Theor Appl Genet (1987) 74:233–241

Cytogenetics of *Lolium perenne*

4. Colchicine induced variation in diploids

L. M. Hague * and R. N. Jones

Department of Agricultural Botany, University College of Wales, Penglais, Aberystwyth, Dyfed SY23 3DD, UK

Received July 25, 1986; Accepted September 30, 1986
Communicated by R. Riley

Summary. A number of diploid inbred lines of *Lolium perenne* were treated with colchicine at the early seedling stage to induce chromosome doubling. In each inbred line the colchicine-treated undoubled diploids were kept as controls, as well as the normal untreated diploids. Comparisons of vegetative growth and development, involving the three treatments within each line, revealed that colchicine treatment of seedlings has long-lasting effects upon plant growth and development independent of chromosome doubling, and that for certain characteristics the effects of chromosome doubling are confounded with other effects of the treatment used to produce tetraploids. This colchicine induced variation in the diploids is transmitted through the seed generations in at least one of the inbred lines. The variation appears to be non-random and also shows a strong genotypic component. In so far as the effects of chromosome doubling could be determined, they showed the usual gigas response but were again strongly influenced by genotype.

Key words: *Lolium perenne* – Chromosome doubling – Colchicine-induced variation

Introduction

To investigate the true effects of chromosome doubling in perenniel ryegrass we have compared colchicine treated and untreated diploids and their derived tetraploids. A basic design fault of previous experiments of this kind has been removed by using homozygous inbred lines, so that we could separate effects due to doubling from those due to gene differences between diploids and colchicine induced tetraploid strains. We have also overcome another deficiency in earlier work by allowing for possible treatment effects due to colchicine itself, by using a double set of controls. In addition to the normal untreated diploids we have also kept the isogenic colchicine treated, but undoubled, diploids. As explained below, the experiment revealed some unexpected findings.

Materials and methods

The material consists of eight inbred lines of *L. perenne* which are of German origin. These "Deutsches Weidelgras", or WD, lines were bred by Utz and Oettler (1978) and the seed material was kindly supplied by Dr. U. Posselt of the State Plant Breeding Institute at the University of Hohenheim. The lines originate from the German ryegrass varieties 'Odenwalder' (lines 003, 038, 064 and 109), 'Odengrun' (206 and 221) and 'Semperweide' (375 and 380); and they were at the S10 generation at least when this work began.

Seeds were germinated on moist filter papers, in Petri dishes, in the autumn of 1981. At a height of 2–3 cm they were treated with colchicine. One batch was immersed in a 0.2% aqueous solution of colchicine at room temperature for 3 h, and another batch was immersed in water. After treatment, and washing, the seedlings were placed onto fresh filter papers and kept in Petri dishes for a further three days before transfer to potting compost in multitrays. The survivors, together with the water-treated controls, were transplanted into 5 inch pots and grown to maturity in an unheated greenhouse in 1982. When the plants flowered, a number of individual heads on each plant from the colchicine-treated individuals were fixed in Carnoy's fluid. Tillers were marked and the heads screened to determine their chromosome number. The majority of plants were found to have a mixture of both diploid and tetraploid tillers, and single tillers were grown again in 1983, by which time most plants were found to be either completely diploid or tetraploid. Both sets of plants from colchicine-

* Present address: Child Health Laboratory, University Hospital of Wales, Heath Park, Cardiff, UK

treated seeds were kept, so each inbred line had normal diploids (2x), colchicine-treated undoubled diploids (C2x) and colchicine-treated tetraploids (C4x).

Single tillers were taken from the 2x, C2x and C4x to produce 15 plants within each line, in the 3rd week of August 1983, for a growth analysis experiment in a heated greenhouse. They were started in multitrays for the first 6 weeks, and were then grown in 5 inch pots. There were 5 plants in each of three replicates which were randomised for lines and treatments. Supplementary lighting was provided to give a 16 h photoperiod. After 13 weeks, recordings were made on leaf widths and tiller numbers. Plants were then cut back for harvest to 3.5 cm above soil level. One week later the plants were repotted into 7 inch pots and moved into an unheated greenhouse in the same randomised layout as previously. Information on heading dates was recorded in May 1984. In August/September 1984 bagged heads were harvested and the plants cut back. Individual tillers were again taken and the plants grown to flowering once more in 1985. The total number of tillers at anthesis was the only character recorded in 1985.

Results

In the growth analysis experiment of 1983–84, nine characters of agronomic importance were recorded. Four of the characters – area of the 5th leaf, tiller number, fresh weight and dry weight – were examined in the early stages of vegetative growth, in the autumn of 1983, and the other five were dealt with at the time of heading, and after the mature heads were harvested, in the summer of 1984. The experiment was incomplete because some treatments and some individual plants were found not to have the expected ploidy level and were rejected. In particular, the C2x treatment was taken out of line 388 and the C4x-treatment was removed from line 038.

