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Characterization of New Tea (*Camellia sinensis* L.) Hybrid Progenies Based on Morphological Traits

B. Thuvaraki[a], M.A.B. Ranatunga[b], J.D. Kottawa-Arachchi[b]* and V.A. Sumanasinghe[a]

[a]Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka,
[b]Plant Breeding Division, Tea Research Institute, Talawakalle, Sri Lanka

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***Email:**
jeevan1188@yahoo.com,
jeevank@tri.lk

ABSTRACT

Hybridization is the main method of obtaining genetic variation and breeding of new cultivars. Two parents selected for their desired characteristics were crossed to create genetic variation. One hundred and eighteen putative hybrids were generated by crossing two diverse parents, TRI 2043 which is characterized with high pubescence density, pigmented leaves, tolerant to blister blight disease, and TRI 3055 a non-pigmented, stem canker tolerant cultivar. A reciprocal cross was also made. Total progeny was characterized for five morphological traits; anthocyanin pigmentation in petiole, leaf vestiture, average number of pubescence of leaf, immature leaf colour, and petiole colour. Average number of pubescence in abaxil of the second leaf varied from 5 to 149 with the mean of 62. Parental cultivar TRI 2043 recorded the highest average number of pubescence and TRI 3055 had the lowest. Among the progenies, 93 individuals contained anthocyanin pigmentation in petiole, which was the characteristic feature of TRI 2043. Population was subjected to genetic analysis and the resultant dendrogram clearly categorized the progenies into four clusters. Significant variation was found among the individuals in the progeny. Forty individuals showed close resemblance with the parent TRI 2043 and 21 individuals grouped with cultivar TRI 3055. Rest of the individuals shared both parental morphological characteristics.

INTRODUCTION

Tea plant belongs to the genus *Camellia* that includes as many as 82 species mainly distributed in the mainland of South-East Asia. The plant was the very first crop used for preparation of non-alcoholic beverage from the ancient times and literature strongly emphasizes its value in disease prevention and as a key component of daily diets (*1-3*). Tea production is one of the major sources of foreign exchange for Sri Lanka. It provides direct and indirect livelihood for over 1.5 million people. Sri Lanka is the world's fourth largest tea producer and the third in export (*4*). Tea industry also plays an important role in Sri Lanka's socioeconomic aspects. Possible ways available for producers to enhance profitability are to fetch attractive prices for their produce by increasing the quality and increasing quantity of the product while reducing the cost of production. In this context, plant breeding plays a vital role in crop improvement strategies.

The scientific approach to tea cultivation in Sri Lanka began after the establishment of the Tea Research Institute of Ceylon in 1925. The mass selection of vigorous seedling plants in the nursery, based on morphological characters, followed by the establishment of seed gardens with these plants by the early planters, marked the beginning of unplanned tea breeding. This approach led to the development of several improved seed populations and can be considered as the first step towards tea improvement prior to the advent of cultivars. Initially, introduction of new cultivars from India and selection methods of tea breeding was practiced followed by large scale multiplication by vegetative cuttings (*5*).

Hybridization is one of the main methods of obtaining genetic variation, and it is an important method of breeding new tea cultivars since the parents as well as the hybridized progeny are heterozygous and heterogeneous (*6*). Two parents selected for their desired characteristics are crossed to introduce genetic variation. After the genetic recombination, all subsequent propagation steps are done by vegetative propagation. In tea, selection of genotypes is carried out in several steps starting from seed progeny and subsequent selections made from the vegetative propagated plants. Then the performance of these accessions is continuously evaluated in 'on station trials' and 'multi locational trials'. Finally accessions with favourable promising results are selected and released as new cultivars (*7*).

This line of work was initiated at the Tea Research Institute in 1961-1962 in order to produce superior cultivars and seed cultivars. Besides the development of superior cultivars, the hybridization programme, carried out during 1980-1987 was aimed at studying floral biology; combining ability; heritability estimates of major attributes such as yield, quality, tolerance to pests, diseases and drought; selection of combiner cultivars (male and female); and clonal compatibility. A large number of controlled hybridization was attempted and some of the progenies were also recommended for planting (*8*).

