Theor. Appl. Genet. 60, 135-140 (1981)

Trisomics of Ryegrass and Their Transmission

E.G.M. Meijer* and B.S. Ahloowalia
Plant Breeding Department, Agricultural Institute, Carlow (Ireland)

Summary. Primary trisomics of perennial ryegrass, *Lolium perenne* L., were studied for meiotic behaviour, fertility, morphology and trisome transmission. Trisomics differed from each other in mean meiotic association, pollen fertility, seed set and morphology. The combined cytomorphological data suggested that the investigated trisomic plants included trisomes 2 to 7. No pollen transmission of trisomes was detected. Female transmission of trisomes ranged from 12% for tri 3 to 37% for tri 4 with a mean of 24% for the six trisomes. Trisome transmission was not related to either chromosome size or trivalent/univalent frequency, although the larger trisomes formed trivalents more frequently than the smaller trisomes.

Key words: Trisomy – Meiosis – Fertility – Morphology – Transmission – *Lolium perenne* L.

Introduction

Perennial ryegrass, *Lolium perenne* L., is a diploid (2n = 14) selfincompatible species. Primary trisomics (2n + 1 = 15) in this species were first reported by Myers (1944) in the progeny of a triploid plant. The trisomics had reduced viability and showed aberrant morphology, e.g. narrow leaves and reduced spikelet number. Essad et al. (1966) reported two primary trisomics, identified as tri-3 and tri-5; however, no details of the phenotypes were given. Ahloowalia (1972) found three primary trisomics in the progeny of a near-triploid (2n = 22). Additional trisomics were obtained by crossing this plant with a diploid. Preliminary studies showed that the trisomics differed in morphology, meiotic behaviour and fertility (Ahloowalia 1976). In this paper, studies are presented on transmission of trisomes in relation to their meiotic behaviour and effects on pollen fertility, seed set and morphology.

* Part of M.Sc.Agr. Thesis, submitted by senior author to the National University of Ireland, Dublin

Materials and Methods

Most trisomics and disomics studied were the progeny of a near-triploid perennial ryegrass *Lolium perenne* L. (2n = 3x + 1 = 22) crossed to diploids of cv. 'Øtofte' and 'Premo'. Two trisomics were isolated in the progeny of a primary trisomic and a double trisomic (2n = 14 + 1 + 1 = 16).

Clones of trisomics, disomic sibs and the parental near-triploid were inter-pollinated with clones of 'Lemtal' annual ryegrass (*Lolium multiflorum Lam.* Seeds of each clone were thrashed manually. Seed set and 100-seed weight were recorded to determine the effect of trisomy on fertility and seed size. Seeds were germinated on blotting paper in the dark at 20°C in an incubator, and germination was recorded after 20 days. Seedlings were transplanted in soil and grown in a glasshouse. A set of trisomic and disomic clones, maintained outdoors during winter, was transferred to 16/8 h light/dark photoperiod at 20/10°C in a growth chamber in the following spring. These clones were compared for heading date with two sets of clones, one maintained with and one without cold-treatment in a glasshouse. Emergence of first spike was scored as the heading date.

Spikes of parental trisomics and their progeny were fixed for 48 h in 6 : 3 : 1 (v/v) ethanol : chloroform : acetic acid fixative mordanted with ferric chloride. Smears of pollen mother cells were stained with 0.5% acetic-carmine and 20-40 cells at the first meiotic metaphase (MI) were analysed for chromosome association. Slides were made permanent by vapour exchange with 95% ethanol and sealed with 'Euparal' (Bradley 1948).

Pollen fertility was determined from clones grown in the glasshouse and under controlled conditions. Pollen grains were stained with 1% alcoholic safranin in glycerol and water (1:2:1, v/v). Empty, partially filled and shrivelled pollen grains were counted as sterile. In some cases, pollen grains were germinated on 20% (w/w) sucrose solution with 10 ppm each of calcium chloride, boric acid and Na_2-EDTA (Ahloowalia 1973) at 21-23°C. Pollen grains showing protrusions or pollen tubes were counted as fertile. Duplicate germination tests were made and over 200 pollen grains per slide were counted.

