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Heritable nature of colchicine induced variation in diploid *Lolium perenne*

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Seedlings of perennial ryegrass from several inbred lines were treated with colchicine, and from the mixoploids produced some diploid tillers were recovered. When these diploid colchicine-treated plants were compared with their isogenic untreated diploids effects on agronomic characters due to the colchicine were discovered. These colchicine-induced changes have remained stable over seven years of vegetative growth and have now been shown to be sexually transmitted through a selfed-seed generation.

INTRODUCTION

In a previous publication we reported on a novel source of genetic variation which was induced by colchicine treatment of seedlings of inbred lines of perennial ryegrass (Hague and Jones, 1987). The variation was found by isolating diploid tillers from mixoploids following colchicine treatment of seedlings. These effects are novel because in other work where colchicine has been used as a chromosome doubling agent the undoubled diploid controls have not been used to check for effects due to the colchicine treatment itself. Previously we found that in the treatment generation there are long lasting effects of the colchicine on several characters of agronomic significance. In this report we confirm the stability of these effects over seven years of vegetative propagation, and we further demonstrate their heritable nature by transmission through a selfed-seed generation.

MATERIALS AND METHODS

The material is the same as that described in a previous paper (Hague and Jones, 1987), the original plants having been maintained by vegetative propagation since their treatment in 1981. There are seven control diploid inbred lines, which are known as the 2x lines, and seven corresponding "isogenic" colchicine-treated diploid lines which are known as the C2x lines. Single tillers were taken from each of 15 2x and 15 C2x plants in each line in August 1987 and these were used as a continuation of the treatment generation used previously for observations on agronomic characters. The tillers were started off in plastic multitrays in John Innes potting compost, in an unheated glasshouse, and grown for 6 weeks. They were then transferred to 5″ pots and observations made on the numbers of vegetative tillers at 3, 5, 7, 9 and 11 weeks, and on fresh and dry weights, at 13 weeks from the date when the tillers were taken. These developmental stages correspond with those used in the 1983–84 experiment. We have labelled these vegetatively propagated plants as the CT0 generation (*i.e.*, the generation in which the colchicine treatment was given). In the autumn of 1988 these plants were seven years old from the date of treatment.

In addition the selfed-seed was threshed from the bagged heads of the plants used in the original study of 1983–84, and these constitute the CT1 generation (*i.e.*, the first selfed-seed generation of the colchicine treated material). The CT0 and CT1 designations are used to describe the generations of both the 2x and C2x lines, notwithstanding the fact that the 2x lines are untreated homozygous lines which were at S10 when the programme began in 1981. The 2x and C2x CT1 seeds were "sown" on moist filter paper in petri dishes in November 1986, and placed in a refrigerator at 4°C for one

week to aid germination. After germinating they were planted in John Innes compost and grown on in plastic multitrays in an unheated glasshouse for 6 weeks. They were then transferred to 5″ pots and grown through to September 1987. After one year of vegetative growth, and chromosome counting, the plants were split down and single diploid tillers were then treated in the same way, and at the corresponding times, as the CT0 material. In the CT1 generation only five lines are represented, as no selfed seed was available for lines 206 and 375. There were 15 2x and 15 C2x plants in each line.

RESULTS

The results for variation in tiller numbers at 11 weeks, and for fresh and dry weights at 13 weeks, for both the CT0 and the CT1 generations, are given as mean values per line in table 1. Analyses of variance of the data, together with that for the CT0 of 1983–84, are summarised in table 2.

The CT0 generation

In every line the C2x plants have a higher mean number of tillers than the isogenic 2x controls,

Table 1 Mean values per line for tiller number and fresh and dry weights, for the 2x and C2x treatments, in the CT0 and CT1 generations of *Lolium perenne* in 1988