An understanding of morphological (*9*), biochemical (*10, 11*) and molecular (*12*) diversity among the accessions is important if the best use is to be made available in plant improvement programmes. Characterization of hybrid progeny is an initial step towards proper utilization of genetic resources. Leaf morphology has an important role in identifying taxa and to study genetic diversity (*13, 14*), variation (*9, 15*), phylogeny and classification (*9, 16, 17*). Use of morphological characters is cost effective when compared with the use of biochemical and molecular markers for preliminarily characterization of a large number of accession to identify morphologically similar groups (*9*) and for simple varietal identification of phenotypically distinguishable cultivars (*18*). However, overlapping of morphological traits, continuous variation of vegetative characteristics and the high degree of plasticity restrain the separation of the progeny into discrete groups. A comprehensive morphological characterization of 203 germplasm accessions was conducted using six quantitative and fourteen qualitative morphological descriptors (*9*). Such studies conducted to characterize the tea hybrid progenies based on their morphological segregation of characters are limited.

The present study was carried out with the objectives of analyzing morphological variations of new tea hybrid progenies, determining the segregation of characters among progenies using morphological descriptors, determining the correlation of the progeny characteristics with their economically important features such as quality, and arbitrating of abundant phenotype resembling parental character.

MATERIALS AND METHODS

Plant materials

The study was conducted at the Tea Research Institute (TRI), Talawakalle, Sri Lanka. One hundred and eighteen putative hybrids generated from crossing programmes during the year 2012 (TRI 3055 x TRI 2043) and year 2013 (TRI 2043 x TRI 3055) were selected for morphological characterization. Two cultivars, TRI 2043 and TRI 3055 (Figure 1), were selected as parents from two distanced ancestor lines and the description of both parents is given in Table 1.

Fig. 1: Active shoots of TRI 2043 (left) and TRI 3055 (right)

Morphological descriptors/markers

Fifty hybrid progenies developed from direct cross and sixty eight hybrid progenies developed from the reciprocal cross were selected for morphological assessment. Prior to morphological studies, field performances of hybrid progenies along with parents were visually assessed. Parental cultivars had been planted in between progenies to minimize experimental errors. Plants were rested for 2 weeks, which allowed free growth of plants in order to obtain sufficient samples with proper stage and plucking was practiced periodically to get sufficient number of newly developed leaf samples. Maturity stage of 'two leaves and a bud samples' was selected from each progeny and used for morphological assessment. All morphological traits were scored as per the guidelines given in Descriptors for Tea (*19, 20*). Five morphological traits such as anthocyanin pigmentation in leaf petiole, leaf vestiture observed on lower surface of the 2nd leaf from the apical bud, leaf pubescence-number of average pubescence recorded on the abaxial of the 2nd leaf near to mid rib, immature leaf colour that has recently unfurled (2nd leaf) and Petiole colour were considered for this study.

Anthocyanin pigmentation in leaf petiole

Leaf samples were subjected to the documentation of anthocyanin pigmentation in petiole. Presence or absence of anthocyanin coloration at the base of petiole was recorded.

Leaf vestiture

The presence or absence of pubescence on the abaxial of the 2nd leaf was checked through microscope and was given a score.

Leaf pubescence

The second leaf from the apical bud of a shoot with two leaves was selected for the determination of leaf pubescence and five leaves were sampled from each cross. A known surface area (7.0174 mm^2) of lower surface of the second leaf near the mid rib was made using a cork borer. The sample was incubated in 95% of ethanol overnight. Bromophenol blue (0.1%) was added and the samples were incubated for 2 hours to stain the surface area. Numbers of pubescence were counted through microscope under the magnification of 20x. Average number of pubescence of 5 samples from each progeny was recorded.

Colour of immature leaf and the petiole

Colour chart published by the Royal Horticultural Society (5th Edition) was used to characterize the immature leaf colour and petiole colour. The second leaf of two leaves and a bud was selected for determination of immature leaf colour and the matching colour was scored for each sample of progeny. While measuring the colour, leaf samples were kept under fluorescence light to maintain uniform exposure. The matching colours of petiole of progenies were scored.

