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GENIC CONTROL OF ANEUPLOIDY IN *PENNISETUM*

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1. INTRODUCTION

Perennial *Pennisetum dubium* is an irregular high polyploid in which chromosome numbers vary from 14 to 84 in root tips, 47 to 74 at MI and 9 to 37 at MII. This variation, which occurs from cell to cell in the same root tip or anther, has been shown to be the result of various mitotic and meiotic abnormalities which are under genetic control. The single perennial hybrid plant, produced from crosses between this species and the regular annual diploid *P. typhoides* ($2n = 14$), has only 21 chromosomes (Gildenhuys and Brix, 1958).

The mitotic behaviour of the 21 chromosomes of this sterile hybrid is nearly normal, but when attempts were made to double the complement by colchicine treatment, abnormalities occurred which give further evidence of genetic control of aneuploidy.

Although many different techniques were employed in attempting to double the chromosome number of the hybrid, none was successful. This was unexpected, since neither Krishnaswamy and Raman (1954) nor ourselves, using different techniques, had great difficulty in obtaining amphidiploids from the hybrid *P. typhoides* ($2n = 14$) $\times$ *P. purpureum* ($2n = 28$). When it became evident that the hybrid between *P. typhoides* and *P. dubium* was reacting differently to colchicine from that between *P. typhoides* and *P. purpureum*, identical treatments were applied to each hybrid to try to determine the nature of the difference between the two.

2. METHODS

Two or three cuttings, each consisting of two to three nodes, were taken from the lower part of mature tillers. These cuttings were totally immersed in a 0.01 per cent. aqueous solution of colchicine for 3 days and then washed and placed in a

nutrient solution. All the root tips which developed at the nodes were removed and fixed every 24 hours over a period of 16 days, after which the cuttings which were still alive were planted in the field. When the cuttings had grown in the field for one year, chromosome counts were again made in the root tips. Because of the failure to double the chromosome number of the hybrid *P. typhoides* × *P. dubium*, cuttings were taken after one year from the previously treated plants of this hybrid and subjected to exactly the same treatment as the original cuttings. Altogether over 100 cuttings of *P. typhoides* × *P. dubium* and over 50 cuttings of the *P. typhoides* × *P. purpureum* hybrid were treated and of these 26 per cent. and 32 per cent. respectively survived.

Chromosome counts were recorded only for intact cells in which there could be no doubt as to the exact number of chromosomes present.

3. OBSERVATIONS

Before treatment no mitotic abnormalities were found in the hybrid *P. typhoides* × *P. purpureum* ($2n = 21$), while in *P. typhoides* × *P. dubium* only 2 cells out of 153 had chromosome numbers differing from 21—these two cells had 24 and 25 chromosomes respectively.

After treatment, most of the root tips of *P. typhoides* × *P. purpureum* had either 21 or 42 chromosomes, but sometimes there was a mixture of 21 and 42, or numbers in the neighbourhood of 84. After one year, the surviving plants either had 21 or 42 chromosomes, indicating that the mixoploids had stabilised at either 21 or 42.

In *P. typhoides* × *P. dubium* on the other hand, the effect of colchicine was quite different. Not only did a wide variation in chromosome number occur from cell to cell, but abnormalities similar to those observed in the polyploid parent (Gildenhuys and Brix, 1958) made their appearance.

In the first 16 days after treatment chromosome numbers varied from 14 to over 42, even in the same root, but, because of the difficulty of making accurate counts, numbers higher than 42 were not recorded. These higher numbers were, however, not always in the neighbourhood of 84, as might have been expected. The most frequent number was 21, which occurred in 32 per cent. of the 47 cells examined (fig. 1A). One year later, the range had narrowed down to 15-25, and by then the vast majority of cells (88 per cent.) contained 21 chromosomes (fig. 1B).

The results of the second treatment were similar to those of the first. Counts made for 16 days after treatment showed that the range of chromosome numbers had again increased to 14 to over 42, with 21 again the most frequent number (27 per cent. of 130 cells—fig. 1C). Four months later this range had narrowed down to 14-25 and of the 59 cells counted 66 per cent. had 21 chromosomes.

After both treatments, tripolar, quadripolar and split spindles, groups of chromosomes, etc., such as have been described in detail (Gildenhuys and Brix, 1958) were observed, but with a much lower frequency than in the polyploid parent. These abnormalities may lead to chromosome elimination and they probably account for the aneuploidy following colchicine treatment.

