MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *Cattleya* HYBRIDS

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By

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(2020-12-001)

THESIS

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2023

DECLARATION

I, hereby declare that this thesis entitled **"Morphological and molecular characterization of *Cattleya* hybrids"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellayani — **Liji Viswanathan**

17.04.2023 — **(2020-12-001)**

CERTIFICATE

Certified that this thesis, entitled **"Morphological and molecular characterization of *Cattleya* hybrids"** is a record of research work done independently by Ms. Liji Viswanathan (2020-12-001) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of Ms. Liji Viswanathan (2020-12-001), a candidate for the degree of **Master of Science in Horticulture** with major field in Floriculture, agree that the thesis entitled **"Morphological and molecular characterization of *Cattleya* hybrids"** may be submitted by Ms. Liji Viswanathan in partial fulfilment of the requirement for the degree.

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Liji Viswanathan

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOS	American Orchid Society
C.	*Cattleya*
CD	Critical difference
cm	Centimeter
cm^2	Square centimeter
CO_2	Carbon dioxide
CRD	Completely Randomized Design
CV	Coefficient of Variation
Df	Degrees of freedom
DNA	Deoxyribonucleic acid
dsDNA	Double –stranded DNA
DUS	Distinctiveness, Uniformity and Stability
EDTA	Ethylene diamine tetraacetic acid
et al.	Co-workers/co-authors
Fig	Figure
g	Gram
GCV	Genotypic Coefficient of Variation
ISSR	Inter Simple Sequence Repeats
K	Potassium
KAU	Kerala Agricultural University

Kb	Kilo Base Pair
kg	Kilogram
l	Litre
Lc.	*Laeliocattleya*
m	Metre
m^{-2}	Per square metre
m^{2}	Square metre
mg	milligram
ml	Millilitre
min	minutes
$\mathbf{N_2}$	Nitrogen
No.	Number
NRCO	National Research Centre for Orchids
ONM	Optical Number of ISSR markers
P	Phosphorus
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
pH	Potential of Hydrogen
PIC	Polymorphic Information Content
PPV and FRA	Protection of Plant Variety and Farmers Rights Authority
RAPD	Random Amplified Polymorphic DNA

RHS	Royal Horticultural society
RNase	Ribonuclease
Rs.	Rupees
ScoT	Start Codon Targeted Primers
Sem	Standard Error of mean
Sl.	Serial
TBE	Tris Borate EDTA
TE	Tris EDTA
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UV	Ultraviolet
v	Volt
viz	Namely

LIST OF SYMBOLS

&	And
@	At the rate of
^{0}C	Degree Celsius
=	Equal to
µ	Micro
/	Per
%	Per cent
₹	Rupees

INTRODUCTION

1. INTRODUCTION

Orchids belong to the family Orchidaceae with more than 25,000 species and over 3,00,000 natural and manmade hybrids. They are well recognised for their stunningly beautiful floswers, which command a very high price on the global market and account for around 10% of all floricultural trade (De, 2020a). *Cattleya* orchids, sometimes referred to as the 'queen of the orchids,' are popular for their showy and brightly coloured flowers.

Cattleyas are found in Mexico and Central America but can also be found worldwide. They have an epiphytic and sympodial character. *Cattleyas* are incredibly resilient and can tolerate changes in temperature, humidity, and even drought-like circumstances to a good extent. They produce spectacular, vibrantly coloured, and occasionally fragrant blossoms. Its great demand for cut flowers and potted plants in the international floriculture market is due to the remarkable beauty of its blossoms. They are widely utilised in both landscape design and floral decoration. *Cattleyas* in miniature sizes are either sold as a single stem or used to create bouquets. Value-added goods like perfumes and dried products are produced with *Cattleyas*.

In India, where there is a considerable demand for orchids, import values have increased relative to export values. Maximum export of orchids was found in 2016-2017 (Rs. 5.23 lakh) followed by 2017-2018 (Rs. 4.89 lakh). According to reports, India imported 1044.36 million tonnes of orchids worth Rs. 2321.84 lakh in 2018–19. *Cattleya* orchids seem to follow the same trend. India still imports a significant portion of *Cattleya* orchids despite having all the ideal conditions for *Cattleya* production. In the year 2016 alone, around 34,564 units of *Cattleya* were imported to India from Thailand, China and France and the average cost of a blooming size *Cattleya* plant ranges from Rs. 850 to Rs. 1500 (Pant *et al*., 2020).This indicates the need for concerted efforts in the country for improving production of *Cattleya* orchids.

Introduced orchid cultivars from Southeast Asian growing regions have been developed, produced, and acclimated to the various agro-climatic conditions, and have taken the lead in India's output of cut flowers. Orchids are Kerala's top priority

floricultural crop due to its high value, high yield per unit area, and suitability for cultivation both in urban and rural areas.

Being a commercially important orchid that is highly priced, easy to grow and can withstand a range of climatic conditions, *Cattleya* orchids must be exploited for commercial cultivation in Kerala. Therefore, there is an imperative need to evaluate the performance of *Cattleya* hybrids for ornamental traits. Assessment of genetic diversity through characterization of the germplasm is important for future crop improvement programmes. Morphological characterization needs to be supported by characterization using molecular markers as the phenotype is affected by environmental factors. Keeping these in view, the experiment was conducted with the following objectives:

- Evaluation of *Cattleya* hybrids for ornamental traits
- Classification based on morphological descriptors and molecular marker profile.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Cattleya orchids must be exploited for commercial cultivation in Kerala since they are a highly valuable, easy to grow orchid that can tolerate a variety of climatic conditions. In this chapter, attempts are made to review the work on morphological variability and use of molecular markers in *Cattleya.*

2.1. VARIABILITY STUDIES

The degree of genetic variability present in the population has a major impact on the outcome of any breeding programme. Understanding the scope of variability contained in a crop species is crucial since it forms the basis for efficient selection.

Within the Orchidaceae family, the most prominent are the orchids of the genus *Cattleya*, characterized by flowers with three sepals and three well-defined petals, one of them modified, known as the labellum (Abraham and Vatsala, 1981).

Barthlott (1981) examined the morphological characteristics of orchids as powerful taxonomic relationship indicators.

Mc Donald (1991) reported that robust hybrids produce bigger, better blooms and are more floriferous with more substantial flowers, highlighting the significance of vegetative vigour in orchids.

