

POLYPLOIDY IN TEA (*CAMELLIA SINENSIS* L.) AND ITS APPLICATION IN TEA BREEDING: A REVIEW

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Tea (*Camellia sinensis* L.) is one of the major plantation crops in the world and, therefore, of major economic importance to a number of countries. So far, tea crop improvement has been mainly achieved through conventional breeding methods that involve selection of superior genotypes from existing local seedling populations, or from progenies obtained by hybridization. However, these breeding methods often have limitations, owing to the plant being a woody perennial and having a highly heterozygous and allogamous nature. Such breeding methods are also slow in achieving results, and cannot keep pace with economic demands.

Breeding and crop-improvement strategies to improve the overall performance of superior, existing cultivars by enhancing a particular character, without changing most of the other traits considered desirable, would overcome the problems faced in classical tea breeding.

In this paper, the use of polyploidy in tea breeding is reviewed, and reasons for its limited application in the developing of promising tea cultivars are discussed. Attempts made in identifying markers, related to morphological and anatomical attributes, to facilitate screening of polyploid genotypes are described. An overview of developing polyploid cultivars, with special emphasis on commercial exploitation, is presented.

Key Words: *Camellia sinensis*, tea, crop improvement, polyploidy, ploidy markers.

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 (Chaudhuri and Bezbaruah, 1985; Amma, 1974). 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 polyploid 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).

Tea (*Camellia sinensis* L.) is one of the major plantation crops in the world and is, therefore, of major economic importance to many developing countries. In order to sustain the tea industry, it is needed to increase productivity while maintaining the quality of made tea. However, 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 abiotic 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 abiotic stresses in existing tea cultivars, without causing changes in the desired parts of the genome (Simura and Inabe, 1952).

In tea, tender leaves are processed to make the final product, and the size of the leaf is one of the major attributes determining final yield. There is a positive correlation between size of the leaf and the yield in tea (Satyanarayan and Sharma, 1982). 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.

Natural polyploids 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.

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.

In the 1930s, the discovery of the effect of colchicine on cell division was a great step forward for mitotic doubling of the chromosome number (Blakeslee and Avery, 1937). Colchicine which is derived from the corms of autumn crocus is found to be the most effective agent in polyploidy induction (Allard, 1960). Colchicine is a narcotic alkaloid, chemically related to morphine and codeine. Its immediate effect on growing tissues, even in very small concentration, is to produce stunting and distortion. 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 TRI 2023, 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. 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).

Attempts that were successful in inducing polyploids using colchicine are detailed in Table 1. Table 1 indicates that the artificial induction of colchipooids in tea is difficult, the highest success rate being only about 30%.

Table 1. Successful attempts reported in induction of colchipooids in tea

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

Morphological, anatomical and cytological markers in polyploid tea

The potential for rapid and early screening of polyploids has not been widely exploited in tea, owing to difficulties in employing time-consuming cytological methods. Hence, the identification of markers related to morphology, anatomy or cytology is of great importance in screening for polyploids, whether they be artificially induced or naturally occurring. In the early years, the ploidy level in tea was confirmed by counting chromosome numbers in meristematic tissue, such as root-tip cells and pollen mother cells

In some other crop species, however, indirect methods have been successfully employed to screen polyploid cultivars. In the light of the findings with other crop species, many attempts have been made for identifying ploidy indicators in tea using morphological, anatomical and cytological attributes.

A detailed study of karyomorphology, together with that of the morphological and anatomical attributes of open-pollinated progenies of triploid tea, was undertaken by Chaudhuri (1979). The results revealed that there was a range of ploidy, from diploidy to pentaploidy, which exhibit wide phenotypic and anatomical variations, such as frequency and size of stomata and sclereids (Chaudhuri and Bezbaruah, 1985). In another study, to assess the effect of the polyploidy level on the morphogenetic attributes of the F1 seedling population, generated from a cross between diploid and tetraploid cultivars, a clear relationship was shown between ploidy levels and morphogenetic variations (Rashid *et al.*, 1985).

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 analyse 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 diploid cultivars (Amma, 1974; Chaudhuri and Bezbaruah, 1985; Wachira, 1994). It was found that triploid plants have a lower stomatal density on the abaxial leaf surface than have diploid cultivars, a few triploid cultivars excepted (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 of correlation between ploidy and stomatal density.

In comparing artificially-induced tetraploids of the Yabukita tea variety with their diploid progenitors, Amma (1974) found that guard-cell and stomata sizes are larger in the former than in the latter. Similarly, Wachira (1994) reported significant differences between diploids and triploids in guard-cell length and breadth. However, he found that the length: breadth ratios of the guard cells were not significantly different between diploids and triploids.