Trisomic identity in a few cases was confirmed from somatic karyotypes by root-tip squash technique (Ahloowalia 1964).

0040-5752/81/0060/0135/$ 1.20

Results

Meiotic Behaviour of Parental Trisomics

Of the twelve trisomics studied, in five plants the trivalent was associated with the nucleolus at diakinesis (Table 1). In plant 15, the extra chromosome was large and nucleolar, suggesting trisomy for chromosome 2 (Fig. 1). The extra chromosomes in plants 38, 68 and 28 were nucleolar and considerably larger than in the other trisomics. Plant 3 was probably trisomic for chromosome 7 as the extra chromosome was the smallest (Fig. 2), and non-nucleolar.

Trisomics showed a wide variation in their chromosome association at MI. The mean number of univalents ranged from 0.48-1.13 and trivalent number ranged from 0.13-0.53 per cell (Table 1). The most common trivalent shape was a V-association, which requires two chiasmata (Fig. 3). The mean chiasmata number of trisomics ranged from 12.9-14.1 per cell and 1.84-2.01 per bivalent (Table 1), but did not differ significantly from that of the disomics.

Trisomics showed segregations of 7:8, 7:1:7 and occasionally 6:3:6, with the univalent(s) often lagging at anaphase I (Fig. 4). Lagging chromosomes divided and formed micro-nuclei in tetrads.

