NOTES AND COMMENTS

BIOCHEMICAL DIFFERENCES BETWEEN SPONTANEOUS AND COLCHICINE-INDUCED AUTOTETRAPLOIDS

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1. Introduction

The visible changes resulting from spontaneous or colchicine-induced chromosome doubling are many. They include changes in fertility and in the sizes and degree of pigmentation of the floral and vegetative parts. In an evaluation of induced polyploidy as a method of crop improvement, Randolph (1941) commented that, owing to cumulative gene action, some constituents are increased following chromosome doubling while others are not affected. He emphasised that a satisfactory evaluation of the physiological differences between diploids and polyploids requires a study of components such as enzymes, hormones, amino and other organic acids. In fact, Noggle (1947) has analysed some of the physiological and chemical differences between the diploid parent line and colchicine-induced tetraploids in rye. He found that tetraploidy could be associated with an increase in total nitrogen, reducing sugar, carotene, riboflavin and potassium but a relative decrease in total and non-reducing sugar and in calcium. Surprisingly, the chlorophyll content of the green tissue of the tetraploid proved to be less than that of the diploid.

Stebbins (1950) emphasised that the morphological and physiological effects of polyploidy depend largely on the nature of the original genotype. But earlier studies have generally assumed that within species or cultivars autotetraploids, whether spontaneous or colchicine-induced, are unlikely to differ much. This assumption has been tested in relation to unidentified flavonoid components in virus-free, diploid ($2n = 2x = 14$) raspberry cultivars and their spontaneous or colchicine-induced autotetraploids.

2. Materials and methods

The tetraploid forms of Malling Exploit and Malling Seedling 69/139 arose as " sporting canes " following propagation of diploid stocks from root cuttings.

Tetraploidy in Malling V and Mylnefield Seedling 4B15 was induced by treating lateral buds with colchicine.

Flavonoid extracts were prepared and chromatograms examined by the methods of Harborne (1959). Minced leaf tissue was plunged into boiling ethanol and simmered for 5 minutes. The chlorophyll-containing extract was filtered and washed repeatedly in petroleum ether. The resulting solution was concentrated before spotting by heating under reduced pressure.

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The extract was applied to Whatman No. 1 paper until a dark brown spot about 1 cm. in diameter was obtained. Two-way ascending runs were made first in BAW (*n*-butanol:glacial acetic:water, 4:1:5 by volume, upper phase) for 3 hours following which the paper was dried and, second, in 15 per cent. acetic acid for 45 minutes. The dried paper was examined under UV light with and without ammonia fuming. Duplicate runs corresponded.

3. Results and discussion

All the cultivars possessed five spots in common including the two large spots basic to the subgenus Idaeobatus (Haskell, 1966). However, the diploids and their respective tetraploids differed in those components which were present in smaller amounts. The differences were significantly greater in the case of the spontaneous tetraploids.

Thus, the tetraploid of Malling Exploit showed three spots which were absent from the diploid while the diploid showed five spots which were not present in the tetraploid (total spot differences = 8). The diploid and tetraploid forms of Malling Seedling 69/139 showed a total of seven spot differences, three spots occurring only in the tetraploid.

In sharp contrast the diploid and colchiploid Malling V each showed only one spot which was absent from the other (total spot differences = 2). Similarly, only three spot differences were found between the diploid and colchiploid Mylnefield Seedling 4B15, two spots being exclusive to the tetraploid.

The difference according to the manner of origin of the tetraploids is highly significant ($t = 18{\cdot}53$ for 6 degrees of freedom).

Thus, it appears that qualitative biochemical changes accompany tetraploidy in raspberry and the extent of these is affected by the manner in which the tetraploids arise.

Colchiploids differ little from their diploid parents. This finding is contrary to expectation in that Foster, Ross and Franzke (1955) have found that colchicine has a mutagenic effect in *Sorghum* even when polyploidy does not ensue. However, Atkinson, Ross and Franzke (1956) reported that after colchicine treatment some *Sorghum* varieties produce tetraploids with few or no gene mutations. It would appear that in raspberry, at least, colchicine has no or only a slight mutagenic effect during polyploid induction (*cf.* Dermen, 1964, for alternative explanation).

The unexpected and comparatively wide differences in relation to minor flavonoid components between spontaneously doubled vegetative tissues and the original diploid need explanation. The material of Malling Exploit, for example, was that of a clone which had been propagated from root-cuttings raised in seed-boxes before field planting; it is not unusual to obtain by this method young plants whose leaves are partially chlorotic or albinotic. It would appear, therefore, that raspberry roots might act as reservoirs of naturally mutated cell clusters which are released into division only when circumstances are suitable for rapid growth and differentiation. This might apply also to clusters of polyploid cells which arise spontaneously by endoploidy in roots and which might be prone to somatic crossing-over.

External conditions are not likely to have influenced these results as Yap and Reichardt (1964) have shown for *Vitis* that chromatogram patterns are probably under genetic control and are little affected by ecological patterns. Further, Geissman (1956) using peach and cherry, found that

even the presence of virus does not affect the presence or absence of flavonoid components in the chromatograms. Furthermore, Stebbins *et al.* (1963) found no differences due to leaf age in *Viola*.

It thus remains to be determined whether the greater departure from the diploid parent of spontaneous, compared with colchicine-induced, tetraploids of raspberry represents a special case relating to the particular mode of vegetative reproduction, or whether such a difference also occurs in other species.

4. Summary

1. In regard to flavonoid constituents, spontaneous tetraploids of raspberry differ more from their parent diploids than do colchiploids.

2. This difference between the two kinds of autopolyploid may depend not on the mode of origin but on the source of the cells.

3. The spontaneous tetraploids arose from root cuttings in which mutant tetraploid cells may accumulate while the artificial polyploids were produced by colchicine treatment of lateral buds.

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