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 Dapeng, 1989; Ahmed and Singh, 1993; Koskey and Wachira, 2000; Ranatunga and Gunasekare, 2002). 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 between chloroplast 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,

rather than by criteria based on the size and density of stomata. It is possible to assume that the attributes, related to anatomical features, are much more precise than the morphological characters which have been used to screen polyploidy genotypes in tea.

Attempts have also been made to study, in confirmed or true polyploid tea cultivars, the variations present in anatomical features related to the reproductive organs. The size of the pollen grain in most of the induced tetraploid cultivars developed in Sri Lanka is higher than that of its diploid counterparts (Gunasekare, 2000). However, a few exceptions have been reported, suggesting that this character does not always reflect the effect of ploidy.

Although extensive work has been done to identify precise markers for ploidy level in tea, it is clear from the above discussion that the results obtained are not consistent. However, among the criteria studied, the number of chloroplasts in the guard cells and the stomatal density can be used with some reliability for ploidy level analysis. These markers may be used for the screening of polyploids from a larger number of tea genotypes, and this procedure could minimize the time and resources needed for subsequent cytological studies. A knowledge of the effect of polyploidy on morphological, anatomical and cytological features of polyploid tea is necessary prior to embarking on a polyploid breeding programme.

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.

With regard to reproductive characters, pollen grains germinated *in vitro* show poorer pollen-tube growth in tetraploid cultivars than in diploid cultivars (Thirukkumaran and Gunasekare, 2001). Only two per cent of the pollen grains of natural triploids were found to be viable, and thousands of pollinations of triploids with triploid pollen, and with pollen from many diploids, failed to set fruit (Bezbaruah, 1971). It had been reported that pollen viability and fertility of triploids are usually poor (Visser, 1969). These findings are supportive of the fact that many polyploid cultivars are unable to set seeds and fruits.

It is believed that polyploidy has been associated with high sterility, owing to aberrant chromosomal synapsis and segregation, during the process of gamete formation (Ye Dapeng, 1989). In most cases, artificially doubled genomes suffer meiotic

irregularities (Hancock, 1997). However, this is less of a problem in a crop like tea, which is grown by means of vegetative propagation for commercial harvesting of the leaf, although when used in subsequent hybridization programmes reduced fertility of polyploids may greatly hamper seed production and fruit development. 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.

Use of polyploids in tea breeding

Polyploidy breeding in tea combines the advantages of hybrid and polyploid vigour. Most of the tea polyploids often lack desirable traits (Bezbaruah, 1968; Sarmah and Bezbaruah, 1984), and polyploidy breeding therefore requires planned hybridization, selection of promising polyploids, and proper evaluation to confirm their performance as potential cultivars. Even among the most successful colchipooids, considerable breeding improvements have to be made before they are released on a commercial scale. For this reason, various attempts have been made to include these polyploids in planned hybridization programmes, with a view of improving their characters further.

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 a triploid 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. Reciprocal crosses were not successful. Progenies of the crosses were tested as cultivars and three of them gave high yield with superior quality (Bezbaruah, 1991). However, there is no indication of the ploidy level of the promising cultivars.

The growth and performance of some Sri Lankan genotypes produced from open-pollinated seedlings of TRI 3069, which is an induced tetraploid of TRI 2025, showed that all the genotypes were diploid (Anon., 1992). This suggests that, although the seedlings were generated from seeds of a confirmed tetraploid seed-bearer, viable seeds are only formed when the embryos are diploid. The clones were of the large-leaf type and, when evaluated with other clones for yield performance, none of the progenies was found to be promising (Anon., 1992). Out of 238 hybrids produced through hybridization between tetraploids and diploids at the Tocklai Experimental Station in Assam, only 79 hybrids were found to be triploid (Barbora *et al.*, 1996).

The conventional method of producing triploids through artificial induction of tetraploids, followed by hybridization with diploid cultivars, is a long process. Oson (1958) used diploidized pollen of immature flowers to pollinate diploid plants for producing triploids. However, there is no evidence that this method has been widely practised in polyploid breeding programmes.

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.

Bezbaruah (1968) reported that some natural triploids and tetraploids are known to produce tea of poor quality. Sarmah and Bezbaruah (1984) also found that polyploids studied were of low quality, even though they were vigorous in growth. The prolific growth in polyploids may be attributed to increased photosynthesis owing to the increase in chloroplast number in the guard cells.

The effects of ploidy on yield and its components have been studied by Amma (1974), Banerjee (1992), Wachira (1994) and Wachira and Ng'etich (1999). It was found that triploid cultivars produced larger and heavier shoots, with fewer harvestable shoots per unit area, compared to diploids. This compensatory effect of shoot mass with shoot number partly explains why triploids yield less than diploids, despite heavier shoot weight (Wachira, 1994). In another study by Singh (1980), it was found that out of the different types of polyploids produced in India, the dry weights of five fully-formed leaves in triploids and tetraploids were higher than that of diploid leaves by 14% and 109%, respectively. Other pentaploids and aneuploids, however, had relatively low leaf dry weights.