Scoring and data analysis

Qualitative multistate morphological traits that depict an array of characters were converted to binary characters based on the variations present in each trait. The presence of phenotypes was given the score of "1" and absence the score "0". Data were analyzed using POPGENE 32 software and a dendrogram was constructed based on Nei's genetic distance analysis by using the software "Tree view".

Results and Discussion

Morphological traits such as leaf vestiture, pubescence in abaxial, anthocyanin pigmentation, immature leaf colour and petiole colour have been determined in both progenies. A parent cultivar, TRI 2043 exhibited unique morphological characteristics such as dark purple pigmentation in young shoots as well as young leaves with dense pubescence. This cultivar had been also recognized to be a highly tolerant cultivar for blister blight disease (*22*). The other parent TRI 3055 showed contrasting morphology such as broad leaves, lack of pigmentation in young leaves and petiole and absence of pubescence (*21*). The various intensities of selected morphological characters indicated that the selected traits of both distanced parental cultivars have been segregating in hybrids progenies.

Morphological characterization of progenies is an important prerequisite to exploit selection process (*7*). Characterization of hybrid progeny is the initial step towards proper utilization of genetic resources in tea breeding programmes. Therefore, the hybrid progenies generated from these parents are believed to be showing higher degree of variation and the better progenies from both crosses can be used for future tea breeding programs. One hundred and eighteen hybrid progenies were generated from crossing the above parents. Number of hybrid progenies survived

Table 1: Characteristic description of parents

Characteristics	TRI 3055	TRI 2043
Pedigree information	Selection of a batch of seeds introduced from ASM 4/10	Accession of TRI selection made from Shan Bansang, seeds from Indo-China.
Morphological characters		
1. Size of leaf	Medium (Length-5-10cm, Width- 3-7cm)	Medium (Length-5-10cm, Width- 3-7cm)
2.Immature leaf colour	Yellow green group 146-A	Brown group 200-A
3.Petiole colour	Yellow green group-144-A	Brown group N-200-A
4.Anthocyanin pigmentation in petiole and young leaf	Absent	Present
5. Vestiture of lower surface.	Glabrous	Pubescent
6. Leaf pubescence	Sparse	Dense

Source: (*20, 21*)

was higher in reciprocal cross than in the direct cross. It may be due to environmental effects as the crosses were made in two different years.

Leaf vestiture

The abaxial of the second leaf of shoots of all hybrid progenies consisted of fine hair like outgrowth "pubescence" which is unicellular in tea. Leaf pubescence was observed in all hybrids (Table 2).

Table 2: Leaf pubescence in parents and progenies.

Cultivars	Leaf pubescence
TRI3055	Absence
TRI2043	Presence
Hybrid progeny	Presence (100%)

Even though the parent TRI 3055 did not contain pubescence in lower surface of leaf, the hybrid progenies developed from the same parent showed the character confirming hybridity. A similar study revealed that the genetic effects governing the inheritance of pubescence in crosses between cowpea with the highly pubescent accession were segregating in the offsprings (*23*).

All the hybrid progenies showed pubescence character underneath the second leaf. The tea accessions with dense pubescence are easier to select, and are of more certain breeding behaviour than glabrous plants. Furthermore, the parental cultivar TRI 2043 is listed as a blister blight resistant cultivar [21] which shows high trichome density. Therefore, the hybrid progenies, which resembled the parent having this character, are likely to show tolerance to blister blight.

Average number of pubescence in lower surface of the 2^{nd} leaf

The pubescence, a heritable character is related to quality of tea. The pubescence type accessions produce better quality orthodox made tea than glabrous ones (*24*). Therefore, the presence or absence of pubescence in lower surface of the second leaf of hybrid progeny was evaluated to determine the segregation of the character to utilize the progenies in the selection process.