Sheehan (1992) found that *Cattleya* had two different flowering habits: type I, which included *Cattleya gigas*, developed new sprouts in the spring and flowers were triggered in the summer, with each sprout flowering separately. *C. trianaei* was a member of Type II and developed spring sprouts but did not bloom until the fall. Spathes dried out during this time, and all sprouts that had matured at this point bloom at once. Such flowering traits were typically passed on to progeny following interspecific crosses.

Rajeevan and Sobhana (1993) assessed 11 species of orchids based on flower characteristics such as inflorescence length, number, size, colour, fragrance, and flowering duration to identify the species suitable for cut flower and pot plants.

Lacerda *et al*. (1995) found that *C. intermedia* was one of the most variable orchid species, and it had a number of traits that plant breeders look for, including colour and stems with several flowers. Additionally, it was one of the *Cattleya* genus's most early-blooming species, flowering for the first time in just three years. These characteristics had prompted breeding efforts to select superior plants.

Sobhana (2000) and Lekharani (2002) reported wide variation in flower count and length and width of the leaves in a variability study conducted in *Dendrobium* orchids.

Pillai (2003) evaluated 15 *Dendrobium* varieties for morphological and floral characters and found that those varieties differed significantly for growth parameters. Significant varietal differences were observed among the varieties for shoot length, length and width of leaves, number of flowers, length and width of flowers.

Roychowdhury *et al.* (2004) found that a wide range of variation was observed among the genotypes in orchids for characters such as the number of shoots per plant, number of leaves per pseudobulb, length and width of the largest leaf, and number of days from leaf emergence to maturity.

In India, *Cattleya* orchids were found suitable for cultivation in valleys and foothills of Arunachal Pradesh, plains of Tripura, Assam, Kerala, Karnataka, Nagaland, West Bengal, Orissa, Sikkim and Western Ghats (Pal and Nagrare, 2006).

Devadas *et al*. (2010) evaluated the rare orchid species, *Renanthera imschootiana* and found that they were with characteristics such as unbranched raceme with a length of 32.2 cm, appealing dominant red-purple (RHS-60A) flowers, and petals tinted greyed orange (RHS-164C) with shade. Having a middle range vase life of 23.7 days and appealing dominant crimson red purple blossom colour, broad lateral sepals offer great breeding value for creating new hybrid variants.

Out of the nine *Cattleya* hybrids evaluated, the maximum longevity of spike was recorded with the variety Ahmad Seikhi (49 days), while the maximum spike length of 35 cm was recorded in Hsinging Catherine. *Cattleya* hybrids *viz*. Queen Sirikhit, Ahmad Seikhi, Guanmiau City, Chinese Beauty Orchid Queen, Blc Mem

Ann Balmores Convess and Hsinging Catherine were found promising for commercial cultivation (NRCO, 2012).

Anand *et al.* (2013) assessed 24 orchid species on vegetative characters and reported that vegetative growth characters in terms of plant height, number of leaves, leaf length, leaf width and habitat play a key role in ultimately deciding crop yield.

The *Cattleya* genus is considered to be the 'Queen of the Orchids' (AOS, 2013) and differs mostly from other orchids by its large flowers arranged in inflorescences, when compared with *Phalaenopsis* and *Dendrobium* orchids

Three novel cultivars (UEL 6, UEL 7 and UEL 8), which express the desired features of mother plants such as bloom size, floral colour, flourishing period, number of flowers, plant height, among others, were successfully created by Faria *et al.* (2013) who researched *Dendrobium* crosses.

Cattleya plants were reported as either unifoliate or bifoliate in nature with elongated pseudobulbs. The present-day hybrid *Cattleyas* belong to the unifoliate group. The flowers are 5 to 15 cm in size and occur in all colours except true blue and black. Unifoliate *Cattleyas* bear up to five flowers per inflorescence whereas bifoliate *Cattleyas* possess 2 to 25 flowers per inflorescence (De *et al*. 2014a).

De *et al*. (2014b), evaluated eight hybrids of *Cattleya* for the development of Distinctiveness, Uniformity and Stability (DUS) test guidelines using common descriptors. Out of the 53 common descriptors developed, plant height, leaf number per pseudobulb, flower width in front view, petal predominant colour, lip predominant colour and lip colour pattern were used for grouping of hybrids.

Burana and Yamane (2017) studied the vase life of *Cattleya* variety 'Lc. Spring Clima × Christina' and found that the flowers were sensitive to ethylene. The vase life was increased by high CO_2 in combination with pre-cooling from 10 days to 14 days.

For the morphological description of the hybrids, 10 plants were evaluated for their vegetative part phytometric characteristics: pseudobulb length and diameter, leaf length and width, and phytometric characteristics of their reproductive part: floral

stem length, flower width and length and number and durability of the flowers (Colombo *et al*., 2017).

A clear understanding on the floral biology is of great importance in orchid breeding due to its structural and functional complications. The important floral characters like flower size, pedicel size, flowering period, number of spikes per plant and spike/stalk length, flower longevity and vase life exhibited a significant variation in quantitative floral characters which was in conformity with the reports of De *et al.* (2019).

De (2020b) evaluated the keeping quality of tropical and subtropical orchid hybrids as effected by different packing materials and found out that *Cattleya* cut spikes had a longevity of 10 days in floral foam and 14 days in plastic vials. The longevity in cellophane paper packing of fully open florets ranged from 14 to 45 days over unpacked florets which had only 7 to 11 days.

Sub-tropical orchids (*Dendrobium* sp.) were evaluated for vegetative characteristics under shadenet circumstances by Pachuau *et al*.(2021). P957, P1006, P1019, Sonia White, Sonia 5N, Somark White, Pink Stripe, and Burana Jade were the kinds used. In terms of plant height (27.1 cm), internodal length (4.5 cm), and shoots per plant, the results showed that variety P1006 was a highly promising variety (8). The Burana Jade variety was an excellent choice for a potted plant because it has the smallest height (13.8 cm), shortest internodes (1.9 cm), and smallest leaves (25.7 cm^2).

2.2. GENETIC PARAMETERS

For any breeding effort, genetic factors like as heritability, genetic advance estimates, and phenotypic and genotypic coefficients of variation are necessary.