The results of the main experiment are given in the form of histograms in Figs. 1–9. These histograms show the patterns of variation in the treatments and lines, for the nine characters concerned, together with treatment means. The 2x means are based on all eight lines used, whereas the C2x and C4x means are based on seven lines only.

Analyses of the results are given in Tables 1–4. Table 1 compares the treatment means. These means were examined by two-way analysis of variance with two factors (lines and treatments), using replicate mean values (except for area of the 5th leaf). In order to take account of the missing treatments in lines 388 and 038, and to use all of the data, the means were examined in three separate pairwise combinations for each character (Table 1). The 2x/C2x comparison is meaningful in that it shows the effect of the colchicine treatment at the same levels of ploidy, and the C2x/C4x comparison shows the effect of chromosome doubling for identical colchicine treatments (on the assumption that colchicine affects 2x and 4x ploidy levels in the same line in the same way). The 2x/C4x comparison, which is the one usually made in this kind of work, is not meaningful in this context because the treatments are confounded.

In compiling Table 1 it became obvious that the characters fell into three distinct groups in their response to the three treatments, and the table is ordered accordingly into response groups I, II and III. The characters are dealt with in this way rather than in the natural sequence in which they were recorded, in order to simplify the interpretation of the results. The same order is used in the other tables, in the figures and in the description of individual characters in the text. In Table 1 the characters in group I show an effect which is due mainly to polyploidy, the character in group II shows a response to colchicine only and those in group III show effects due to both colchicine and chromosome doubling. The pattern is particularly clearcut if the table is read without the 2x/C4x comparison.

Table 1. Summary of analyses of variance to test for differences in the means of treatments in the growth analysis experiment of 1983–84. A one-way classification, on individual plant data, was used for the area of the 5th leaf, and a two-way analysis on replicate means was used for the other eight characters

	Character	Comparison of means		
		2x/C2x	2x/C4x	C2x/C4x
I	1. Area of 5th leaf 2. Heading date 3. No. flowering heads 4. Florets/mid spike	NS	Significant at either ** or *** level	
II	5. Length of head	***	***	NS
III	6. Tiller number 8. Dry weight	***	***	***
	7. Fresh weight 9. Spikelets/ear	***	NS	***

Table 2. Comparison of 2x/C2x treatments within lines

	Characters	Lines						
		003	064	109	206	221	375	038
I	1. Area of 5th leaf	.	.	**	.	.	.	*
	2. Heading date	**	.	.	.	*	.	.
	3. No. flowering heads	.	.	.	.	**	.	.
	4. Florets/mid spike	.	.	.	**	.	**	.
II	5. Length of head	.	.	**	**	.	.	.
III	6. Tiller number	**	.	.	.	**	**	**
	7. Fresh weight 8. Dry weight	**	.	.	.	**	.	**
	9. Spikelets/ear	*	.	**	.	**	.	**

Table 3. Comparisons of 2x/C4x treatments within lines

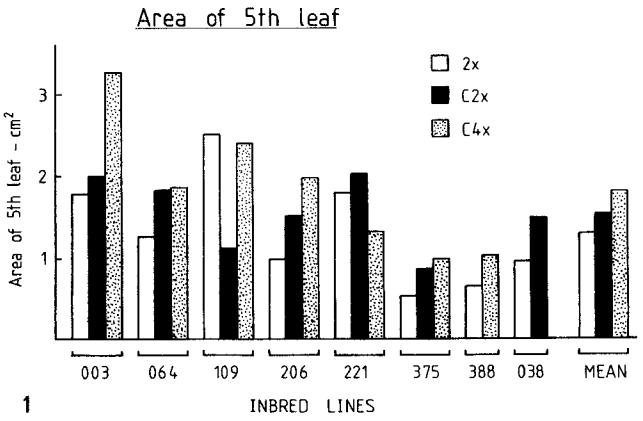
	Characters	Lines						
		003	064	109	206	221	375	388
I	1. Area of 5th leaf	**	.	.	**	.	.	.
	2. Heading date	**	**	.	*	.	**	**
	3. No. flowering heads	**	.	**	**	**	**	**
	4. Florets/mid spike	.	**	.	.	.	**	**
II	5. Length of head	.	.	**	**	.	.	**
III	6. Tiller number	**	.	**	**	.	**	**
	7. Fresh weight	.	*	**	**	.	.	**
	8. Dry weight	.	.	**	**	.	.	**
	9. Spikelets/ear	.	*	.	.	**	**	.