Average number of pubescence was analyzed through univariate procedure. Minimum and maximum values, means, and other statistical parameters of leaf pubescence in direct crosses and reciprocal crosses are given in Table 3. Leaf pubescence in direct crosses varied from 5 to 126 with a mean of 72.5. The range of leaf pubescence in reciprocal crosses was 6.25 to 149.2 and with a mean of 60. With the absence of leaf pubescence in parental cultivar TRI 3055, this character may have been originated from other parent TRI 2043 as a prominent phenotype segregating among hybrid progenies. Coefficient of variation and standard deviations between progenies in direct cross were lower than that of reciprocal cross. The degree of pubescence on the abaxial of the leaf is, however, more important for quality of made tea. A strong correlation between the ordered arrangement of pubescence and quality was established and suggested that, the pubescence is indeed a factor in tea quality and hence of significance in selection for quality (*24*).

Table 3: Basic statistics of leaf pubescence in progenies

Basic statistics	**Direct cross (TRI 3055 x TRI 2043)**	**Reciprocal cross (TRI 2043 x TRI 3055)**
Range	5-126	6.25-149.2
Mean value	72.5	60.1
Median	68.7	56.9
Mode	44.0	59.5
Coefficient of variation	35.7	46.7
Standard deviation	25.9	28.1
Skewness	0.1	0.8

Distribution of leaf pubescence in direct cross and reciprocal cross is given in Figure 2 and Figure 3 respectively.

Most of the hybrid progenies resembled TRI 2043 characters such as pigmentation in leaf petiole, immature leaf colour, leaf pubescence, etc. Therefore, those progenies

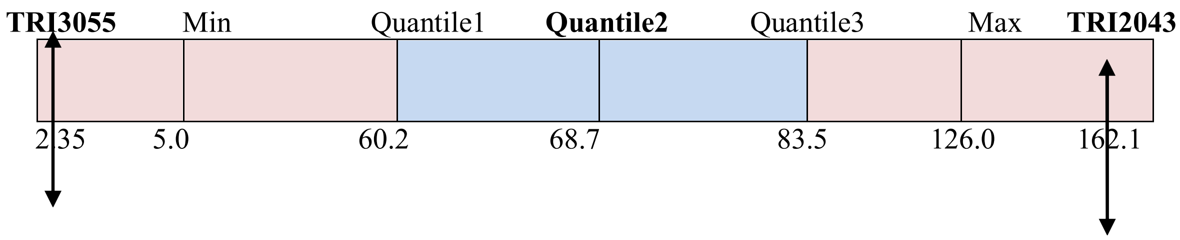

Fig. 2: Distribution of leaf pubescence in direct cross

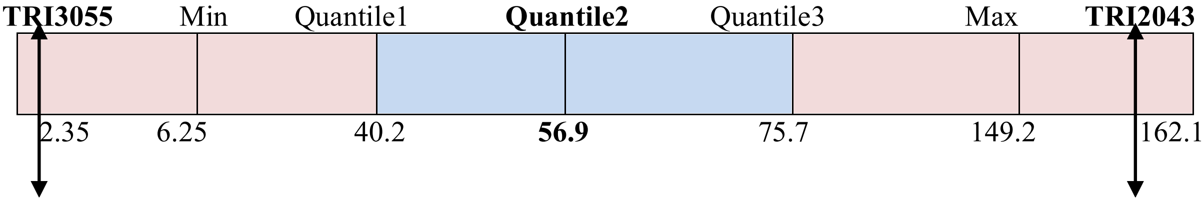

Fig. 3: Distribution of leaf pubescence in reciprocal cross

can be selected for future breeding programmes. Since TRI 2043 is considered as blister blight tolerant cultivar, the hybrid progenies resembling that parent may also have such a character. The Figure 4 shows that none of progenies exceeded both parents in pubescent character. No hybrid was indicated to be an outlier. Therefore, the character, pubescence is segregating among progenies.

Anthocyanin pigmentation

Anthocyanin has been widely used as a chemical marker in characterization of several tea cultivars since it is an easily observable phenotypic trait. In addition, anthocyanins are also powerful antioxidants. Tea made from anthocyanin-rich cultivars can be considered specialty tea with high antioxidant activity (*25*). Anthocyanin pigmentation in the red flowers is used as a taxonomic marker in determination of genetic diversity of wild species in section *Camellia* (*26*). Hence, it has a potential to be used as a selection criterion in tea breeding programmes.