		Tiller number—11 weeks		Fresh weight—13 weeks		Dry weight—13 weeks	
Lines	($n = 15$)	CT0	CT1	CT0	CT1	CT0	CT1
003	2x	10·3 ± 0·71	11·7 ± 1·45	0·47 ± 0·05	0·63 ± 0·10	0·14 ± 0·02	0·22 ± 0·03
	C2x	11·3 ± 0·75	9·3 ± 1·36	0·77 ± 0·06	0·49 ± 0·10	0·24 ± 0·02	0·16 ± 0·03
038	2x	9·5 ± 0·72	12·9 ± 1·52	0·47 ± 0·05	0·78 ± 0·12	0·16 ± 0·02	0·22 ± 0·04
	C2x	10·9 ± 1·02	23·2 ± 1·96	0·55 ± 0·07	2·06 ± 0·27	0·17 ± 0·02	0·58 ± 0·07
064	2x	9·1 ± 0·73	6·4 ± 0·83	0·39 ± 0·06	0·27 ± 0·04	0·11 ± 0·02	0·08 ± 0·01
	C2x	10·9 ± 0·77	8·8 ± 1·82	0·59 ± 0·05	0·45 ± 0·13	0·19 ± 0·02	0·14 ± 0·03
109	2x	7·3 ± 0·73	6·4 ± 0·61	0·62 ± 0·05	0·44 ± 0·04	0·19 ± 0·01	0·11 ± 0·01
	C2x	9·5 ± 0·77	9·0 ± 0·73	1·16 ± 0·11	0·73 ± 0·06	0·30 ± 0·02	0·20 ± 0·02
206	2x	5·2 ± 0·38	—	0·38 ± 0·04	—	0·17 ± 0·01	—
	C2x	6·0 ± 0·36	—	0·53 ± 0·05	—	0·22 ± 0·02	—
221	2x	2·5 ± 0·26	3·0 ± 0·35	0·17 ± 0·03	0·48 ± 0·08	0·07 ± 0·01	0·12 ± 0·02
	C2x	3·9 ± 0·25	4·9 ± 0·64	0·41 ± 0·04	0·75 ± 0·12	0·17 ± 0·01	0·02 ± 0·03
375	2x	5·5 ± 0·52	—	0·18 ± 0·03	—	0·06 ± 0·01	—
	C2x	6·8 ± 1·00	—	0·20 ± 0·04	—	0·09 ± 0·02	—
Mean	2x	7·1	8·1	0·38	0·52	0·13	0·15
	C2x	8·5	11·0	0·60	0·90	0·20	0·26

Table 2 Summary of one-way analyses of variance to test for differences in means of the 2x and C2x treatments within lines, and for the overall means of the lines, for the three agronomic characters used. A summary of the relevant part of the 1983–84 analysis is given for comparison, as well as those for the CT0 and CT1 generations in 1988

	Line							
Character	003	064	109	206	221	375	038	Mean
CT0 generation 1983–84 ($n = 15$)								
Tiller no.	**	ns	ns	ns	**	**	**	***
Fresh wt.	**	ns	ns	ns	**	ns	**	***
Dry wt.	**	ns	ns	ns	**	ns	**	***
CT0 generation 1988 ($n = 15$)								
Tiller no.	ns	ns	ns	ns	**	ns	ns	**
Fresh wt.	**	**	***	*	***	ns	ns	**
Dry wt.	**	**	***	*	**	ns	ns	**
CT1 generation 1988 ($n = 15$)								
Tiller no.	ns	ns	*	—	ns	—	**	*
Fresh wt.	ns	ns	**	—	*	—	**	**
Dry wt.	ns	ns	**	—	*	—	**	**

although the difference is only significant in line 221. The overall means (7·1 for the 2x and 8·5 for the C2x) differ significantly at the 1·0 per cent level, and on average the 2x plants have 83·5 per cent of the tiller number of the C2x.

For fresh weights and dry weights the patterns are virtually identical, and the differences between treatments are more pronounced than those for tiller number. Five of the seven lines show significant effects in both cases, and the mean difference over all lines is again significant at the 1·0 per cent level for both. In the case of fresh weight the 2x lines have a mean (0·38 g) of 63 per cent of the C2x (0·60 g), and the biggest difference is in line 109 where the 2x and C2x values are 0·62 g and 1·16 g/plant respectively. Corresponding dry weight differences are 65 per cent for the means and 0·11 g and 0·19 g for the 2x and C2x of line 064.

In the CT0 generation the differences between the 2x and C2x treatments remain undiminished after 7 years of vegetative propagation from the date of treatment as seedlings in 1981, despite the fact that the plants are heavily infected with viruses and in a degenerating state of health. As table 2 shows there is some inconsistency in the pattern from year to year, and there appears to be a strong genotype-environment interaction as well as genotypic effects between the lines.

The CT1 generation

The analysis in table 2 shows a significant increase in tillering in C2x, compared with the 2x, in lines 109 and 038, and also in the means of all lines. Line 038 displays the most marked effect where the C2x (23·2 tillers) has almost double the number of the 2x (12·9). Overall the 2x have 73 per cent of the tillers of the C2x, so the difference between the two treatments is greater than that in the CT0 material.

