

The results are summarized in Table 2.

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