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A SIMPLE TECHNIQUE FOR THE INDUCTION OF POLYPLOIDS IN TEA

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Tetraploid plants of five clones of tea were isolated by treating the meristematic tissues of terminal buds of actively-growing shoots with 0.2% and 0.5% colchicine in 1.0% agar for two to seven days.

INTRODUCTION

The cultivated forms of tea in the genus *Camellia* typified by *C. sinensis* var. *assamica* and *C. sinensis* var. *sinensis* and their hybrids are mostly diploids with 2n = 30 chromosomes. A few instances of natural polyploids have been reported in the literature (Karasawa 1932; Bezbaruah 1970). In this survey of chromosome numbers we discovered two triploid clones with 2n=45 chromosomes among a group of 120 clones. Naturally evolved polyploids are not commonly found. There has recently been increasing interest in the production of polyploid breeding lines of tea. Katsuo (1966) described an elaborate method where he applied 0.2% colchicine to axillary buds on etiolated tea shoots and treated them in the dark. He isolated only one wholly tetraploid shoot from 292 treated shoots, while a number of chimeric shoots were also identified. Amma (1974) discovered one tetraploid plant among several diploid plants irradiated with x-rays. It appears from these reports that polyploids in tea are induced only with difficulty.

The products of changes in chromosome number may result in improvements in the usefulness of the crop itself, or provide easier access to otherwise unattainable genetic variation. An important feature of the tea plant is that it can be vegetatively propagated. This makes it possible to utilise directly any variants which have useful combinations of characters. Polyploidy, can therefore be regarded as an additional source of genetic variation which may improve the overall performance of existing diploid clones or enhance a particular character while retaining most of the characters of the diploid progenitor. For instance, Simura & Inaba (1952) reported that triploid forms of tea were hardier and more resistant to the cold conditions prevailing in Japan than diploids and a clone which is widely recommended for planting in South India is reported to be a natural triploid (Jayasuriya & Govindarajulu).

Tea is highly self incompatible, but this sterility is of little or no consequence commercially as the economic product is vegetative. However, self incompatibility has been a handicap in tea breeding programmes. It offers a serious barrier to the production of homozygous lines and genetical studies in tea have therefore, been scanty. The nature of self incompatibility in tea has been reported as gametophytic (Tomo *et al.* 1956; Rogers 1975). This conclusion is based on studies of diploid varieties of tea. The study of this phenomenon at the level of tetraploidy may provide further confirmation that the incompatibility reaction in tea acts through the gametes.

The aim of this investigation was to evolve a simple technique of applying colchicine and to reliably isolate wholly tetraploid plants of tea. The technique could be used to develop a large number of tetraploid clones for cytogenetic studies and to serve as a nucleus for the production of triploids and other higher polyploids in tea.

MATERIALS AND METHODS

1 *Treatment with colchicine*

Active shoots with 4-5 expanded leaves developing from pruned bushes were selected for treatment. Five shoots of each of the diploid clones TRI 2023, 2024,2025 and 2026 and DT 95 were treated with 0.2% and 0.5% colchicine for 2-7 days. The terminal bud was carefully dissected to expose the meristematic tissue which was then covered with a 1.0% block of agar impregnated with 0.2% or 0.5% colchicine and contained in a gelatine capsule. At the end of the treatment period, the capsule was carefully removed, the treated region washed with a spray of water and allowed to regenerate. The activity of the treated meristem was enhanced by periodically removing all the axillary buds arising from below the point of treatment. After the terminal bud had grown out and produced about 5-6 mature leaves, the shoots were cut just below the point of treatment and removed for propagation. In the majority of the treated shoots only one or two leaves arising immediately above the point of treatment showed signs of morphological aberration, while leaves higher up on the shoots appeared normal. Accordingly, in each shoot 2-3 successive nodes from immediately above the point of treatment were propagated for further observations.

All the surviving plants were grown in the field and a new generation of cuttings from each of the surviving plants were propagated in the nursery. Root tips collected from these cuttings were cytologically examined for chromosome numbers.

2. *Cytological Procedure*

The root tips were washed thoroughly in distilled water and prefixed in a solution of 0.2% colchicine or a saturated solution of p-dichlorobenzene for 3 ½ to 4 hours. Washed several times in distilled water and fixed in 3 absolute alcohol: 1 acetic acid mixture, and stored overnight at 5-7°C. The root tips were washed again, hydrolysed in N-HCl at 60°C for 15 min, and stained in Feulgan. Temporary squash preparations were examined at a magnification of 1600 under phase contrast, and chromosome counts were made.