Fresh and dry weights again give the same pattern. There is significantly more vegetative production in the C2x in lines 109, 221 and 038, and the overall means are also significant ($P < 0{\cdot}01$). The 2x overall means as a percentage of the C2x are 58 per cent and 57 per cent for the fresh and dry weights respectively, and in line 038 which has the biggest difference the C2x has more than 2·6 times the fresh and the dry weight of the 2x.

It will be seen in table 1 that the CT1 values, for all three characters, are larger than those of the CT0 in both 2x and C2x treatments. This is due to the fact that the CT0 plants were seven years old in 1988 whereas the CT1 were in their first year of growth from seed.

The genotypic component

It is clear from the data in table 1 that there is considerable variation in the values of the agronomic characters between lines. This variation is to be expected as a consequence of gene segregation during inbreeding and production of the lines, but there is also considerable interaction with seasonal effects which applies to both the 2x and C2x treatments.

In the CT0 2x controls the range of variation for tiller number goes from 10·3 in line 003 down to 2·5 in line 221, and the variation for fresh and dry weights are of a similar order of magnitude. When comparisons are made with the previous experiments of 1984 and 1985 (Hague and Jones, 1987) the order of ranking of the lines is not entirely consistent, even though the plants have been maintained throughout by vegetative propagation. For tiller numbers line 038 is consistently high, line 221 consistently low and the other lines change their relative order. This interaction between lines and seasons applies also to the fresh and dry weight data. In the CT0 C2x the variation between lines is likewise extensive for all three characters, and it shows an equally strong interaction with seasons.

Comparisons of line mean values of the CT0 with the CT1 generations by regression analysis gives no significance for any of the characters in either the 2x or the C2x treatments. Only five lines are available for these comparisons, and this is probably insufficient to demonstrate heritability in this precise way especially in view of the strong interaction of these characters with environmental effects.

DISCUSSION

In the previous publication in this series it was firmly established that a short burst of colchicine treatment (0·2 per cent for 3 h) applied to one week old seedlings of inbred *L. perenne* induced durable and directional changes in a number of agronomically useful characters. The effects were described in detail in the treatment generation and were shown to be stable over several years of vegetative reproduction. Preliminary evidence was also given which suggested that the effects were of a heritable nature in sexual reproduction. In this report the evidence is provided to firmly establish that these directional changes, at least in terms of

tiller numbers and fresh and dry weights of leaf material, are transmitted through the sexual cycle of reproduction, and that in this sense they are heritable. For tillers, the C2x plants in the CT0 generations have 121 per cent the mean number of the 2x, and in the CT1 the corresponding mean value is 136 per cent. The mean percentage increase of the C2x over the 2x is similar for fresh and dry weights, and for fresh weight the value is 167 per cent in the CT0 and 173 per cent in the CT1. The average level of change is therefore considerable, and is undiminished between the treatment generation and one that follows.

The mutagenic effects of colchicine, in terms of major gene changes, are well known. Burnham (1962) reviews the early literature and a more detailed discussion on this question was also included in our previous report (Hague and Jones, 1987). A more recent report, concerning major gene mutations for desynapsis in *Lathyrus* is given by Khawaja and Ellis, 1987.

In the *Lolium* inbred lines we have not observed any obvious qualitative changes in either the CT0 or the CT1 generation plants. The changes we have so far found are all for quantiative characters. The variation is non-random and the changes are always in one direction in the affected lines, or else they are nonsignificant. It is possible that we are seeing a manifestation of heterozygosity brought about by multiple point mutations at numerous loci, in which case we should expect some evidence of gene segregation in the CT1 and later generations. If this explanation holds then we might also anticipate that other mutagens would bring about similar effects.

The changes appear to act at a fundamental level on many aspects of plant development, particularly on tillering and on the capacity for vegetative growth. Another possibility is that some alterations have been induced in genome organisation, such as the amplification of ribosomal RNA cistrons, or other families of repeated sequences of the kind described in some flax genotrophs (see Walbot and Cullis, 1985; and Cullis 1986 for review and references).

The nature and causes of the induced changes in perennial ryegrass are unknown. The precise treatment conditions are also unknown, and we have yet to ascertain the degree to which multiple treatments, and treatments on a range of other species, may be effective. We have also to consider the expression of these effects at the ceullular, organelle and molecular levels, and this work is in progress.

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