High heritability along with high genetic advance as percent of mean in characters suggested that the genotypic variations for such characters are probably due to high additive genetic effects whereas environmental effects had least effect on such characters (Johnson *et al*., 1955).

Selection based on characters with high heritability and genetic advance would be more effective and are generally controlled by additive gene action and hence amenable to genetic improvement through selection (Panse, 1957).

Allard (1960) found that, compared to hybrid breeding, intraspecific crossings are more limited in their ability to improve species (interspecific and intergeneric crossing). This results from the genetic gain shown in flower shape, substance, and texture, which is typically higher at first and tends to have lower values in subsequent generations.

The extremely heterozygous nature of multigeneric hybrids was demonstrated by Kamemoto (1983), who reported that even reciprocal crossings produced offspring that differed in vegetative features and flower production.

Based on 16 floral and vegetative morphological characteristics, Balfour and Linder (1990) investigated intraspecific variation of individuals from seven groups of populations of *Disa uniflora*.

Dendrobium aggregatum and a few other species that are grown in the plains of West Bengal were subjected to genetic study by Rehman *et al*. (1993). For inflorescence length, number of flowers per inflorescence, number of flowers per inflorescence, and flower size, high genetic advance was seen. These features also had significant heritability and genetic advance estimates, which suggested successful selection based on these traits.

The degree of genetic variety within a species and how it is distributed both within and among populations offer insights into the variables that control inbreeding, gene flow, and variation maintenance. Geographic range has a positive relationship with genetic diversity at the species level but no influence on how genetic variation is distributed among populations (Ayres and Ryan, 1999).

Sobhana (2000) performed genetic studies on *Dendrobium* hybrids. Number of shoots per plant, days for floret opening, and number of flowers per spike all showed high genetic diversity. Most of the characters ranged from having low to high heritabilities. All floral traits associated with flower size showed the highest

heritability. It was also reported that plant height had significant positive phenotypic correlation with petal length and width and length of the largest leaf. Flower number per peduncle had a significant negative correlation with flower length and width. The length of flower had significant positive correlation with width of the flower, which is an important attribute of cut flower selection.

In a study conducted by Salamini *et al*. (2002), the sequence diversity at genes affecting important phenotypes is known to be significantly decreased by domestication. The frequency of alleles regulating traits of interest rose in populations subjected to continuous selection, where only the best individuals were favoured for reproduction. This resulted in a loss of variety in crop plants.

Pridgeon *et al.* (2005) reported one of the key traits of the *Cattleya* genus is the potential for interspecific or intergeneric crosses, such as those with *Brassavola, Epidendrum, Encyclia, Laelia, or Sophronitis*, with many of these now being *Cattleya* (Van den Berg, 2014). This makes it possible to create and grow intergeneric/inter specific hybrids, which are a source of variety for traits including plant growth, flower colour, and inflorescence shape.

In *Brassocattleya*, the varieties Deesse and Hortland were reported to produce many hybrids in the *Cattleya* alliance. The trigeneric hybrid *Brassolaeliocattleya* was well known for its varieties like Edwin Chong, Golden Myth, Herons Ghyll, Molflora, Norman Bay, Nugget *etc*. which were proven as parent plants for hybridization work (Bhattacharjee and Das, 2008).

A cultivar called *Laelia* 'Brazilian Girl Rosa' was produced by Cardoso (2010) by an interspecific/intergeneric cross between *Cattleya* 'White Dream' and *Laelia* 'Rubin'. *Brassolaeliocattleya* is a hybrid of three genera (*Brassavola, Laelia, and Cattleya*), however it is unclear how much each genus contributed to *Cattleya* hybrid improvement as an ornamental and horticultural plant. *Brassavola, Laelia, and Sophronitis*, however, contribute to the creation of compact size hybrids in their research.

Neto and Vieira (2011) reported that in wild and bred *C. intermedia* accessions, the genetic variability including flower shape and number, precocity, texture and the thickness of floral parts as the result of plant domestication and the use of superior genotypes in the crop improvement programme.

Number of leaves per pseudobulb had negative correlation with good floral attributes. So plants with unifoliate leaves produced larger flowers and took minimum days from emergence to maturity of inflorescence (De *et al*., 2014a)**.**

High degrees of heterozygosity are also significant sources of variation that can be used to select various desired traits in breeding programmes (Fridman, 2014). Although such goals have only been the focus of a relatively small number of breeding efforts for ornamental plants, including orchids, this includes a decrease in the juvenile stage and resistance to pests and diseases.

Cardoso *et al.* (2016) reported two flowering types in *Cattleya*, one of which produced flowers once a year and the other of which produced flowers multiple times. Such flowering characteristics were found transmitting to the descendants following interspecific crosses.Though ordinary varieties of *Cattleya* are seasonal, a superior cultivar *Brassolaeliocattleya* BR501, produced flowers four times a year. The durability of opened flowers was around 20–25 days in the plant.

Rahi (2017) found that the extensive range of variation in genotypes may be due to the higher order monogeneric, bigeneric, or multigeneric hybrid genotypes used in the study.

2.3. GENETIC DIVERGENCE ANALYSIS

Geburek (1997) reported that knowledge on genetic diversity is the baseline for conservation. In order to support the conservation of wild orchids, information on the levels and patterns of genetic diversity of these plants must be gathered through comparative population studies utilising both the allozyme and RAPD approaches.

An effective method for differentiating across plant kinds is DNA fingerprinting. The limits of morphological and biochemical characterizations,

particularly for closely related varieties, are eliminated by the use of many and highly polymorphic DNA markers (Sun and Wong, 2001).

Research using molecular systematics has brought a continuing revolution to plant taxonomy. For instance, studies on *Laeliinae* (Van den Berg *et al*., 2000) and *Cattleya* genera (Dressler and Higgins, 2003) showed that *Cattleya* is not clearly delimited.

The populations of all *Cattleya* variations were extremely varied, according to Rego *et al.* (2009), which examined the genetic diversity among eleven *C.violacea* types from the Amazonian Region (three Brazil Amazonian States and one Venezuelan Amazonian region) with variable flower colour. The conservation of orchids is therefore more difficult than the conservation of many other plants because a greater number of population locations need to be safeguarded in order to sustain the complete genetic diversity at the species level for their long-term survival. This demonstrated the need for the biological diversity of the genus *Cattleya* and the Orchidaceae family to be conserved both in situ and ex situ in the Amazon region.