4. DISCUSSION

The two perennial hybrids, whilst similar in their mitotic behaviour, react in a strikingly different manner to treatment with colchicine. They have a common female parent in the regular annual diploid *P. typhoides*

and have therefore the same cytoplasm. The male parents are, however, very different. *P. purpureum* is a regular tetraploid and the hybrid with *P. typhoides* behaves as a regular amphidiploid when the chromosome complement is doubled (Krishnaswamy and Raman, 1954). *P. dubium*, on the other hand, is an irregular high polyploid which, although a facultative aposporic apomict (Gildenhuys and Brix, 1959), can produce fertile

FIG. 1.—Frequency distributions of different chromosome complements per cell in root tips of the hybrid *P. typhoides* $\times$ *P. dubium* after treatment with colchicine.

A. First sixteen days after treatment.

pollen grains with only 14 chromosomes. The aneuploidy in this species, which has a most frequent chromosome number of $2n = 66$, is due to various mitotic and meiotic abnormalities. The frequency of these abnormalities varies from plant to plant and can, therefore, be assumed to be under genetic control (Gildenhuys and Brix, 1958). In its untreated hybrid with 21 chromosomes, the genes or gene complexes which control these abnormalities do not express themselves, but when the chromosome

number is doubled, even temporarily, the abnormalities again make their appearance. It would seem then that a higher gene dosage or a higher chromosome number is necessary before the abnormalities show themselves.

An explanation may lie in the relationship between the two parental species. There appears to be little affinity between *P. typhoides* and *P. dubium* (Gildenhuys, 1958), so that the hybrid is in fact a very wide cross.

FIG. 1.

B. One year after treatment.

It is possible that a cytoplasmic barrier to the expression of genes in low dosage may occur in such a wide cross, but on doubling the chromosome number, and hence the gene dosage, this barrier is overcome and the threshold necessary for chromosome elimination is passed. Once the process of elimination becomes operative, it will continue even to the extent of producing cells with less than the original 21 chromosomes, the number of chromosomes remaining possibly depending upon which chromosomes

are eliminated. As times goes on, cells with highly reduced chromosome numbers are evidently eliminated, probably through competition, as is shown by the tendency to stabilise at 21 (cf. figs. 1A, 1B, 1C). This is probably the "balanced" number for the hybrid, in the same way as $2n = 66$ could be considered to be the "balanced" number for the polyploid parent. In the latter, which produces seed, the *status quo* is maintained by apomixis.

Whatever the mechanism which causes aneuploidy to be expressed in

FIG. 1.

C. First sixteen days after second treatment.

the treated and not in the untreated cuttings of *P. typhoides* × *P. dubium*, the origin of the difference in response to colchicine between this hybrid and the hybrid *P. typhoides* × *P. purpureum* seems clear. The two hybrids have the same species as female parent and thus have cytoplasm and one set of chromosomes in common, so that the main difference between the two must therefore lie in the chromosomes they received from their male parents. Each received 14 chromosomes, the one from an irregular polyploid in which aneuploidy is genetically controlled, the other from a regular tetraploid (in which aneuploidy has not been reported). The aneuploidy occurring in the treated hybrid *P. typhoides* × *P. dubium* and which is absent

in treated *P. typhoides* × *P. purpureum* must therefore be assumed to be due to genes carried in the 14 chromosomes received from the polyploid parent. With these genes functioning when the chromosome number is raised, it may be impossible permanently to double the chromosome complement of such a hybrid.

5. SUMMARY

1. Two hybrids, *P. typhoides* × *P. purpureum* and *P. typhoides* × *P. dubium*, both with 21 somatic chromosomes, behave normally in mitosis.

2. When they are treated with colchicine the former produces $2n = 21$ and $2n = 42$ plants. In the latter, various mitotic abnormalities, similar to abnormalities occurring in its polyploid parent, appear and lead to chromosome numbers varying from 14 to 42, even in the same root. After one year this hybrid stabilises, through chromosome elimination and the elimination of highly reduced cells, at more or less $2n = 21$.

3. The irregular behaviour of the latter hybrid after treatment with colchicine is considered to be due to the action of genes inherited from its aneuploid *P. dubium* parent and which only express themselves when in double dosage.

4. Through genic control it may therefore be impossible permanently to double the somatic chromosome complement of *P. typhoides* × *P. dubium*.

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