Although it was shown that polyploids in tea could enhance and maximize yield (Jayasuriya and Govindarajulu, 1975; Kulasegaram, 1980; Sharma and Ranganathan, 1986), this is not always the case, as there are instances where increased polyploidy was demonstrated to depress the productivity (Banerjee, 1992; Wachira, 1994). However, it is clear from these studies that, though increased ploidy depressed yields significantly, a significant difference in production could also be observed among

genotypes of the same ploidy level. In certain cases, triploids out-yielded diploids, which indicate the potential for selecting or developing high-yielding polyploid cultivars.

It has been found that rooting ability, leaf size and leaf dry weights of triploids and tetraploids were higher than that of diploids. However, pentaploids and aneuploids are poor rooters and have smaller leaves than those of diploids, triploids and tetraploids (Banerjee, 1992).

Commercial exploitation of polyploid teas in Sri Lanka has led to the release of two commercial cultivars. The first cultivar, TRI 3069, which is an induced tetraploid of TRI 2025, has been accepted in commercial plantations and possesses many improved traits compared to TRI 2025. The second cultivar, HS 10A, which is a natural triploid selected from a seedling-tea population on Hethersett Estate, was found to be better adapted to high elevations than diploid cultivars (Kulasegaram, 1980). In addition, some open-pollinated and controlled-pollinated progenies, generated from polyploid clones, have shown promising performances in clone evaluation trials. These clones are now being assessed for various characteristics before being released as potential clones for commercial-scale planting in Sri Lanka.

It has been reported that triploid forms of tea were hardier and more resistant to cold conditions than diploids (Simura and Inabe, 1952), and a clone which is widely recommended for planting in South India is reported to be a natural triploid (Jayasuriya and Govindarajulu, 1975).

Commercially-acceptable polyploid tea cultivars developed in some tea-growing countries are detailed in Table 2. It is apparent from Table 2 that only eight polyploids have found their way into cultivation, including one artificially-induced polyploid.

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

Country	Polyploid cultivar	Level of ploidy	Type of polyploidy	Promising characteristic(s)	Reference
India	Sundaram	3n	Natural	High yield and quality	Venkataramani (1969); Sharma and Ranganathan(1986)
India	UPASI 3	3n	Natural	High yield and overall quality	Satyanarayana and Sharma (1993)
India	UPASI 20	3n	Natural	Moderate yield, highly tolerant to drought	-do-
India	TV 29	3n	Natural	High quality	Barbora <i>et al.</i> (1996)
Japan	Not known	3n	Natural	Hardier and cold resistant	Simura and Inabe (1952)
Kenya	382/1	3n	Natural	High yield	Wachira (1994)
Sri Lanka	TRI 3069	4n	Artificial	High yield and drought tolerant	Kulasegaram (1980)
Sri Lanka	HS 10A	3n	Natural	Cold resistant	-do-

CONCLUSIONS

Several points on the future use of polyploids in practical tea-breeding emerge from this review. In tea, induced polyploids have not achieved much commercially. There may be several reasons why artificial polyploids have been underutilized in tea-breeding programmes. Primarily, as tea is a late-flowering, woody perennial, the development of induced polyploids to give cultivars is a long and tedious process.

It is apparent, however, that naturally-evolved, desirable polyploids are common in tea populations around the world. It is necessary to evaluate and characterize these polyploids in order to identify suitable materials for use in future breeding programmes. As the tea plant is vegetatively propagated, it is possible to utilize directly any desirable variants through mass propagation of selected polyploids.

Induced polyploids also lack some desirable traits found in natural polyploid genotypes. Moreover, it has become clear that the doubling of chromosome number is much more likely to produce types that are inferior to their diploid progenitors and rarely endowed with superior characters. Although induced polyploids cannot be expected to produce striking improvements in an established, highly-selected crop like tea, it does provide novel genetic variability, which could be an additional source

for breeding. Therefore, it is worthwhile continuing these non-conventional breeding methods for obtaining elite genotypes in tea, as polyploid-breeding in tea is still in its early stages, although the possibilities are immense. The attitude towards polyploidy may gradually change with increasing experience and its use as a raw material in plant-breeding programmes.

Identification and confirmation of polyploids through cytological methods are tedious. On the other hand, the markers studied, in relation to morphological and anatomical features, are not accurate enough to clearly differentiate polyploid tea genotypes, with the sole exception of the number of chloroplasts in guard cells.

However, the use of the flow-cytometry technique, to estimate genome size by measuring DNA content, has so far not been applied to tea ploidy determination. Such an application would carry tea polyploid breeding a step further.

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