Pinheiro *et al*. (2012) observed high levels of polymorphism in *Cattleya labiata* in a study with 130 *Cattleya* species using RAPD and ISSR markers. This study helped to understand the genetic structure of the species and to define strategies for conservation and breeding programmes

In order to estimate genetic diversity, Fajardo *et al*. (2014) examined the polymorphic information content (PIC) and optimal number of ISSR markers (ONM) for five *Laeliinae* orchids. For the purpose of examining genetic diversity and differentiation, the phylogenetic relationships between *Cattleya granulosa*, an endangered Brazilian orchid, and four other native Brazilian species (*Brassavola tuberculata, Cattleya bicolor, Cattleya labiata, and Cattleya schofieldiana*) were examined. *C. labiata* had the lowest level of genetic diversity (H E = 0.13) and *C. bicolor* had the highest level (H E = 0.219) among the five species under study. . Of the five studied species, *C. bicolor* exhibited the highest level of genetic diversity (H_E = 0.219), while *C. labiata* exhibited the lowest level (H_E = 0.132). Analysis of molecular variance revealed that 23.26% of species differ genetically from one

another. The ISSR data's principal component analysis (PCA) revealed that unifoliate and bifoliolate species have genetically distinct populations. Additionally, PCA showed a strong relationship between *C. granulosa and C. schofieldiana,* a species that many scientists believe to be a subspecies of *C. granulosa*. Thus concluded that ISSR markers are a possible tool for phylogenetic reconstruction of closely related species.

Hartati (2017) studied the genetic diversity of some important species of genus *Coelogyne* spp., performed using inter simple sequence repeats (ISSR) molecular marker. The DNA of six orchid species from the genus of *Coleogyne* spp was extracted and used as samples in a PCR amplification process with ten ISSR primers. This study revealed that the six orchid species under examination had similarity coefficients ranging from 0.32 to 0.70, indicating that the genetic diversity of the species was dispersed between 0.30 and 0.68, and polymorphic amplification bands as high as 98.9%.

Qiang *et al*. (2020) studied the genetic diversity of *Cattleya* germplasms, and the results of ISSR-PCR analysis showed that there was abundant genetic diversity among them. The 18 *Cattleya* germplasm resources were divided into seven genera, which were *Cattleya*, *Crispae*, *Schomburgkoidea*, *Falcata*, *Intermedia*, *Rhizantha* and *Guarianthe.*

Gholami *et al*.(2021) studied the genetic diversity, population structure, and phylogeny of Iranian orchids using inter-simple sequence repeat (ISSR) markers to find markers associated with phenotypic traits. Six major groups were identified by the UPGMA genetic similarity dendrogram using Jaccard coefficients (r = 0.973). According to the Bayesian clustering method, the division of the accessions into eight groups produced the data with the highest likelihood. Based on the multiple association study, features connected to flowers and tubers had strong correlations together and were linked to specific ISSR bands. Overall, ISSR markers identified the variability among accessions within each population and species and were beneficial for differentiating and clarifying the relationships among species and populations gathered from geographically distinct areas.

Tikendra *et al*. (2021a) conducted genetic variation study in 11 populations of *D. chrysotoxum* using 20 different inter-simple sequence repeats (ISSR) and 9 start codon targeted (SCoT) primers. Out of 199 bands produced by ISSR markers, 196 bands were polymorphic, resulting in high polymorphism (98.49%). SCoT markers, however, produced 111 polymorphic fragments generating 100% polymorphism.

Dendrobium moschatum's molecular genetic homogeneity was evaluated by Tikendra *et al*. (2021b). High genetic closeness between mother and micropropagated plants was also found by using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based dendrograms. The categorization of plants according to UPGMA dendrograms was further confirmed by Principal Coordinate Analysis (PCoA). The markers' matrices' correlation analysis showed that ISSR markers were more effective than RAPD markers. This is the first account of genetic homogeneity testing utilising molecular markers on *Dendrobium moschatum* that has been propagated in vitro.

Inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) molecular markers have recently been utilised for the classification of plants such as Ilex aquifolium L. (Tsaktsira *et al*., 2021). These markers are free from environmental influences, have an infinite number of obtained polymorphisms, and only a little amount of DNA is needed for analysis.

In comparison to other techniques, ISSR marker is popular due to its simplicity, speed, convenience of use, and affordability. Examples include examining the homogeneity of *Anoectochilus elatus* Lindl. regenerated - Genetic diversity of jewel orchid 4817 from somatic embryogenesis or screening the somaclonal variation of *Ludisia discolour* A.Rich. for long-term conservation) (Sherif *et al*. 2018; Rajan *et al*., 2022). More recently, a study from Malaysia revealed the use of this marker in validating the genetic stability of the in vitro grown plants to the mother plant, a gem orchid native to Sabah, Malaysia (David *et al*., 2022).

Tran *et al*. (2022) investigated the genetic diversity of jewel orchids in Vietnam using RAPD and ISSR markers to provide basic data conservation of genetic resources and the development of this medicinal plant. A total of 20 jewel orchid

samples were genetically characterized by using 10 RAPD and 10 ISSR primers. Combined RAPD and ISSR markers produced similarity coefficients ranging from 0.53 to 0.83. Based on the PIC values, the primers used by both markers are highly informative. However, the topology of cluster analysis of 20 jewel orchid accessions does not correspond to the taxa studied. The findings could be potential to employ in future classification, conservation, and development of this plant.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study was conducted to evaluate the performance of *Cattleya* hybrids based on growth and flowering attributes and to characterize them using morphological descriptors and molecular markers. The investigation was carried out in the Department of Floriculture and Landscaping, College of Agriculture, Vellayani, Thiruvananthapuram during November 2021 to October 2022.

Details of materials used and methodologies adopted during this investigation are presented below.

3.1. EVALUATION OF THE HYBRIDS AND MORPHOLOGICAL CHARACTERIZATION

3.1.1. Experimental Material

Near flowering size plants of twelve *Cattleya* hybrids were selected for studying their morphological and floral characters for one year.

The details of the genotypes used in the study are given in Table 1.

