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Polyploidyand it's Applications in Tea (*Camellia sinensis L.*) Breeding; Review

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ABSTRACT

Non-conventional methods, such as polyploidy breeding, may induce more vigour and some degree of resistance to biotic and a biotic stresses in existing tea cultivars, without causing changes in the desired parts of the genome. Polyploids can be developed through natural and artificial induction.Naturally occurring polyploids in tea may arise either through spontaneous chromosome doubling in somatic tissues, or through the occurrence of unreduced gametes. Artificially polyploidy can be induced in plants by using colchicine that many geneticists and plant breeders assumed it to be a new path for the rapid development of novel and superior types of crop cultivars including tea.Since Polyploidy breeding in tea combines the advantages of hybrid and polyploid vigour must be utilized in detail to cope up with the existing environment change

Keywords: *Breeding, colchicine, natural, polyploid, tea*

INTRODUCTION

Polyploidy plays a major role in increasing the amount of genetic material in plants, and results in a change in the chromosome number. Polyploids have proportionally larger cells than their diploid counterparts; hence it is expected to contribute to larger plant size and higher yields (Allard, 1960). In tea, gigantism in various morphological characters has been found to be associated with tetraploids (Amma, 1974; Chaudhuri and Bezbaruah, 1985). Apart from increasing the biomass of crops (Allard, 1960), induced polyploids could be used as an additional source of genetic variation to improve the overall performance of existing diploids, or to enhance particular characters such as the shoot size trait (Wachira and Kiplangat, 1991), while retaining most of the characteristics of the diploid progenitor. In addition, they could also be used as a source of breeding material for the production of secondary polyploidy cultivars by crossing with diploids (Singh, 1980). Although the value of polyploids in overcoming barriers to inter-specific gene introgression has been recognized in plant species (Allard, 1960), this has not been fully exploited in perennial crops (Wachira and Ng'etich, 1999).Many improved, high-yielding tea cultivars possess one or a few undesirable traits such as susceptibility to pests and diseases, which preclude their extensive use in commercial planting. Therefore to develop promising cultivars, breeding strategies, which combine high yield, good cup quality and resistance to biotic and a biotic stresses, are required to obtain high productivity and to reduce the cost of production. However, owing to the highly heterozygous and self-incompatible nature of the tea plant, introduction of a specific trait of interest into a proven cultivar, solely through conventional methods, has some limitations. On the other hand, non-conventional methods, such as polyploidy breeding, may induce more vigour and some degree of resistance to biotic and a biotic stresses in existing tea cultivars, without causing changes in the desired parts of the genome (Simura and Inabe, 1952). There is a positive correlation between size of the leaf and the yield in tea. Therefore, the generation of polyploid tea genotypes with bigger leaves may be useful in developing high-yielding tea cultivars. Further, in asexually-propagated perennial crops such as tea, where the vegetative organs are of economic value, polyploidy breeding can be used effectively in their genetic improvement (Gunasekara and Ranatunga, 2003).

LITERATURE REVIEW

Natural Polyploid in Tea

Several studies carried out on chromosome number in *Camellia sinensis* have revealed that

it is a diploid (2n = 30) (Morinago *et al.*, 1929; Barua, 1989). Janaki Ammal (1952) has however reported the occurrence of intra-specific polyploids of tea. Subsequently, intra- and inter-specific polyploids of *Camellia* were reported by Kondo (1977), and most of these polyploid genotypes were found to be naturally occurring in tea populations (Bezbaruah, 1971; Jayasuriya and Govindarajulu, 1975). Naturally occurring polyploids in tea may arise either through spontaneous chromosome doubling in somatic tissues, or through the occurrence of unreduced gametes. Natural polyploids are more common in Japanese tea varieties than in tea populations from other areas (Banerjee, 1992; Simura and Inabe, 1952). Natural polyploids have also been found in tea populations in Kenya (Wachira and Kiplangat, 1991). Although some naturally-evolved triploids, tetraploids, pentaploids and aneuploids, resulting from open-pollinated progenies, are found in tea populations in India (Bezbaruah, 1968), these polyploids are reported to exist at low frequencies (Singh, 1982). In Sri Lanka, cultivars such as HS/10A and GF 5/01 were confirmed as triploids (Anandappa, 1973). These natural triploid cultivars are selections made on tea estates from seedling populations. Although it seems that polyploids occur in natural tea populations, there is no estimate available of the frequency of their occurrence (Gunasekara and Ranatunga, 2003).