In another study, the young shoot characters such as pigmentation in young leaves was used for diversity analysis of *Camellia* spp. (*27*). All individuals of the progeny were characterized morphologically for presence or absence of purple pigments as well as intensity of the pigments using Royal Horticultural Society (RHS) colour chart. Colour variation of tea shoots throughout the population confirmed transgressive segregation for anthocyanin pigments. Anthocyanin concentration of this population revealed that there was deviation from Mendalian segregation for the anthocyanin pigmentation. Therefore, there is no major gene inheritance of the trait, but a polygenic inheritance can be observed and this trait can be considered as governed by quantitative trait loci. (*25*). Orthodox tea made from anthocyanin rich-tea was higher in antioxidants, astringent and had a better mouth feel (*25*).

TRI 2043 is a red pigmented cultivar used for silver-tip production. Anthocyanin is contributing to taste as a moderately astringent sensation to silvertips. Therefore, the present study was conducted to select the hybrid progenies with high anthocyanin pigmentation. Majority of the hybrid progenies showed anthocyanin pigmentation in petiole (Figure 5). The degree of pigmentation varied between progenies. Compared to the direct cross, reciprocal cross progenies contained higher anthocyanin pigmentation. This is due to the maternal (TRI 2043) inheritance of this character. According to the results, colour variation of tea shoots throughout the population confirmed a transgressive segregation for anthocyanin pigments.

Immature leaf colour

Chlorophylls, carotenoids and anthocyanins play a major role in determination of leaf colour. Anthocyanin is responsible for the reddish brown colour of leaves and flowers. The immature leaf colour showed considerable variations among hybrid progenies. The colour variation among hybrid progenies indicated that different levels of gene expression occur in hybrid progenies. Although anthocyanin was absent in parent TRI 3055, the hybrid progeny showed various intensities of reddish-brown pigmentation. Therefore, the parent TRI 2043 having pigmentation could be the dominant parent for segregation of anthocyanin. Furthermore, the variation of pigment contents in tea has also been attributed to environmental differences such as shade level, fertilizer (*28*) and cultivar differences (*10*).

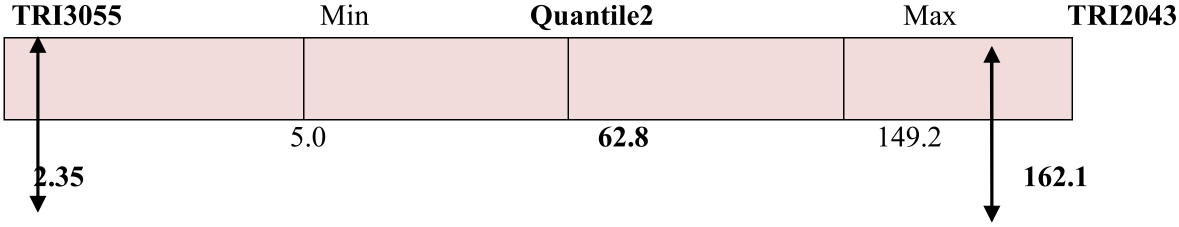

Fig. 4: Distribution of leaf pubescence in both cross combinations

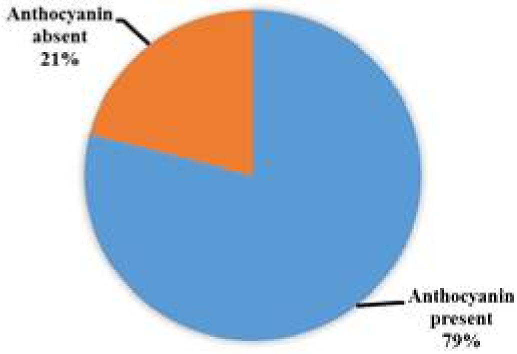

Fig. 5: Anthocyanin pigmentation in all hybrid progenies

The degradation of chlorophylls into pheophytin and pheophorbide was higher in orthodox teas and was responsible for appearance and blackness (*28*). Conversely, chlorophyll *a* and *b* were positively correlated with the appearance and the total quality score in tea [28]. The carotenoids play an important role as aroma precursors in black tea. Most of the cultivars having light green leaves produce high quality black tea (*10*). Colour of the immature 2nd leaf of hybrid progenies was recorded to relate with the quality. Royal Horticultural Society (RHS) colour chart was used as a standard reference to record the colour. The chart is essential for accurately identifying the colours of immature leaf. The chart is equally useful to compare the colour of leaves among hybrid progenies.