Table 4. Comparison of C2x/C4x treatments within lines

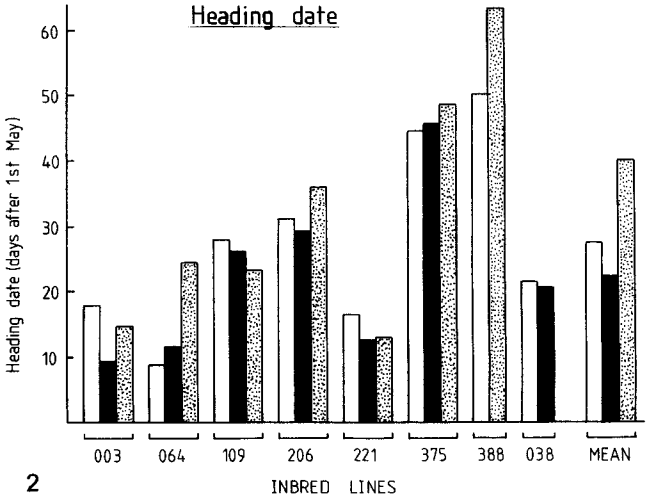
	Characters	Lines					
		003	064	109	206	221	375
I	1. Area of 5th leaf	**	.	**	.	.	.
	2. Heading date	**	**	.	.	.	*
	3. No. flowering heads	**	.	**	**	.	**
	4. Florets/mid spike	.	*	.	.	.	**
II	5. Length of head	.	.	**	.	.	.
III	6. Tiller number	**	**	**	**	**	**
	7. Fresh weight 8. Dry weight	**	.	*	.	**	.
	9. Spikelets/ear	**	**	.	.	.	**

The analyses summarised in Table 1 give levels of significance for treatment means only. Details of items for replicates, and between lines variation are not given. As it happens, the item for replicates is non-significant in almost every case, and the item for interactions between lines and treatments is significant in almost every case. There is thus a strong genotypic component to the variation. For a given character there is a differential response of the lines to the treatments involved, as can be clearly seen in Figs. 1–9. The between lines item is also highly significant for every character, confirming the strong genotypic component in the pattern of variation. This between line variation is to be expected in view of the inbred nature of the lines, and the fact that each line is comprised of a number of pure breeding individuals with unique and distinctive phenotypic traits.

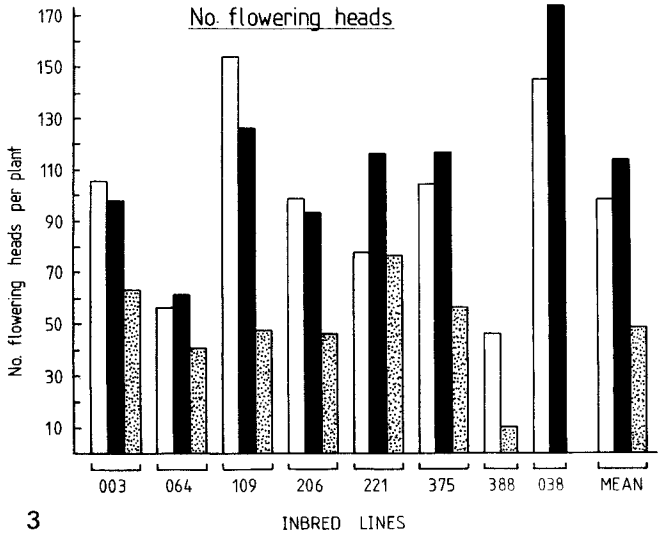
Variation between treatments within individual lines was analysed by one-way analysis of variance using individual plant data. A separate analysis was