3.1.2. Layout and conduct of the experiment

The experimental plants were grown in mud pots with the potting mixture containing tile bits, chopped coconut husk or charcoal as per the Package of Practices of Kerala Agricultural University (KAU, 2016). All plants received uniform cultural practices. Artificial shade of 50 per cent was provided with polypropylene agro-shade netting. The experiment was laid out in completely randomized block design with five replications.

The plants were sprayed with the supernatant liquid of cowdung slurry at fortnightly interval. During the vegetative phase, fertilizer mixture of N, P and K were applied in the ratio of 3:1:1. During the blooming phase, this ratio was 1:2:2. The mixture was applied twice a week @2-3g per litre.

Observations were recorded as per the guidelines of the DUS (Distinctiveness, Uniformity and Stability) on *Cattleya* by the Protection of Plant Variety and Farmers Rights Authority (PPV&FRA), Govt. of India on the following characters.

3.1.3. Morphological Characters

3.1.3.1. Quantitative characters

3.1.3.1.1. Plant height (cm)

Total height of the plant was measured from the base to the growing apex and expressed in centimeters.

3.1.3.1.2. Number of shoots per plant

Number of shoots produced per plant was recorded at monthly interval.

3.1.3.1.3. Number of leaves per pseudobulb

Number of leaves per each pseudobulb recorded monthly.

3.1.3.1.4. Length of the largest leaf (cm)

Length of the largest leaf in each plant was recorded and expressed in centimeters.

3.1.3.1.5. Width of the largest leaf (cm)

Width of the largest leaf in each plant was recorded and expressed in centimeters.

3.1.3.1.6. Days from emergence to maturity of leaves

Number of days taken from emergence to maturity of leaves was recorded.

3.1.3.2. Pseudo qualitative characters

The following characters were recorded visually.

3.1.3.2.1. Nature of pseudo bulb

Nature of pseudo bulb was recorded as either cylindric, clavate or globular.

3.1.3.2.2. Leaf shape

Shape of the largest leaf was recorded as either narrow oblong, ligulate, elliptic or ovate.

3.1.3.2.3. Leaf colour pattern

Leaf colour pattern was recorded either as uniformly green on both sides, green upside/purple beneath, spotted or streaked.

3.1.3.2.4. Dorsal sepal shape

Dorsal sepal shape was recorded either as oblong, lanceolate, elliptic or ovate.

3.1.3.2.5. Dorsal sepal apex

Dorsal sepal shape was recorded either as acute, notched or obtuse.

Plate 1. General view of the experimental plants

Table 1. Details of *Cattleya* genotypes used in the study

Sl.No.	Abbreviations	*Cattleya* hybrids
1	TS	*Rhyncholaeliocattleya* Tawan Shine
2	B	*Rhyncattleanthe* Burana Beauty
3	TB	*Rhyncholaeliocattleya* Taichung Beauty
4	MY	*Rhyncholaeliocattleya* Mahina Yahiro
5	PW	*Rhyncholaeliocattleya* Petch Wangnam Khiew
6	CD	*Cattleya* Chocolate Drops Volcano Queen
7	ID	*Rhyncholaeliocattleya* Irene Dopkin
8	H X M	*Rhyncholaeliocattleya* Haadyayi Delight x Mary Song
9	A X N	*Cattleya* Aurantiaca x Netrasiri Beauty
10	T	*Cattleya* Tipo
11	MA	*Rhyncholaeliocattleya* Memoria Anna Balmores
12	MS	*Rhyncholaeliocattleya* Morning Stars

3.1.3.2.6. Dorsal sepal curvature

Dorsal sepal shape was recorded either as incurved, reflex or straight.

3.1.3.2.7. Lateral sepal shape

Lateral sepal shape was recorded either as oblong, lanceolate, elliptic or ovate.

3.1.3.2.8. Lateral sepal apex

Lateral sepal apex was recorded either as acute, notched or obtuse.

3.1.3.2.9. Lateral sepal curvature

Lateral sepal curvature was recorded either as incurved, reflex or straight.

3.1.3.2.10. Petal shape

Petal shape was recorded either as oblong, lanceolate, elliptic, ovate or round.

3.1.3.2.11. Petal curvature

Petal curvature was recorded either as incurved, reflex or straight.

3.1.3.2.12. Petal margin

Petal margin was recorded either as entire, undulate or undulate crisped.

3.1.4 Floral characters

3.1.4.1. Quantitative characters

3.1.4.1.1. Inflorescence number per pseudobulb

Inflorescence number per pseudobulb was recorded either as one or two or more.

3.1.4.1.2. Inflorescence length (cm)

Inflorescence length from base of peduncle to the tip of the flowers was recorded and expressed in centimeters.

3.1.4.1.3. Peduncle sheath length (cm)

Peduncle sheath length of the plants was recorded and expressed in centimeters.

3.1.4.1.4. Peduncle length (cm)

Peduncle length of the plants was recorded and expressed in centimeters.

3.1.4.1.5. Flower number per peduncle

Flower number per peduncle was counted and recorded.

3.1.4.1.6. Flower length from tip of dorsal sepal to tip of lip (cm)

Flower length from tip of dorsal sepal to tip of lip was recorded in the spread out position and expressed in centimeters.

Rlc.Petch Wangnum Khiew

***C.* Aurantiaca x Netrasiri Beauty**

Rlc.Memoria Anna Balmores

Rlc.Morning Star

Rlc.Mahina Yahiro

Plate 2. *Cattleya* hybrids used for the study

Rth. Burana Beauty

C. Tipo

Rlc.Haadyayi Delight x Mary Song

Rlc.Taichung Beauty

Rlc.Irene dopkin

Plate 2. *Cattleya* hybrids used for the study (Continued)

3.1.4.1.7. Flower width in front view (cm)

Flower width in front view was recorded in the spread out position and expressed in centimeters.

3.1.4.1.8. Days from emergence to opening of the Inflorescence

Days from emergence to opening of the Inflorescence was observed and recorded.

3.1.4.1.9. Flower longevity on the plant

Flower longevity on the plant was recorded by observing the number of days the flowers remained in the plant without fading.

3.1.4.1.10. Dorsal sepal length (cm)

Dorsal sepal length was recorded in the spread out position and expressed in centimeters.

3.1.4.1.11. Dorsal sepal width (cm)

Dorsal sepal width was recorded in the spread out position and expressed in centimeters.