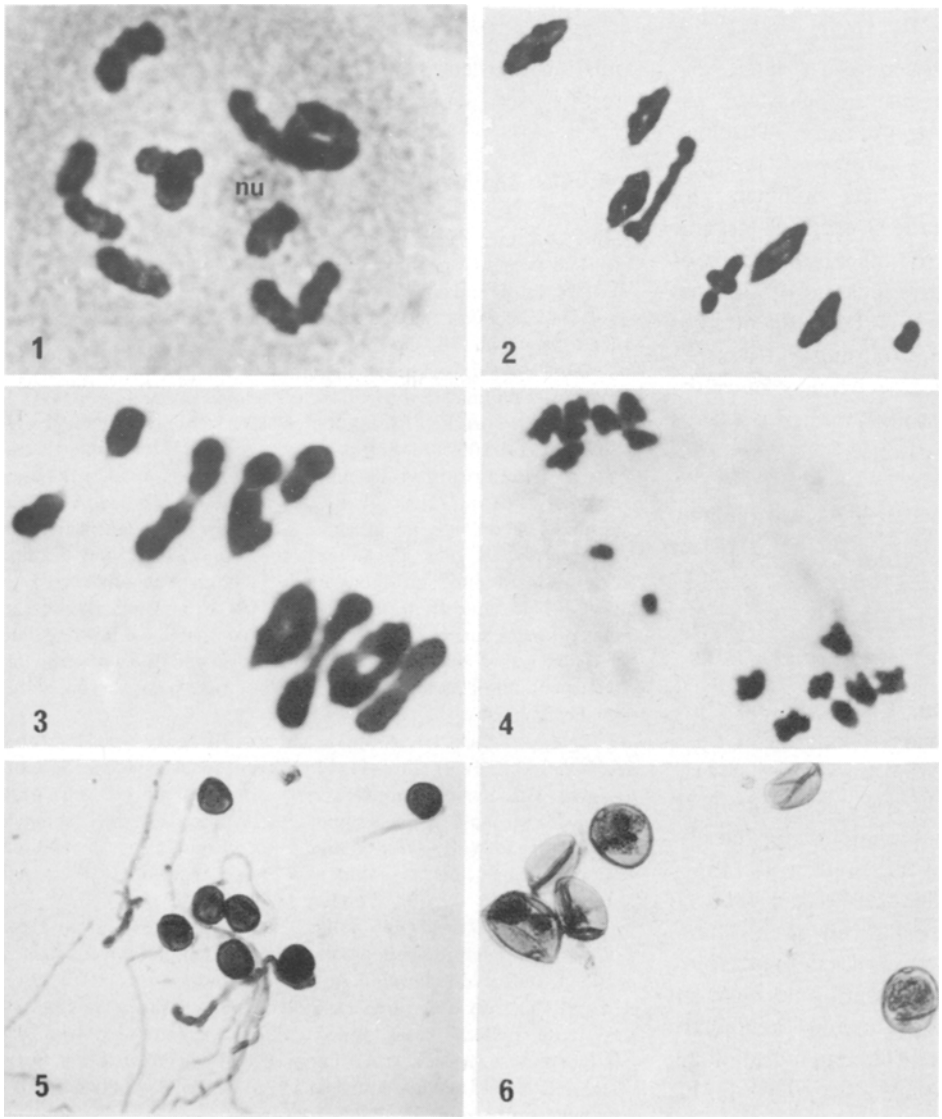

Fig. 1-6. Microsporogenesis in ryegrass trisomics: 1 Diakinesis, 1 III + 6 II, trivalent associated with nucleolus (nu) (tri 2). 2 Metaphase I, 7 II + 1 I (tri-7). 3 Metaphase I, 1 III + 6 II (tri-3). 4 Anaphase I, 7:7 segregation with lagging chromatids. 5 Germinated pollen grains (tri-6). 6 Sterile pollen

Table 1. Chromosome association and chiasma number at metaphase I in trisomics and disomic-sibs

Plant no.	Mean chromosome association/cell			Mean no. of chiasmata		Nucleolar association of trisome[a]	Tentative identity of trisome
	I	II	III	/cell	/bivalent		
Trisomics (2n = 15)							
54	0.78	6.63	0.33	13.1	1.85	NN	5
26	0.93	6.70	0.23	13.3	1.92	NN	5
25	0.80	6.80	0.20	13.0	1.85	NN	6
57	0.65	6.65	0.35	14.1	2.01	NN	6
4	0.73	6.73	0.28	13.2	1.88	NN	6
63	0.60	6.63	0.38	13.9	1.99	NN	7
3	1.13	6.78	0.13	13.3	1.92	NN	7
15	0.48	6.48	0.53	13.9	1.97	N	2
28	0.65	6.58	0.40	12.9	1.84	N	2
38	0.48	6.53	0.48	13.1	1.86	N	3
68	0.55	6.55	0.45	13.8	1.97	N	3
9	0.70	6.70	0.30	13.2	1.88	N	4
Disomic-sibs							
17	–	7.00	–	13.4	1.91	–	–
19	–	7.00	–	13.4	1.91	–	–
51	–	7.00	–	13.5	1.93	–	–

[a] NN = non-nucleolar; N = nucleolar

Fertility

Pollen stainability of trisomics ranged from 11-81% as compared with 86% for disomics (Table 2). Pollen stainability of plants 54, 4 and 9, was 11, 23 and 38% respectively when grown in the glasshouse (Table 2) but was considerably higher (being 70,70 and 78% respectively) when grown in the growth chamber. Such differences were not observed for other plants. Of the four trisomics investigated, plant 25 showed 68% pollen germination (Fig. 5) and 81% stainability which was as high as that of the disomics (Table 2). In the remaining trisomics, pollen germination was lower than pollen stainability (Table 2). Some trisomics produced pollen of varying size. In plants with low fertility, most pollen grains were collapsed and shrivelled (Fig. 6).

Seed set on trisomics was low, averaging 7 seeds per spike and ranged from 3-21 as compared to 31 and 32 in the two disomic-sibs (Table 2). Most seeds produced by trisomics were either empty or partially filled. Some trisomics had reduced 100-seed weight (Table 2).