Artificial Induction of Polyploids

When it was discovered, in 1937, that polyploidy can be induced in plants by using colchicine, many geneticists and plant breeders assumed it to be a new path for the rapid development of novel and superior types of crop cultivars. It was thought that induced autopolyploids might lead to valuable genetic variants in crops. This has led to the synthesis of induced polyploids in a large number of crop species. Colchicine inhibits mitosis in cells by interfering with the structure of the mitotic spindle, thus resulting in formation of cells with a doubled chromosome number. As in many other crops, colchicine has been used to induce artificial polyploids in tea. Sebasthiampillai (1976) was able to produce tetraploid plants from five Sri Lankan tea cultivars, namely TRI2023, 2024, 2025, 2026 and DT 95, by treating the meristematic tissues of the terminal bud with colchicine impregnated in agar, for 2-7 days. He further found that some cultivars were resistant to the activity of colchicine, and concentrations higher than 0.5% would be required for the induction of polyploids in their case, indicating a differential response of clones to colchicine treatment(Table 1). The ploidy level of the resultant, induced polyploid was confirmed as tetraploid from cytological examination of root-tip cells. Attempts to induce polyploids using ethyl methane sulphonate (EMS) at the Tocklai Experimental Station, Jorhat, Assam in India,were not successful. However, more than 170 and 70 polyploids were subsequently developed through hybridization and colchicine treatment, respectively (Singh, 1999).Chemically induced polyploidy could increase tea production by 40%-60% in Pakistan (Hasnain et al.,2015).

Table1. *Successful attempts reported in induction of colchiploids in tea*

Material used	Treatment	% Success	Reference
Axillary buds of etiolated shoots	Cotton wool moistened with 0.2% colchicine and treated in the dark	13.0%	Katsuo (1966)
Terminal buds of active shootsdeveloping from pruned bushes	Agar impregnated with 0.2% or 0.5% colchicine for 5-6 days	13.5%	Sebastiampillai(1976)
Terminal buds	Immersion in 1-2% aqueous colchicines solution for 5-7 days	6 -17%	Goswani andSharma(1979)
Flower buds	0.05% colchicine - injection and drop application for 2-6 days	30.0%	Osone (1958)

Source: *Gunasekara and Ranatunga, 2003*

Morphological, Anatomical and Cytological Markers in Polyploidy Tea

The determination of leaf area at plucking of eight triploid and six diploid Kenyan clones showed that, leaf area was larger in triploids than in diploids, with exception of the diploid clone, S15/10, which had a similar leaf area to triploids. In the same study, it was shown that the leaves of diploid clones extend more quickly than those of triploid clones (Ng'etich and Wachira, 1992). It appears, however, that these phenotypical or morphological attributes do not truly reflect the level of ploidy, and therefore

cannot be used for efficient screening of polyploid genotypes of tea. The reason for this may be that the attributes considered are more affected by environmental factors than by genotype.

Some studies have attempted to analyze the effects of ploidy on stomatal density, and it has been found that stomatal density can be used as a marker in differentiating between polyploid and dip loid cultivars (Amma, 1974; Chaudhuri and Bezbaruah, 1985; Wachira, 1994). It was found that triploid plants have a lower stomatal density on the baxial leaf surface than have diploid cultivars, a few triploid cultivars different (Wachira, 1994). It is apparent from these results that this marker could not always be used as a reliable marker for identification of polyploids in tea, and Chaudhuri and Bezbaruah (1985) had indeed reported that there is a lack o f correlation between ploidy and stomatal density. Among the other anatomical and cytological features studied, chloroplast number in the guard cells has been identified as a reliable ploidy marker in tea, and this has been proved by many workers using tea populations of diverse origin (Ye,1989; Koskey and Wachira,2000). Chen and Ye (1989) found an increase in the number of chloroplasts in the guard cells of the triploid and tetraploid cultivars of tea. In the study by Koskey and Wachira (2000), the ratio of the guard-cell chloroplast numbers in diploids, triploids and tetraploids was found to be 2:3:4, which is the same as the ratio of their chromosome numbers (30: 45: 60). The same trend in chloroplast count was reported also by Ahmed and Singh (1993). From these studies, it is possible to derive a relationship betweenchloroplast number and the level of polyploidy in tea. The findings indicate that the ploidy level of tea could be accurately and rapidly identified by the chloroplast-count method.

More precise cytological techniques used in identifying polyploids include chromosome counting in pollen mother cells, root-tip cells, and meristematic tissue cells at the shoot tip. Wachira and Muoki (1997) devised a new cytological technique to assess the activity of nucleoli and nucleolus-organizing regions, based on a comparison of the results of silver-staining of polyploids and diploids. Their study revealed that the mode of nucleolar number corresponded to multiples of the somatic cell number, and was a good marker for ploidy.Several polyploid tea cultivars have been converted into seed bearers and used for creating natural hybrids, but information on the resultant progenies are not widely available (Gunasekara and Ranatunga, 2003).