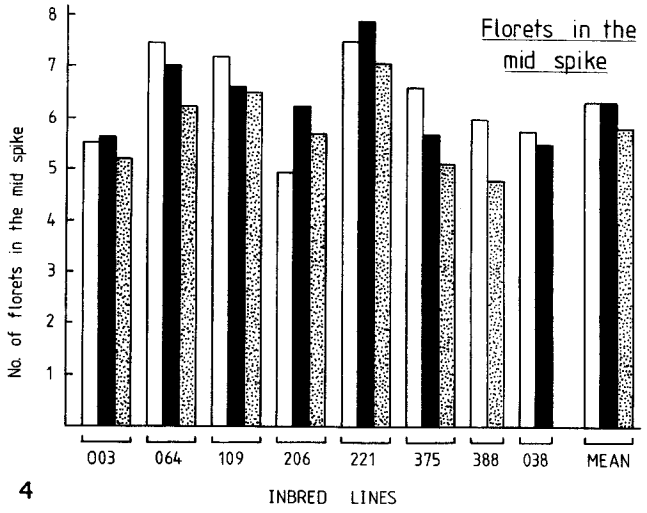
1



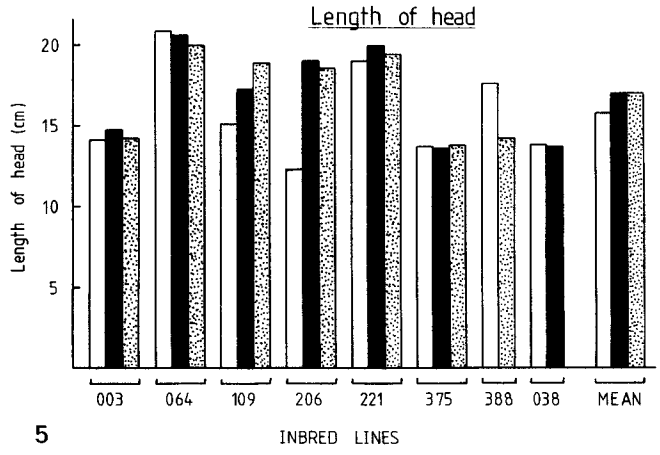
2



3

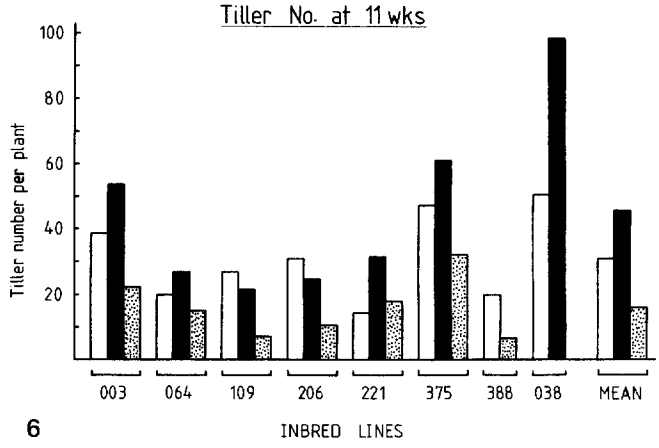


4

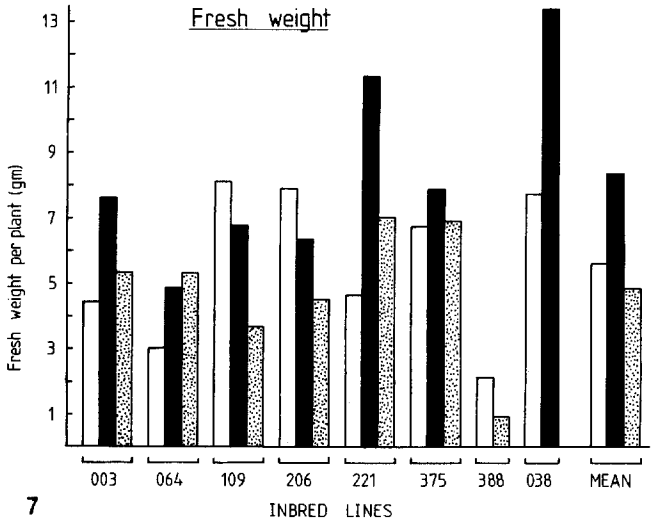


5

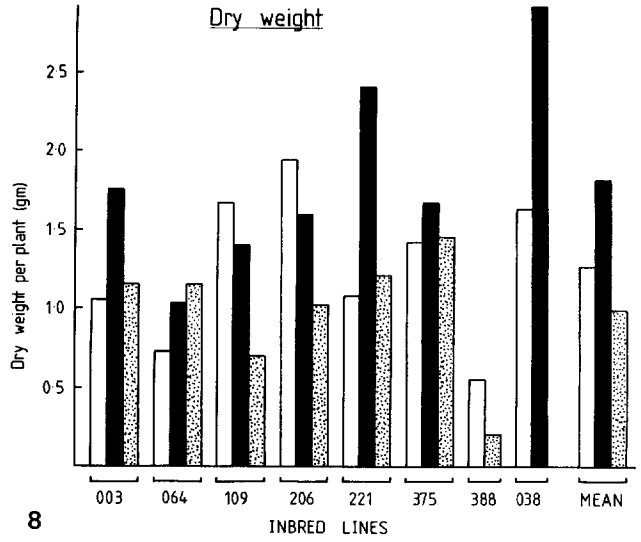
Figs. 1–9. Histograms showing the patterns of variation in the nine characters used in the growth anlaysis experiment in 1983–84, together with mean values for the three treatments applied to each character



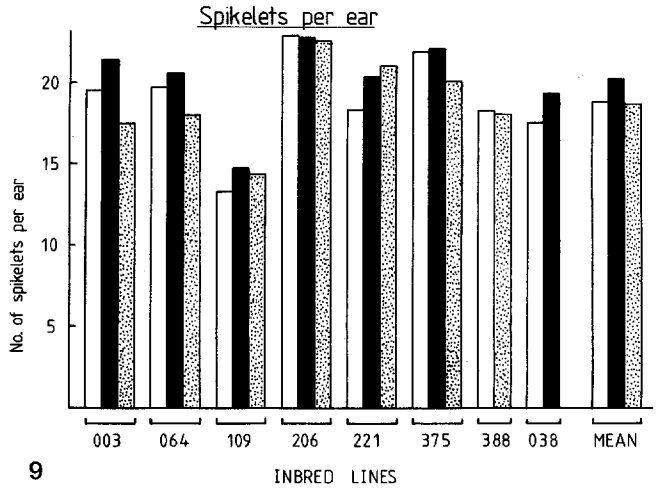
6



7



8



9

performed for each character for each of the three treatment comparisons, and the outcome of these analysis are given in Tables 2, 3 and 4, for 2x/C2x, 2x/C4x and C2x/C4x respectively. These analyses show in detail how the variation between treatments within lines contributes to the analysis of means given in Table 1. They can be 'read' in conjunction with the histograms in Figs. 1–9.