Plant Morphology

Trisomics differed from each other in several morphological characters, e.g. plant height, flag-leaf length, spike exertion, number of spikelets, spike shape and heading date. For most characters, trisomics showed a greater variation

Table 2. Fertility of open-pollinated trisomics and disomic-sibs in glasshouse

Plant no.	Trisome identity	Pollen stainability (%)	Pollen germination (%)	No. of seeds per spike	Hundred-seed wt. (mg)	Seed germination (%)
Trisomics (2n = 15)						
54	5	11	–	4	181	74
26	5	59	18	3	115	39
25	6	81	68	21	116	67
57	6	38	–	4	–	64
4	6	23	–	8	139	60
63	7	68	–	4	204	89
3	7	41	–	3	–	59
15	2	33	–	5	124	34
28	2	78	52	7	164	50
38	3	26	–	7	–	91
68	3	18	–	13	147	64
9	4	38	18	9	210	89
Disomic-sibs (2n = 14)						
17		80	70	32	170	72
19		83	–	31	180	89
51		90	79	–	–	–
'Lemtal' (annual)[a]		86	–	–	233	86

[a] Mean of five plants

than the disomics. Most trisomic plants could be distinguished from disomic-sibs by their leaf length and width, thickness and compactness of spikes, spikelet size and stemthickness (Fig. 8).

Trisomics were as vigorous as the disomic-sibs when grown in the glasshouse during summer or in the growth chamber at 20/10°C, 16/8h light/dark photoperiod. However, during winter, glasshouse grown trisomics were less vigorous than the disomics.

The relative heading date of each trisomic was virtually constant under different environments. Three late heading trisomics did not flower without cold treatment (Table 3).

Transmission of Trisomes

Seed-progenies of eight trisomics, likely representing trisome 2-7, were investigated for trisome transmission. Seed germination of trisomics ranged from 34-91% (mean 65%) as compared to 72% and 89% for the disomic-sibs (Table 2).

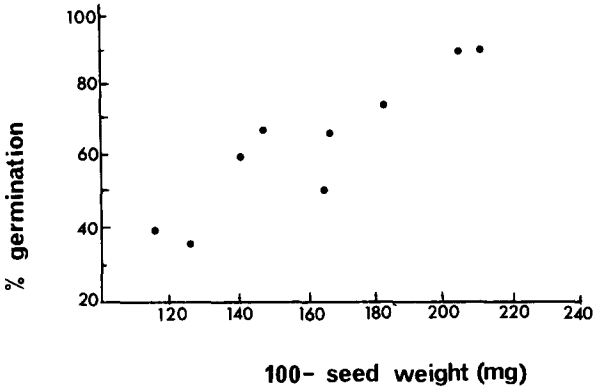

Fig. 7. Relationship between seed weight and germination of trisomics

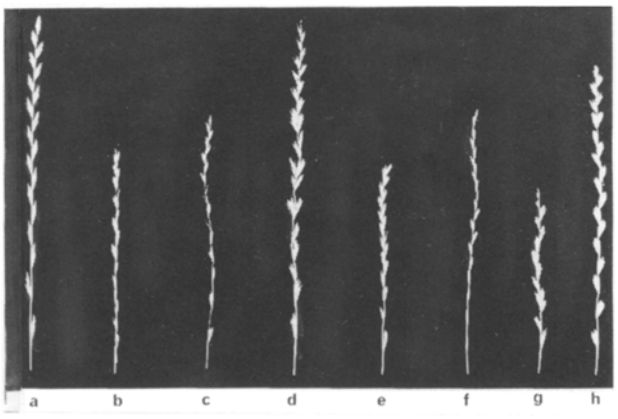

Fig. 8. Representative spikes. *a* disomic; *b*, *h* trisomic 6; *c* tri 4; *d* tri 2; *e* tri 5; *f* and *g* tri 3

Seed weight and germination were significantly correlated ($r = +0.73, P < 0.025$) (Fig. 7).

Of the 160 plants examined, 39 (24%) were trisomics (Table 4). All trisomics were primary except one telotrisomic which was recovered in the progeny of plant 57. The trisomic frequency in the individual progenies ranged from 12% for plant 68 (tri 3) to 37% for plant 9 (tri 4) (Table 4).