Petiole colour

Petiole colour of hybrid progenies was categorized into five different groups. While considering the combination of both crosses, yellow green colour was shown by majority of the progenies. Therefore, hybrid progenies resembled the parent TRI 3055.

Cluster analysis on morphological traits

Genetic distances were calculated for morphological traits and a dendrogram was constructed using the software package "Tree View". Dendrograms were developed separately for each cross.

Progenies were grouped into four different clusters (Figure 8). Progeny number 36 and 43 precisely resembled the parental cultivar TRI 2043. This was due to the leaf vestiture (absence only in this cultivar) of that parent. The progenies, which morphologically resembled the parent TRI 2043, were believed to show the characteristics of that parent. The hybrids that were not grouped with both parents are believed to share intermediate characteristics.

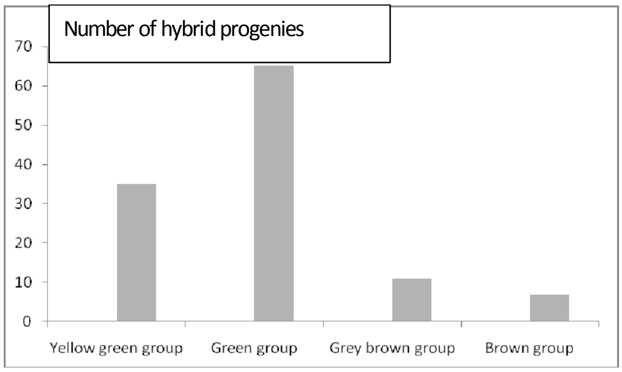

Fig. 6: Immature leaf colour of hybrid progenies.

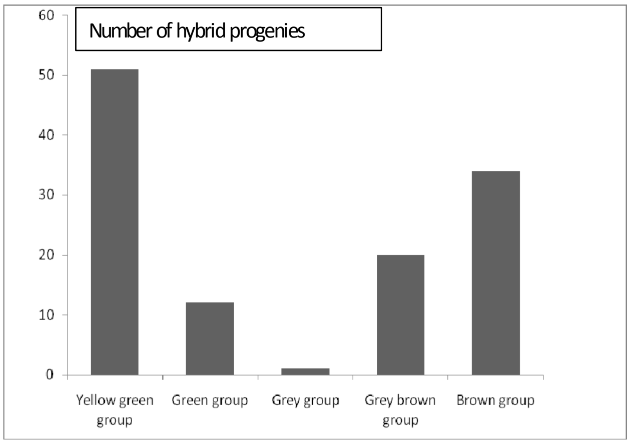

Fig. 7: Petiole colour of all hybrid progenies

Reciprocal progenies were grouped into 4 different clusters. Five progenies (Hybrid progeny 72, 90, 93,125 and 153) closely resembled the parent TRI 2043. Parental cultivar TRI 3055 did not match with any progeny. Majority of the hybrids were not grouped with both parents. They shared intermediate characters of both parents.