Area of the fifth leaf (cm²)

The area of the fifth leaf on each plant was determined using a Crump area meter. The fifth leaf was measured when the seventh leaf was half-way emerged from its leaf sheath, so that all plants were at an equivalent stage of development when the recordings were made. Leaves were removed early on in the experiment, before the plants were transferred to pots in a replicated layout, so that the analysis in Table 1 was made on individual plant data rather than a replicate means.

The analysis of means for this character, as well as for the other three characters in group I, indicates that the main treatment differences are due to chromosome doubling (Table 1, Fig. 1). There are significant differences between the mean values for the 2x (1.30 cm^2) and the C4x (1.82 cm^2), and between the C2x (1.54 cm^2) and the C4x. The 2x and the C2x do not differ significantly.

Heading date

Heading date was taken as days after the 1st May 1984, and was recorded as the date on which three heads had three florets emerged from their leaf sheaths. The

pattern of variation shown in Fig. 2 suggests that this character is primarily determined by genotype, and that chromosome doubling is also an important factor. The tetraploids (+31.95 days) are significantly later than both the normal diploids (+27.41) and the C2x colchicine-treated diploids (+22.12), and there is no significant difference between the means of the 2x and the C2x (Table 1).

Number of flowering heads

This character shows a strong effect due to chromosome doubling (Fig. 3, means). The tetraploids have about half the number (48.6) of heads of that found in the 2x (98.6) and C2x (113.7), and the differences are highly significant (Table 1). The 2x and C2x do not differ in their mean values.

Number of florets in the mid spike

The mean numbers of florets in the mid spike for the 2x, C2x and C4x treatments are 6.36, 6.36 and 5.79 respectively, and the C4x has significantly fewer than either the 2x or C2x (Table 1). The main effect on the means, therefore, is due to chromosome doubling.

The number of florets in the mid spike was multiplied by the number of spikelets/ear (see later) to give an estimate of the number of florets/ear. This character shows a pattern of variation which is virtually identical to that for the number of florets in the mid spike.

Length of head (cm)

Head length was measured on a sample of five heads from each plant. The means for the treatments are 15.77 (2x), 16.98 (C2x) and 16.99 (C4x). The 2x are significantly shorter than both the C2x and the C4x, and the C2x and C4x do not differ (Table 1). This is the only character for which the C2x and the C4x do not differ, indicating that variation in head length may be largely a colchicine-induced effect with little influence due to polyploidy.

Tiller number

The number of vegetative tillers in each plant was counted at two-weekly intervals, beginning one week after the start of the experiment. Only the 5th tiller count at week 11 was used in the analysis, and this is the only set of the tiller number data given here (Fig. 6). In the earlier counts the trends observed simply became more pronounced over time.

The analysis in Table 1 shows that the mean number of tillers differs between all three treatments. In the normal diploids there is a mean of 31 tillers per plant; the C2x has a mean of 45.3, and the C4x are the lowest at 15.74. The increased number in the C2x treatment, over the 2x, is consistent in that the C2x have significantly more tillers (lines 003, 221, 375 and 038) or else the difference is non-significant (Table 2). The effect is particularly striking in line 038 where the C2x plants have nearly twice as many tillers as the 2x (98.10 and 50.53, respectively). Likewise the C4x have consistently fewer tillers than the normal diploids, and this difference is significant in five of the seven lines (Table 3). The difference in mean between the C2x and the C4x, within lines, is significant in every case (Table 4).

This character, like the other three in this group, appears to be influenced by both the colchicine treatment and the chromosome doubling.

Fresh weight (gm)

The vegetative material was harvested on the 22nd of November, 1983, thirteen weeks after the start of the experiment, and the fresh weight per plant was determined. As the analysis (Table 1) and the histograms (Fig. 7) show the mean of the C2x treatment is greater than that of both the 2x and the C4x; but the 2x and C4x are not different. Means for the 2x, C2x and C4x are 5.57, 8.29 and 4.81, respectively.

Dry weight (gm)

After weighing, the fresh material was oven dried at 80 °C for a minimum of 8 h and then reweighed to obtain the dry weights for each plant. The pattern of variation in this character is very similar to that for the fresh weights (Table 1, Fig. 8), except that the differences between all three treatments are significant. The means are 1.26 (2x), 1.83 (C2x) and 0.99 (C4x).

Number of spikelets/ear

The number of spikelets per ear was counted in a sample of five heads per plant after the mature inflorescences had been harvested in 1984. The pattern of variation in the mean treatment values is identical to that for fresh weight (Table 1), and the numbers for the 2x, C2x and C4x treatments are 18.92, 20.14 and 18.82 respectively. Values for 2x and C4x are identical.