3.1.4.1.12. Lateral sepal length (cm)

Lateral sepal length was recorded in the spread out position and expressed in centimeters.

3.1.4.1.13. Lateral sepal width (cm)

Lateral sepal width was recorded in the spread out position and expressed in centimeters.

3.1.4.1.14. Petal length (cm)

Petal length was recorded in the spread out position and expressed in centimeters.

3.1.4.1.15. Petal width (cm)

Petal width was recorded in the spread out position and expressed in centimeters.

3.1.4.1.16. Lip length (cm)

Lip length was recorded in the spread out position and expressed in centimeters.

3.1.4.1.17. Lip width (cm)

Lip width was recorded in the spread out position and expressed in centimeters.

3.1.4.1.18. Column length (cm)

Column length was recorded in the spread out position and expressed in centimeters.

3.1.4.1.19. Length of flower pedicel (cm)

Length of flower pedicel was recorded in the spread out position and expressed in centimeters.

3.1.4.1.20. Vase life of flowers (days)

Flower stalk was placed in measured quantity of tap water taken in test tubes and mouth of the test tube was covered by cling film. Appearance of wilting or colour fading was noticed and days taken for the appearance of senescence symptoms was recorded as vase life.

3.1.4.2. Qualitative Characters

For the assessment of colour characteristics, the Royal Horticultural Society (RHS) colour chart was used.

3.1.4.2.1. Sepal dominant colour

Sepal dominant colour was recorded either as white, yellow or purple.

3.1.4.2.2. Sepal colour pattern inside

Sepal colour pattern inside was recorded either as uniform, spotted or shaded /striped.

3.1.4.2.3. Petal predominant colour

Petal predominant colour was recorded either as either as white, yellow or purple.

3.1.4.2.4. Petal colour pattern

Petal colour pattern was recorded either as uniform, spotted or shaded /striped.

3.1.4.2.5. Lip predominant colour

Lip predominant colour was recorded either as either as white, yellow or purple.

3.1.4.2.6. Lip colour

Lip colour was recorded either as yellow or purple with one or two numbers.

3.1.4.2.7. Column colour pattern

Column colour pattern was recorded either as uniform, spotted or shaded /striped/streaked.

3.1.4.3. Pseudoqualitative Characters

3.1.4.3.1. Lip shape

Lip shape was recorded either as oblong or lanceolate.

3.1.4.3.2. Lip lateral lobe shape

Lip lateral lobe shape was recorded either as oblong or round.

3.1.4.3.3. Lip lateral lobe margin

Lip lateral lobe margin was recorded either as entire, crisped or undulate crisped.

3.1.4.3.4. Lip mid lobe shape

Lip mid lobe shape was recorded either as oblong or round.

3.1.4.3.5. Lip mid lobe margin

Lip mid lobe margin was recorded either as entire, crisped or undulate crisped.

3.1.4.3.6. Lip surface inside

Lip surface inside was recorded either as glabrous/pubescent.

3.1.4.3.7. Flowering season

Flowering season was recorded either as winter or rainy.

3.2. MOLECULAR CHARACTERIZATION

The twelve hybrids of *Cattleya* used in the experiment were studied for molecular characterization. The investigations were carried out in the Rajiv Gandhi Centre for Biotechnology, Trivandrum. The materials used and methodology followed are described below.

3.2.1. DNA isolation using NucleoSpin® Plant II Kit (Macherey-Nagel)

Leaf samples were collected from young new leaves of *Cattleya* plants. About 100 mg of the tissue was homogenized using liquid nitrogen and the powdered tissue was transferred to a microcentrifuge tube. Four hundred microlitres of buffer PL1 was

added and vortexed for 1 minute. Ten microlitres of RNase A solution was added and inverted to mix. The homogenate was incubated at 65^{o}C for 10 minutes. The lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for 2 minutes. The flow through liquid was collected and the filter was discarded. Four hundred and fifty microlitres of buffer PC was added and mixed well. The solution was transferred to a Nucleospin Plant II column, centrifuged for 1 minute and the flow through liquid was discarded. Four hundred microlitre buffer PW1 was added to the column, centrifuged at 11000 x g for 1 minute and flow though liquid was discarded. Then 700 µl PW2 was added, centrifuged at 11000 x g and flow through liquid was discarded. Finally 200 µl of PW2 was added and centrifuged at 11000 x g for 2 minutes to dry the silica membrane. The column was transferred to a new 1.7 ml tube and 50 µl of buffer PE was added and incubated at 65^{o}C for 5 minutes. The column was then centrifuged at 11000 x g for 1 minute to elute the DNA. The eluted DNA was stored at 4^{o}C.

3.2.2. Agarose Gel Electrophoresis for DNA quality check

The quality was checked using agarose gel electrophoresis. 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.2.3. Quantification using Quantifluor

1 µl of isolated DNA was added to the 200 µl of Quantiflour (which contains a fluorescent DNA-binding dye that enables sensitive quantitation of small amounts of double-stranded DNA (dsDNA) in solution) in a 0.5ml tube and mixed by tapping and short spin was given and kept in the fluorometer (which uses fluorescent labelled dyes for quantification of DNA) for quantification of the DNA.

3.2.4. ISSR PCR analysis

PCR amplification reactions were carried out in a 20 µl reaction volume.

2x DyNAzyme II PCR Master Mix	:	10 µl
Primer (10 µM)	:	1 µl
DW	:	7 µl
DNA	:	2 µl

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) with ten ISSR primers (Table 2.)

PCR amplification profile

95 ºC	-	5.00 min	
94 ºC	-	0.45 min	35 cycles
52 ºC	-	1.00 min	
72 ºC	-	1.30 min	
72 ºC	-	10.00 min	
4 ºC	-	∞	

3.2.5. Agarose gel electrophoresis of PCR products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was 1 kb plus DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.3. STATISTICAL ANALYSIS

The data collected were subjected to Analysis of Variance (ANOVA) to test for significant differences among the genotypes (Panse and Sukhatme,1967). Variance component analysis and cluster analysis (Singh and Chaudhary, 1985) were the statistical analyses done in the present study. GrapesAgri1 was utilised for analysis of data (Gopinath *et al*., 2021).