CONCLUSIONS

Of the 118 hybrid progeny, all the hybrids showed leaf vestiture in the abaxial of the 2nd leaf. None of the hybrid exceeded the average number of pubescence of either parent. Morphological traits correlated with quality of tea and

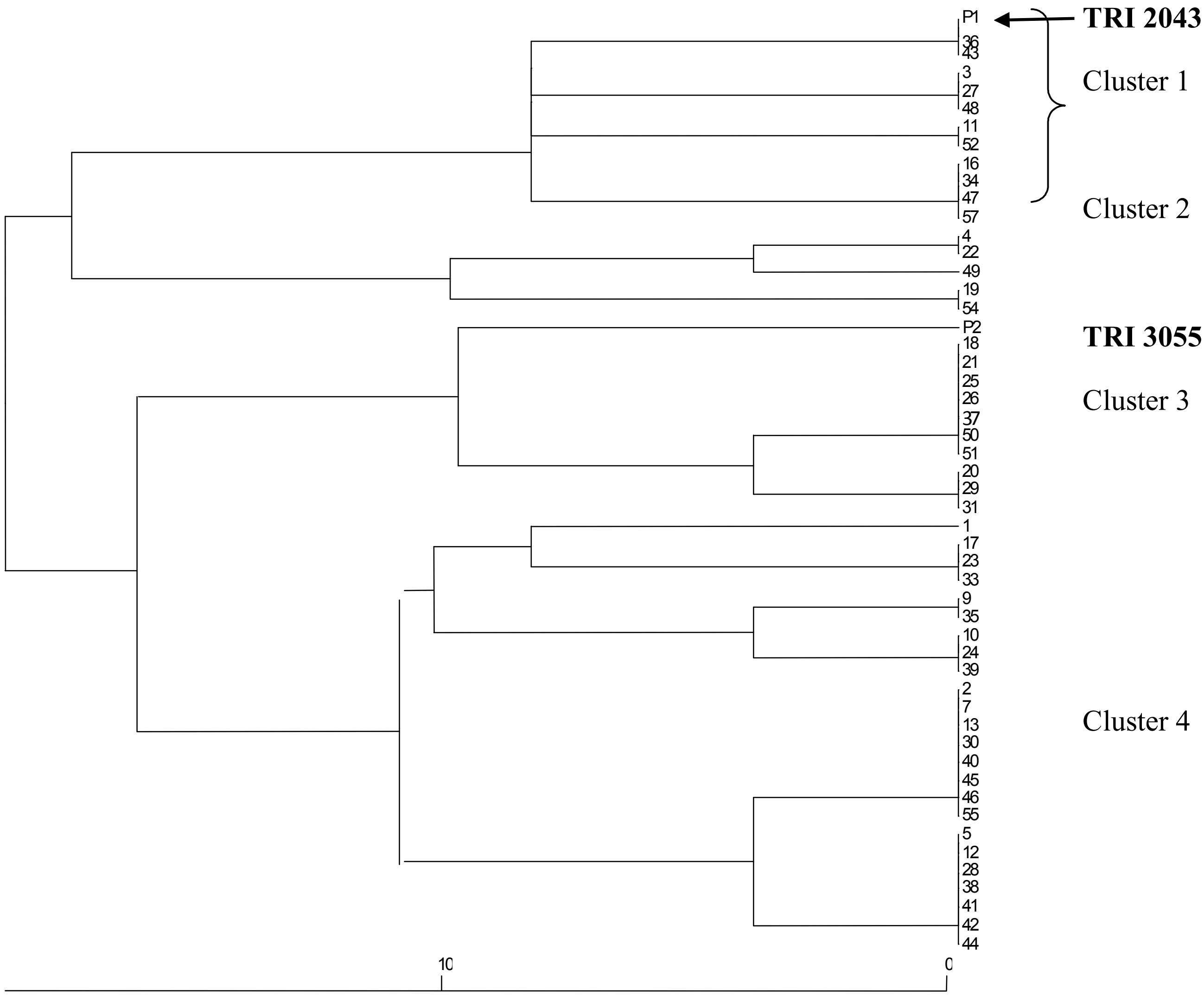

Fig. 8: Dendrogram developed for direct cross

resistance to diseases were segregating in progenies and could be correlated with quality of tea and blister blight tolerance. The majority of progenies showed anthocyanin pigmentation in their petiole and leaf pubescence. This characteristic feature can be correlated with antioxidant activity.