Tiller number in 1985

The plants from the growth analysis experiment were kept, at the end of 1984, and repotted as single tillers in the unheated greenhouse. Line 388 was lost over the winter. In the summer of 1985 the tillers were counted again, when the plants were at anthesis. At the time of this counting, the plants were in their 3rd season of growth and approaching 4 years of age from the time of colchicine treatment in 1981. The counts were made on

all of the tillers both vegetative and flowering, and the results are shown in the histograms in Fig. 10. Each treatment contained 10 plants and the data were analysed using a one-way classification.

As Fig. 10 shows, the three treatments are all different. Mean values are 41.33 (2x), 59.88 (C2x) and 27.33 (C4x), and pairwise comparisons show significance for all three pairs, $P < 0.001$ for 2x/C2x, $P < 0.05$ for 2x/C4x and $P < 0.001$ for C2x/C4x. The variation is due to both colchicine effects and chromosome doubling, as it was for the tiller numbers in 1983.

Seed transmission of the colchicine effect

In three of the inbred lines information is available on the transmission of the colchicine effect on tiller number through the sexual cycle, by selfing, in 2x and C2x plants. The seed used came from self-pollination of plants grown in 1983, and it was planted as unreplicated trials in multitrays in a heated greenhouse at the end of 1983. There were 20 plants in each treatment for each of the lines 003, 064 and 109. The results of the tests are given in Table 5. As the table shows, the differences between the means of the 2x and C2x treatments are significant in line 003, but not in lines 064 and 109. It will be recalled that in the growth analysis experiment the 2x/C2x comparison within lines also gave significance for tiller number differences in line 003, but lines 064 and 109 were non-significant.

The first generation of C2x plants, from the selfed seed of line 003, came into head and was bagged so that selfing took place to produce a second generation of selfed C2x seed. This second generation was grown in the heated greenhouse in the same way, in comparison with normal diploid seed, and the tillers counted again. Again the C2x plants had more tillers than the normal diploid, a mean of 5.6 compared to 3.7 ($t = 6.45^{***}$).

In one inbred line at least it seems fairly clear that the colchicine effect on tiller number is transmitted undiminished through at least two selfed seed generations. This finding is important because it removes any element of doubt that the effects may be due to mixoploidy in the C2x treatment.

Summary and interpretation of results

In order to summarise and interpret the results it is helpful to present Table 1 again in an altered form which shows only the two meaningful comparisons, and which includes symbols to show the direction of change in number, size and timing of the characters for the two treatments within each pair. The revised summary is given in Table 6.

The mean values for the four characters in group I differ because of effects due to chromosome doubling,

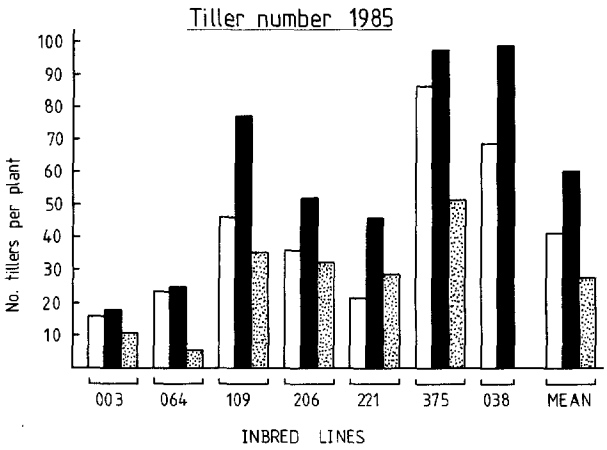


Fig. 10. Histograms showing the variation in tiller numbers in the seven lines grown in 1985, together with mean values for the three treatments applied to the lines

Table 5. Tiller numbers at six weeks (003) and nine weeks (064, 109) of age in the progeny plants grown from selfed seed of normal diploids and first generation selfed seed of C2x plants in three of the inbred lines

Line	No. of tillers		*t*-tests
	2x	C2x	
003	8.25	11.90	$t = 3.38^{**}$
064	21.95	21.75	$t = 0.16$, NS
109	10.80	10.85	$t = 0.09$, NS

Table 6. Summary of meaningful comparisons between treatment means, together with signs showing the direction of number or size relationships of character difference between pairs of treatments. The symbols indicate the relationships of the second treatment to the first one in each pair; e.g. for 2x/C2x the > sign means that the C2x has more than, or larger than the 2x for the character concerned

	Character	Comparison of means	
		2x/C2x	C2x/C4x
I	1. Area of 5th leaf	NS	** >
	2. Heading date		
	3. No. flowering heads	NS	*** <
	4. Florets/mid spike		
II	5. Length of head	*** >	NS
III	6. Tiller no. 11 wks	*** >	*** <
	7. Fresh weight		
	8. Dry weight		
	9. Spikelets/ear		
	10. Tiller No. 1985		

the single character in group II differs in means due to colchicine and the other four, in group III have responded to both treatments. In group III it can also be seen that the effects due to colchicine are in the opposite direction from those due to tetraploidy.