Table 3. Flowering response of trisomics and disomic-sibs grown under different environments

Plant no.	Trisome identity	No. of days to heading[a]		
		1	2	3[b]
Trisomics (2n = 15)				
54	5	30	39	39
26	5	30	34	39
25	6	19	17	19
57	6	35	45	39
4	6	14	19	18
63	7	55	61	NH
3	7	11	11	17
15	2	52	54	NH
28	2	17	19	17
38	3	39	32	NH
68	3	44	53	NH
9	4	32	45	46
Disomic-sibs (2n = 14)				
17	–	30	39	33
1	–	24	24	27
18	–	26	19	26
51	–	24	17	17

[a] Number of days from April 1
[b] 1 = growth chamber 16/8h, light/dark photoperiod, 20/10°C; 2 = cold-treatment, glasshouse grown; 3 = no cold-treatment, glasshouse grown, NH = no heads

Table 4. Female transmission of trisomes

Parental trisomics plant no.	Trisome identity	Progeny		Trisomic transmission (%)
		Plant examined (no.)	Trisomics (no.)	
26	5	9	2	22
25	6	31	7	23
57	6	14	3	21
4	6	15	4	27
63	7	25	5	20
15	2	12	2	17
68	3	16	2	12
9	4	38	14	37

No double trisomic or tetrasomics were recovered on inter-pollination of trisomics. No trisomics were found in the progeny of disomics on inter-pollination with trisomics, suggesting that the extra chromosomes were not pollen transmitted.

Discussion

The karyotype of ryegrass consists of three nucleolar and four non-nucleolar chromosomes and most chromosomes differ in relative length and position of the centromere (Essad et al. 1966). These differences might be reflected in the meiotic behaviour of the trisomes and may affect their transmission.

The combined cyto-morphological data suggested that probably six out of the seven possible trisomics were present among the twelve trisomic plants investigated. In five plants the extra chromosome was nucleolar and in seven, non-nucleolar. The mean meiotic association in trisomics showed that trisomics could be divided into two groups: 1. those with high trivalent but low univalent number, i.e. plants 15, 28, 38 and 68, all with a nucleolar trisome. 2. Others with low trivalent but high univalent number. This group included all non-nucleolar trisomics except plant 9. In plant 15 the nucleolar trisome was relatively large and likely trisome 2. The other plants with nucleolar trisomes and high trivalent frequency were different since the extra chromosome was relatively smaller than in plant 15 and formed trivalents less frequently, suggesting trisomy for chromosome 3 or 4. Accordingly, the nucleolar trisome with the lowest trivalent association (plant 9) would be trisome 4. Among the non-nucleolar trisomics, plant 3 was identified as trisome 7, since the extra chromosome was the smallest and its identity was later confirmed from somatic karyotype. This plant also had the lowest trivalent frequency and markedly differed from the other trisomics in morphology. The remaining plants, in which the trisomes were non-nucleolar, would then be trisomic for chromosome 5 or 6, since none of them carried trisome 1, the largest of the non-nucleolar group. These plants showed similar meiotic association but differed in pollen fertility, seed set, 100-seed weight and morphology. On this basis, the trisome in plant 54 and 26 was likely trisome 5 with median centromere. Plants 4, 25 and 57 would be then trisomic for chromosome 6.

Female transmission of the nucleolar chromosomes was distinctly different from that of the non-nucleolar chromosomes. Among the nucleolar group, trisomic 4 had the highest transmission (37%), and trisome 3 the lowest (12%). Trisomic 4 had a higher seed weight than the other trisomics. Transmission of the non-nucleolar trisomes 5-7 was similar to each other, although they differed in their trivalent/univalent number per cell, seed set and seed weight. A relationship between trivalent frequency and transmission has been reported in trisomics of *Medicago falcata* x *M. sativa* hybrids (Buss and Cleveland 1978) and *Avena strigosa* (Rajhathy 1975). In *Zea mays* (Einset 1943) high transmission was related to the frequency of trivalent formation and chromosome length. Although, the relatively larger chromosomes (tri 2-4) showed high trivalent association, no relationship between chromosome size or trivalent frequency and transmission was detected in the present study. On the other hand, trisomic 2 (plant 15) with the highest trivalent number had a rather low transmission as compared to trisomic 6 (plant 4) which involved a small chromosome with low trivalent frequency. Trisomy did not affect either chiasmata number or pairing of other chromosomes and most univalents arose from breakdown of trivalents. Thus trisome size and pairing had little or no detectable effect on trisome transmission.