Use of Polyploidy in Tea Breeding

Polyploidy breeding in tea combines the advantages of hybrid and polyploid vigour**.** High yielding polyploid clones, which possess low quality traits, have been improved through hybridization with a diploid cultivar of high quality traits (Sarmah and Bezbaruah,1984). Triploids have been artificially produced by hybridizing tetraploid tea with diploids in Japan (Osone,1958), in India (Chaudhuri,1979), and in Bangladesh (Rashid *et al.*,1985). It has been shown that it is possible to combine good cup quality, with the superior vigour and hardiness of the polyploids, by crossing tetraploids with high-quality diploid clones, and then selecting elite clones from the triploid progeny for commercialization. Open-pollinated progenies of triploids were found inferior in cup quality, though their growth was vigorous (Bezbaruah, 1976). Subsequently, these polyploids were utilized for breeding triploids, by crossing with high-quality diploid clones as the male parent.Certain studies indicated that natural polyploids found in South India possess attributes for high yield and quality (Sharma and Ranganathan, 1985).

Commercial Exploitation of Polyploid Cultivars

Although many studies have been attempted to identify natural polyploids and to synthesize artificial polyploids, reports on their performance and trait evaluation are scarce. After the discovery of natural polyploids from tea populations in various countries (Karasawa, 1932; Bezbaruah 1971; Amma, 1974; Katsuo, 1966; Sebasthiampillai, 1976), natural polyploids were included in cultivar selection programmes to identify desirable agronomic traits. Certain studies indicated that natural polyploids found in South India possess attributes for high yield and quality (Sharma and Ranganathan, 1985). On the other hand, Banerjee (1992) has reported that though polyploids show high vigour and resistance to environmental stresses, they do not always contribute towards high yields (Gunasekara and Ranatunga, 2003).

Table.2: *Commercial exploitation of polyploid tea cultivars in the world and their improved and promising characteristics*

Country	cultivar Polyploid	Level of ploidy	type of polyploidy	Promising characteristic(s)	Reference
India	Sundaram	3n	Natural	High yield andquality	Venkataramani (1969);
India	UPASI3	3n	Natural	High yield andOverall quality	Satyanarayana and Sharma (1993)
India	UPASI20	3n	Natural	Moderate yield,highly tolerantto drought	-do-
India	TV 29	3n	Natural	High quality	Barbora *et al.* (1996)
Japan	Not known	3n	Natural	Hardier andcold resistant	Simura and Inabe (1952)
Kenya	382/1	3n	Natural	High yield	Wachira (1994)
Sri Lanka	TRI 3069	4n	Artificial	High yield anddrought tolerant	Kulasegaram (1980)
Sri Lanka	HS 10A	3n	Natural	Cold resistant	-do-

Source: *Gunasekara and Ranatunga, 2003*

Effect of Ploidy Level on Tea

Polyploidy may cause phenotypic variation in the same *Camellia* species. Triploids, in general, are morevigorous, hardier and cold tolerant than diploids (Simurah, 1956a; Hasnain *et al*., 2015). Some triploids and aneoploids had been found to have superior vigour (Hasnain *et al*., 2015). Morphological and anatomical studies on polyploids revealed awide range of phenotypic variations and anatomical characteristics like frequency and size of stomata and sclereids (Chaudhurai, 1979). Chromosome number is also correlated to the pollen size in genus *Camellia* (Ackerman and Kondo, 1980) and to the stomatical guard cells of leaf the mean number/stomata being 21.9±2. 07 for diploids, 32.5±3.15 for triploids and 41.4±4.26 for tetraploids (Ahmed and Sing, 1993; Hasnain *et al*., 2015). A simple and effective method to distinguish the polyploidy of tea is to count the chloroplast numbers per guard cell. Chloroplast numbers per guard cell of diploid, triploid and tetraploid were generally equal or a slightly less than 16, 24 and 32, respectively (Dapeng, 1989).Genetic variations or mutations of diploid tea plants into polyploids are therefore expected to improve the vigour and hardness. Out of different types of tea polyploids produced so far, dry weights, leaf size and rooting ability of triploids were higher by 14% and 109%, respectively, over diploids (Hasnain *et al*., 2015). Pentapoids and aneuploids were, however, poor rooters and had smaller leaves than diploids, triploids and tetraploids. Consequently breeding might have to be concentrated mostly on the production of vigorous triploids (3n=3x= 45) or perhaps tetraploids (2n=4x=60), providing that the quality aspects do not deteriorate (Singh, 1980; Hasnain *et al*., 2015).

CONCLUSION

Polyploidy plays a major role in increasing the amount of genetic material in plants, results in a change in the chromosome number and give proportionally larger cells than their diploid counterparts; hence it is expected to contribute to larger plant size and higher yields. Since Polyploidy breeding in tea combines the advantages of hybrid and polyploid vigour must be utilized in detail to cope up the existing environment change.

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