Another important aspect to note, which can be observed from Table 2 and Figs. 1–10, is that the colchicine effect in Groups II and III is *non-random.* When the 2x/C2x comparisons are studied for individual lines it can be seen that for each character the difference between the two treatments is either significant in the *same direction,* or else it is non-significant. In other words, the colchicine appears to induce directional changes rather than random mutations.

As far as polyploidy is concerned the differences between lines are again consistent. They are either significant in the same direction or else they are non-significant (Table 4, Figs. 1–10). Much of the contrasting variation in treatments between different lines, in Figs. 1–10, is therefore due to error, and the real differences are in line with the treatment means.

In one line at least the colchicine effect is transmitted through two selfed-seed generations.

Table 2 shows that lines 003, 221 and 038 are the most sensitive to the colchicine effect, and that lines 064 and 206 are the least sensitive. Line 003 responds most to chromosome doubling and line 206 is the least affected (Table 4). The characters which are most sensitive to colchicine are tiller number and spikelets per ear (Table 2), and the one least affected is the number of flowering heads. In contrast the number of flowering heads gives the greatest response to chromosome doubling, while area of the 5th leaf gives the least.

Discussion

As far as we can see the outcome of chromosome doubling in this material is broadly in line with previous results, (Myers 1939, 1947; Hill and Myers 1944; Shalygin 1941; Wit 1959; Ahloowalia 1967; Van Bogaert 1975) but there are two new elements which warrant further consideration.

In the first place, there are quite significant differences in relation to genotype. This genotypic variation affects all of the characters and its expression gives different results for different aspects of growth and development. The average level of response also comprises a wide range of individual line effects, and conceals quite marked performance differences of certain genotypes. In the utilisation of polyploidy therefore particular attention should be paid to the genotype response – as far as this is possible in an outbreeding species.

Secondly, it is evident that the true effects of chromosome doubling in *Lolium perenne* remain unknown. For several characters the effects of doubling are confounded with the colchicine treatment, and this element appears to have been overlooked thus far. It is true that some investigators have compared C2x with C4x treatments, and this has been done where clonal pairs of plants have been used, from individual mixoploids, in an attempt to secure isogenic clones at both ploidy levels from heterozygous material (e.g. Hill and Myers 1944; Wit 1959). But in these cases the normal diploids could not be used, so the colchicine effect would have passed unnoticed. In most other cases investigators have seen no reason to use C2x controls in their experiments, even though they have drawn attention to the differential response of varieties to colchicine treatment (Ahloowalia 1967). One difficulty with the present analysis is that we still do not have an ideal comparison between normal 2x lines and their *normal* autotetraploids. To achieve this we would need to double the lines by some natural method if one could be found. The signs so far suggest that such natural autotetraploids should be inferior to those made with colchicine, at least in the CO generation, because of the opposing effects of colchicine and chromosome doubling. In other words, for several of the characters described here, we need to subtract out a fair proportion of the C4x performance in order to determine that residual part which is due to the tetraploidy alone. The fundamental truth, at this preliminary stage, is that the C2x treatment in *L. perenne* appears to offer a better prospect for plant improvement than do the C4x autotetraploids.

The colchicine effect found in this inbred material of perennial ryegrass appears to be strongly persistent, but as yet its genetic basis and mode of action remain unknown – except to say that by the very nature of the material any form of selection among the treated seedlings can be ruled out. It has long been known, however, that colchicine has mutagenic properties, and there is also a wealth of literature concerning its physiological and biochemical effects on both plant and animal tissues. The subject has been reviewed in detail by Dermen (1940) and by Eigsti and Dustin (1954). Of particular interest, in relation to the present work, is the comprehensive analysis carried out on colchicine-induced mutation in *Sorghum* (Franzke and Ross 1957; Erichsen et al. 1962; Foster et al. 1961ab; Sanders et al. 1962). In *Sorghum* colchicine treatment of seeds induces point mutations at many loci distributed over several chromosomes. Reciprocal crosses confirmed a nuclear basis to the induced heritabale changes. Multiple mutational events attributable to colchicine have also been described in barley (Gilbert and Patterson 1965) and to a lesser extent in lettuce (Eenink and Groenwold 1981). The possibility exists in *L. perenne* therefore that induced gene mutation occurring at many loci in the inbred lines could restore a measure of heterozygosity and give rise to heterotic effects. Such a mechanism would account for the directional aspect of the changes, and experiments could no doubt be designed to test for this possibility. In any event we have not observed any

qualitative variation in our treated lines, and have not as yet found any evidence to indicate that major gene mutations have occurred. Another possibility to consider is that of DNA sequence amplification of the kind associated with environmentally induced heritable changes in flax (Durrant 1962; Evans 1968; Cullis 1979, 1983) and various other kinds of genome change associated with stress conditions in plants (Walbot and Cullis 1985). To what extent the effects observed here are a manifestation of changes in the self-assembly of tubulin molecules, and of the cytoskeletal properties of cells, is also unknown at this stage. The answer of this question will be resolved at a later stage when reciprocal crosses have been made between the 2x and C2x treatments within lines.