Table 2. ISSR Primers used for molecular characterization

Sl. No.	Primer Name	Nucleotide sequence
1	810	5’ GA GA GA GA GA GA GA GA T 3’
2	812	5’ GA GA GA GA GA GA GA GA A 3’
3	814	5’ CT CT CT CT CT CT CT CT CTA 3’
4	818	5’ CA CA CA CA CA CA CA CA G 3
5	836	5’ AG AG AG AG AG AG AG AG YA 3’
6	840	5’ GA GA GA GA GA GA GA GA YT 3’
7	842	5’ GA GA GA GA GA GA GA GA YG 3’
8	843	5’ CT CT CT CT CT CT CT CT CT RA 3’
9	862	5’ AG CA GC AG CA GC AG CA GC 3’
10	885	5’ BHB GA GA GA GA GA GA GA 3’

3.3.1. Analysis of Variance

Analysis of variance with two variables X and Y was measured in genotypes 'g' developed in completely randomised design with 'r' replication and is given as below

Source	Degrees of freedom	Mean Square		
		X	Y	XY
Between genotypes	(g-1)	G_{XX}	G_{YY}	G_{XY}
Error	(r-1)(g-1)	E_{XX}	E_{YY}	E_{XY}

3.3.2. Coefficient of Variation

Variability that existed in the population for various characters were apportioned using the estimates of coefficient of variation.

For the character $X_{i,}$

Phenotypic coefficient of variation, PCV $= \frac{\sigma pi}{xi} x\ 100$

Genotypic coefficient of variation, GCV $= \frac{\sigma gi}{xi} x\ 100$

Environmental coefficient of variation, ECV $= \frac{\sigma ei}{xi} x\ 100$

Where σ_{pi}, σ_{gi} and $\sigma_{ei}$are the phenotypic, genotypic and environmental standard deviations respectively.

3.3.3. Heritability Coefficient

Jain (1982) proposed the mathematical relationship of variance estimates on computation of heritability, which is usually expressed as a percentage.

Heritability (broad sense), $H^2 = \frac{\sigma gi\ 2}{\sigma pi2} x\ 100$

The heritability % were categorized as suggested by Robinson *et al.* (1949) namely, low (0-30), moderate (30-60) and high (above 60) .

3.3.4. Genetic Advance Under Selection

Genetic advance as percentage of mean was calculated as per the formula given by Lush (1949).

Genetic advance,GA = $\frac{kH\ 2\ \sigma pi}{xi}$ x 100

H^2 - heritability in broad sense

σ_{pi} - phenotypic standard deviation

k -selection differential that is 2.06 % in case of 5 % selection in large samples (Miller *et al*., 1958).

Genetic advance as percentage were categorized into low (< 20 %) and high (> 20%) as suggested by Robinson *et al*. (1949).

3.3.4. Correlation Analysis

The correlation coefficients (phenotypic, genotypic and environmental) between two characters denoted as i and j were worked out as

Genotypic correlation (r_{gij}) $\frac{\sigma gij}{\sigma gix\sigma gj}$

Phenotypic correlation (r_{pij}) $\frac{\sigma pij}{\sigma pix\sigma pj}$

Environmental correlation (r_{eij}) $\frac{\sigma eij}{\sigma eix\sigma ej}$

Where σg_{ij}, σp_{ij} and $\sigma e_{ij}$are the genotypic, phenotypic and environmental covariances between the characters i and j.

3.3.5. Cluster Analysis

Genetic divergence was studied using Average method and Euclidean Distance measures.

3.3.6. Data analysis

The reproducible bands were scored for their presence (+) or absence (-) for all the characters studied. The similarity matrices was developed using the data obtained by ISSR primers 12 *cattleya* genotypes using "SIMQUAL" sub- programme of software NTSYS-pc. The allelic diversity was used to produce a dendrogram using 'cluster tree analysis' sub programme of same software which revealed the genetic relationship between the *cattleya* genotypes.

The association between the various genotypes was assessed from the dendrogram obtained from 10 ISSR primers (polymorphic) data using "SIMQUAL" sub- programme of software NTSYS-pc.

RESULTS

4. RESULTS

The recorded observations were statistically analysed during November 2021 to October 2022 and the results of the present study on morphological and molecular characterization of *Cattleya* hybrids are presented below.

4.1. EVALUATION OF THE HYBRIDS AND MORPHOLOGICAL CHARACTERIZATION

4.1.1. Analysis of variance of morphological quantitative characters

The mean performance of all the genotypes for the morphological characters are presented in Table 3. The analysis of variance revealed significant differences among the genotypes for all of the studied morphological characters, with the exception of plant height, number of shoots per plant, number of leaves per pseudobulb, length and width of the largest leaf, and number of days from leaf emergence to maturity.

Plant height was observed highest for the genotype Taichung Beauty (12.04 cm) and lowest for the genotype Chocolate Drops Volcano Queen (7.53 cm). The genotype Chocolate Drops Volcano Queen (15.7 nos) recorded the highest value for the character, number of shoots per plant which was on par with Haadyayi Delight x Mary Song and *Cattleya* Tipo while the lowest value was recorded for the genotype (4.5 nos). The highest value for the character, length of the largest leaf was noticed in the genotype (20.29 cm) which was on par with Tawan Shine, Petch Wangnam Khiew, Memoria Anna Balmores Taichung Beauty and Morning Stars. The lowest was reported in Burana Beauty (10.07 cm). For the character, width of the largest leaf, the highest mean value was observed for the genotype Petch Wangnam Khiew (5.23 cm) which was on par with Taichung Beauty, Tawan Shine, Memoria Anna Balmores, *Cattleya* Tipo, Morning Stars and Irene Dopkin except Mahina Yahiro, Haadyayi Delight x Mary Song and Chocolate Drops Volcano Queen whereas the lowest value was exhibited by the genotype Aurantiaca x Netrasiri Beauty (2.81cm). For the character, days from emergence to maturity of leaves, the genotype Burana Beauty recorded the highest value (55 days) which was on par with Burana Beauty, *Cattleya* Tipo and Taichung Beauty, and the genotype Haadyayi Delight x Mary Song and Chocolate Drops Volcano Queen (30.67 days) recorded the lowest value.

4.1.2. Analysis of variance of floral quantitative characters

The mean performance of all the genotypes with respect to floral characters are presented in Table 4.