Mean female transmission (24%) of ryegrass trisomes studied was similar to that reported for other diploid gramineae, e.g., *Hordeum spontaneum* (Tsuchiya 1960), *H. vulgare* (Tsuchiya 1967) and *Secale cereale* (Kamanoi and Jenkens 1962), but somewhat higher than the trisomes of *Avena strigosa* (Rajhathy 1975). None of the trisomes studied was pollen transmitted. Although larger chromosomes have a greater physical chance to pair and form trivalents than the smaller chromosomes (Einset 1943), neither female transmission nor pollen fertility was related to trivalent frequency in the trisomics studied.

In general, trisomy did not affect heading date. However, it is likely that the extra chromosome in plants 15, 63 and 68 delayed heading. Late heading was reported for trisomics of *Pennisetum americanum* (Gill et al. 1970) and *Avena strigosa* (Rajhathy 1975). A delayed heading in plants of self-incompatible species like ryegrass would selectively reduce transmission of a trisome due to lack of pollination and reduced fecundity.

Literature

Ahloowalia, B.S. (1964): A root-tip squash technique for screening chromosome number in *Lolium*. Euphytica 14, 170-172

Ahloowalia, B.S. (1972): Trisomics and aneuploids of ryegrass. Theor. Appl. Genet. 42, 363-367

Ahloowalia, B.S. (1973): Germination in vitro of ryegrass pollen grains. Euphytica 22, 575-581

Ahloowalia, B.S. (1976): Trisomics of perennial ryegrass. pp. 27-28 Res. Rep. Plant Sci. Crop Husb. Agric. Inst. Dublin 1976

Bradley, M.V. (1948): A method of making aceto-carmine squashes permanent without removal of the cover slip. Stain Technol. 23, 41-44

Buss, G.R.; Cleveland, R.W. (1978): Meiosis of trisomics of diploid alfalfa Crop Sci. 11, 808-810

Einset, J. (1943): Chromosome length in relation to transmission frequency of maize trisomics. Genetics 28, 349-364

Essad, S.; Arnoux, J.; Maia, N. (1966): Controle de validité des caryogrammes. Application au caryotype de *Lolium perenne* L. Chromosoma 20, 202-220

Gill, B.S.; Virmany, S.S.; Minocha, J.L. (1970): Primary simple trisomics in pearl millet. Can. J. Genet. Cytol. 12, 474-483

Kamanoi, M.; Jenkins, B.C. (1962): Trisomics in common rye. Seiken Ziho 13, 118-123

Myers, W.M. (1944): Cytological studies of a triploid perennial ryegrass and its progeny. J. Hered. 35, 17-23

Rajhathy, T. (1975): Trisomics of *Avena strigosa*. Can. J. Genet. Cytol. 17, 151-166

Tsuchiya, T. (1960): Cytogenetic studies of trisomics in barley. Jpn. J. Bot. 17, 177-213

Tsuchiya, T. (1967): The establishment of a trisomic series in a two-rowed cultivated variety of barley. Can. J. Genet. Cytol. 9, 667-684

Received November 7, 1980
Accepted March 28, 1981
Communicated by P.L. Pfahler

Mr. E.G.M. Meijer
Max-Planck-Institut
für Züchtungsforschung
D-5000 Köln 30
(Federal Republic of Germany)

Dr. B.S. Ahloowalia
Plant Breeding Department
The Agricultural Institute
Oak Park Research Centre
Carlow (Ireland)