On a final point of discussion it should also be mentioned that colchicine can affect characters associated with chromosome pairing behaviour at meiosis. One of the earliest reports on such effects comes from Sparrow (1942) working on *Antirrhinum majus*. Young cuttings of *Antirrhinum* were immersed in colchicine for varying periods of time and then taken out and grown on to flowering. A small increase in the number of univalents was found in treated plants some 15 weeks after the time when the treatment was given. Finch and Bennett (1979) reported a similar small effect in barley treated as seedlings.

Studies are in hand to determine the genetic basis of the colchicine-induced variation in diploid inbred lines of *L. perenne*, and to monitor its longer term effects.

Acknowledgements. We wish to thank Dr. E.L. Breese and Mr. Eric Stevens of the Welsh Plant Breeding Station for advice on the design of the growth analysis experiment. Dr. Breese also read the manuscript. We thank Mrs. Mared Breese for her invaluable technical assistance and our colleague Dr. G.M. Evans for advice on statistical matters.

The work was supported by Grant No. AG 2/85 from the AFRC.

References

Ahloowalia BS (1967) Colchicine induced polyploids in ryegrass. Euphytica 16:49–60

Cullis CA (1979) Quantitative variation of ribosomal RNA genes in flax genotrophs. Heredity 42:237–246

Cullis CA (1983) Environmentally induced DNA changes in plants. CRC Crit Rev Plant Sci 1:117–131

Dermen H (1940) Colchicine polyploidy and technique. Bot Rev 6:599–635

Durrant A (1962) The environmental induction of heritable change in *Linum*. Heredity 17:27–61

Eenink AH, Groenwold R (1981) Increase of genetic variation in some lettuce cultivars by induction of mutations: a technical note. Euphytica 30:735–737

Eigsti OJ, Dustin P (1954) Colchicine in agriculture, medicine, biology and chemistry. Iowa State College Press, Ames, Iowa, 470 pp.

Erichsen AW, Franzke CJ, Sanders ME, Ross JG (1962) Colchicine-induced mutations in Sorghums which affect qualitative characters. J Hered 53:304–308

Evans GM (1968) Nuclear changes in flax. Heredity 23:25–38

Finch RA, Bennett MD (1979) Meiotic stability in control and newly colchicine-induced dihaploid barley. Can J Genet Cytol 21:33–35

Foster AE, Ross JG, Franzke CJ (1961a) Proximity of mutated genes in colchicine-induced mutants of Sorghum. Crop Sci 1:72–75

Foster AE, Ross JG, Franzke CJ (1961b) Estimates of the number of mutated genes in a colchicine-induced mutant of Sorghum. Crop Sci 1:272–276

Franzke CJ, Ross JG (1957) A lineal series of mutants induced by colchicine treatment. J Hered 48:47–50

Gilbert SK, Patterson FL (1965) Colchicine-induced mutants of 'Decatur' barley. Crop Sci 5:44–47

Hill HD, Myers WM (1944) Isolation of diploid and tetraploid clones from mixoploid plants of ryegrass (*Lolium perenne* L.) produced by treatment of germinating seeds with colchicine. J Hered 35:359–361

Myers WM (1939) Colchicine-induced tetraploidy in perennial ryegrass. J Hered 30:499–504

Myers WM (1947) Cytology and genetics of forage grasses. Bot Rev 13:319–367

Sanders ME, Franzke CJ, Ross JG (1962) Genetic studies of nontrue-breeding mutants in Sorghum obtained after colchicine treatment. Crop Sci 2:387–390

Shalygin IN (1941) Production of tetraploids in *Lolium* by treating germinating seeds with colchicine. CR Dokl Acad Sci URSS 30:527–529

Sparrow AH (1942) Colchicine induced univalents in diploid *Antirrhinum majus* L. Science 96:363–364

Utz HF, Oettler G (1978) Performance of inbred lines and their top crosses in perennial ryegrass (*Lolium perenne* L.). Z Pflanzenzücht 80:223–229

Van Bogaert G (1975) A comparison between colchicine-induced tetraploid and diploid cultivars of *Lolium* species. In: Nuesch B (ed) Ploidy in fodder plants. Eucarpia Rep Meet Fodder Crops Sect, Zürich, pp 101–110

Walbot V, Cullis CA (1985) Rapid genomic change in higher plants. Annu Rev Plant Physiol 36:317–396

Wit F (1959) Chromosome doubling and the improvement of grasses. Genet Agrar 11:97–115