Generated result showed that majority of the hybrid progenies did not resemble both parental cultivars and showed variations. They were morphologically uniform and sharing both parental characters. Hybrid progenies 36, 43, 72, 90, 93,125,153 closely resembled the parental cultivar TRI 2043. They could be blister blight resistant cultivars. These progenies are suggested for use as alternative cultivars for silvertip production. None of the hybrid progeny morphologically resembled the parental cultivar TRI 3055. This was due to the special vestiture character of this parent.

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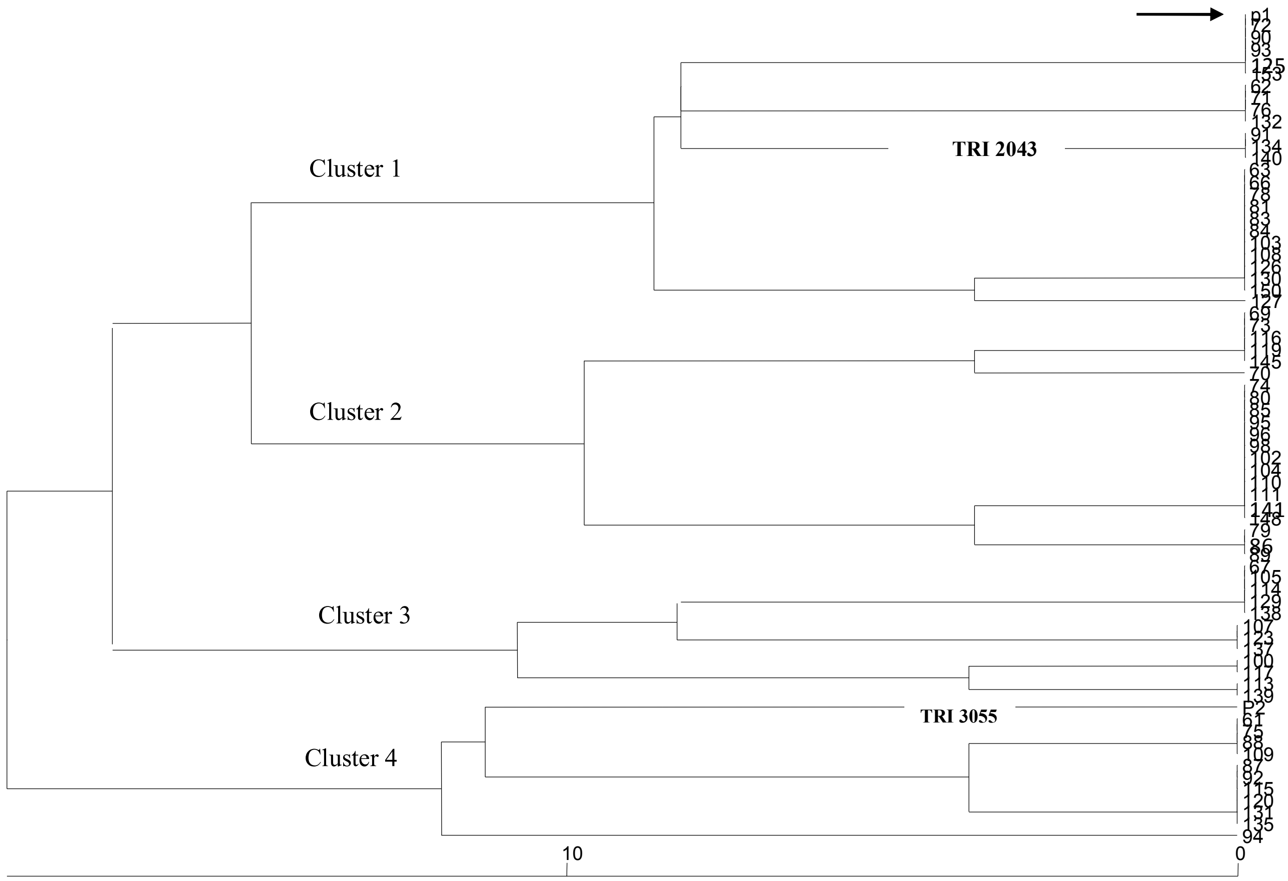

Fig. 9: Dendrogram developed for reciprocal cross

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