RESULTS AND DISCUSSION

From a total of 95 shoots which were treated, 12 wholly tetraploid plants were isolated (Table 1). The clonal progeny derived from each of the tetraploids were cytologically examined again and confirmed as tetraploidds. Apart from these 12 tetraploids, one plant of clone TRI 2023 was found to contain both diploid and tetraploid cells in different root tips. This plant was therefore a chromosomal chimera which on later examination had reverted back to its diploid state.

The results (Table 1) indicated that both concentrations of colchicine used were effective in inducing tetraploids in tea. The survival of the shoots was highest at a colchicine concentration of 0.2% treated for four days, but this treatment was ineffective in inducing tetraploidy in any of the clones. Although the use of 0.2% colchicine for five and seven days produced some tetraploids in the treated shoots,

TABLE 1—*Numbers of surviving shoots and induced tetraploids out of 5 shoots per treatment in 5 clones*

Colchicine Treatment	DT 95		TRI 2023		TRI 2024		TRI 2025		TRI 2026	
	No. of survivors	No. of tetraploids	No. of survivors	No. of tetraploids	No. of survivors	No. of tetraploids	No. of survivors	No. of tetraploids	No. of survivors	No. of tetraploids
0.2%—4 days ..	—	—	5	0	4	0	—	—	5	0
0.2%—5 days ..	—	—	3	1	3	1	—	—	3	9
0.2%—7 days ..	—	—	3	1	2	0	—	—	2	1+1*
0.5%—3 days ..	3	1	3	0	2	0	3	1	2	1
0.5%—6 days ..	3	1	3	2	2	0	3	1	2	1
TOTAL ..	6	2	17	4	13	1	6	2	14	3

* Mixoploid plant reverted back to a diploid.

the higher concentration of 0.5% colchicine was superior in inducing tetraploidy in tea. The most consistent treatment which produced tetraploids was 0.5% colchicine for six days, but this treatment failed to produce any tetraploids in the survivors of clone TRI 2024. The production of a single tetraploid plant of TRI 2024 when treated with 0.2% colchicine for five days, therefore. appears to be fortuitous. It is more likely that TRI 2024 is more resistant to the activity of colchicine and concentrations higher than 0.5% may be required to induce tetraploidy in such clones.

Differential sensitivity to colchicine has been reported in other plant species. Ahloowalia (1967) noted that varieties of rye grass treated with colchicine responded differentially with respect to their survival rate and their tendency to become polyploids. The variability or the ease with which tetraploids were isolated in clone TRI 2023 as against TRI 2024 for the same treatment is suggestive of differential response of clones to colchicine. The question of whether genetical factors influence their sensitivity to colchicine is yet to be determined.

The mean survival of the treated shoots (Table 1) at the highest concentration of 0.5% colchicine for all clones was about 50%. High survival rate in tea is not an important consideration, the criterion being the production of at least one polyploid plant. A clonal population of this single polyploid plant can be readily established by vegetative propagation. This advantage in tea, over sexually propagated crop plants, prompts the use of even higher concentrations of colchicine than has been used in this study to increase further the chances of obtaining polyploids.

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REFERENCES

AHLOOWALIA, B. S. (1967). *Euphytica* **16**, 49-60.
AMMA, S. (1974). *Study of Tea*, No. 46, 1-6.
BEZBARUAH, H. P. (1970). *Annual Scientific Report for* 1969-70, Tea Research Association, N. E. India.
JAYASURIYA, P. and GOVINDARAJULU, V. Handbook of tea culture. *United Planters Association of Southern India, Tea Research Station.*
KATSUO, K. (1966). *Study of Tea*, No. 33, 1-4.
KARASAWA, K. (1932). *Botanical Magazine*, Tokyo **46**, 458-460.
ROGERS, S. (1975). *Tea Quarterly* **45** (In the press).
SIMURA, T. and INABA, T. (1953). *Chagyo Kenkyu Hokoku/Tea Research Journal* **28**, 5, 7 (Plant Breeding Abstracts, 1955, 3300).
TOMO, M. FUCHINOUE, Y., and YAMANE, H. (1956). *Japanese Journal of Breeding* **5**, 247-253.

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