Out of the twelve *Cattleya* hybrids, flowering was observed in 10 hybrids such as B, TB, MY, ID, A X N, PW, MS, H X M, MA and T during the experiment. The genotypes showed significant differences in the floral characters such as pseudo bulb length at flowering (cm), pseudo bulb breadth at broadest part at flowering (cm), inflorescence number per pseudobulb, inflorescence length (cm), peduncle sheath length (cm), peduncle length (cm), flower number per peduncle, flower length from tip of dorsal sepal to tip of lip (cm), flower width in front view (cm), days from emergence to opening of the inflorescence, flower longevity on the plant, dorsal sepal length (cm), dorsal sepal width (cm), lateral sepal length(cm), lateral sepal width(cm), petal length (cm), petal width (cm), lip length (cm), lip width (cm), column length (cm), length of flower pedicel (cm), vase life of flowers (days).

For the characters, such as plant height from base to tip of the flowering shoot, peduncle length and days from emergence to opening of inflorescence, the highest value was observed for the genotype A X N (13.66 cm, 9.66 cm and 15.33 days respectively) and the lowest value for the first character was noticed in the genotype B (8.83 cm) and others were in MY (5.43 cm and 6 days) respectively. The genotype H X M noted the highest value for the characters pseudobulb length at flowering (12 cm) and column length (2.5 cm) and the lowest value was obtained for the genotypes TB (7.3 cm) and A X N (1.2 cm) respectively. For the character, pseudobulb breadth at flowering, the highest value was observed for the genotype MY (2.96 cm) and lowest value was exhibited by the genotype A X N (1 cm). The characters, inflorescence number per pseudobulb (3.66 nos) and flower number per peduncle (3.33 nos) was recorded the highest for the genotype T and the lowest value (one) was obtained for all other genotypes except in A X N and B. Length of flower pedicel (7.43 cm), flower length (7.33 cm) and inflorescence length (13.83 cm) was observed the highest for the genotype MS and the lowest value was noticed in the genotypes B (4.16 cm), A X N (5 cm) and MY (10.5 cm). The genotype ID (8.06 cm) noted the highest value for the character peduncle sheath length and lowest was for the genotype A X N (4 cm).

Table 3. Variability in morphological quantitative characters of the twelve Cattleya hybrids

Genotypes	Plant height (cm)	Number of shoots per plant	Length of the largest leaf (cm)	Width of the largest leaf (cm)	Number of days from emergence to maturity of leaf
TS	10.05	8.73^{bc}	17.36^{abcd}	4.78^{a}	33.33^{c}
B	7.73	7.00^{c}	10.07^{f}	3.31^{d}	55^{a}
TB	12.04	7.30^{c}	20.29^{a}	4.68^{ab}	48.33^{ab}
MY	8.08	7.63^{c}	15.64^{cd}	3.45^{cd}	35.33^{c}
PW	10	8.43^{bc}	18.23^{abc}	5.23^{a}	36.67^{bc}
CD	7.53	15.70^{a}	11.20^{ef}	3.02^{d}	30.67^{c}
ID	7.77	4.53^{c}	14.11^{de}	4.39^{abc}	31.67^{c}
H X M	8.78	12.70^{ab}	16.09^{cd}	3.73^{bcd}	30.67^{c}
A X N	8.36	5.40^{c}	14.86^{cde}	2.81^{d}	32.33^{c}
T	9.74	12.63^{ab}	16.4^{bcd}	4.33^{abc}	48.33^{ab}
MA	10.02	4.50^{c}	17.20^{abcd}	4.33^{abc}	32.33^{c}
MS	10.42	8.77^{bc}	19.96^{ab}	4.88^{a}	53.33^{a}
SE(m)	1.067	1.6370	1.317	0.346	4.284
SE(d)	1.509^{NS}	2.3150	1.863	0.49	6.058
CD(5%)		4.7790	3.845	1.011	12.504

Plant height-not significant

Table 4. Variability in floral quantitative characters of the *Cattleya* hybrids

Genotype	PHBT	PL	PB	IN	IL	PSL	PL	FN	FL	FW	DEOI
B	8.83^{g}	8.00^{fg}	1.06^{g}	1.66^{c}	10.83^{de}	6.83^{cd}	8.16^{b}	1.66^{cd}	6.16^{e}	4.83^{h}	13.67^{b}
TB	10.66^{e}	7.83^{g}	2.46^{bc}	1.00^{c}	11.00^{de}	6.93^{c}	5.96^{fg}	1.00^{d}	6.93^{cd}	9.93^{de}	7.00^{e}
MY	12.33^{cd}	8.83^{efg}	2.96^{a}	1^{c}	10.5^{e}	6.43^{d}	5.43^{h}	1.00^{d}	6.86^{cd}	10.46^{cd}	6.00^{e}
PW	13.16^{ab}	11.23^{ab}	2.70^{ab}	1^{c}	11.33^{cd}	6.73^{cd}	7.7^{c}	1.00^{d}	13.4^{a}	12.76^{a}	10.33^{c}
ID	9.66^{f}	9.03^{ef}	2.06^{de}	1^{c}	10.83^{de}	8.06^{a}	5.53^{gh}	2.00^{bc}	7.23^{bc}	10.50^{c}	7.00^{e}
H X M	12.83^{bc}	12.00^{a}	2.30^{cd}	1^{c}	11.63^{c}	5.00^{e}	6.16^{ef}	2.00^{bc}	5.5^{f}	9.66^{ef}	11.33^{c}
A X N	13.66^{a}	8.66^{efg}	1.00^{g}	2.66^{b}	12.66^{b}	4.00^{f}	9.66^{a}	2.66^{ab}	5^{g}	5.66^{g}	15.33^{a}
T	10.16^{ef}	9.16^{de}	1.66^{f}	3.66^{a}	12.83^{b}	6.66^{cd}	5.83^{fgh}	3.33^{a}	6.66^{d}	9.33^{f}	6.33^{e}
MA	12.16^{d}	10.66^{bc}	1.83^{ef}	1^{c}	13.66^{a}	7.83^{ab}	6.5^{de}	1.00^{d}	7.16^{bc}	11.66^{b}	8.33^{d}
MS	13.16^{ab}	10.13^{cd}	2.13^{de}	1^{c}	13.83^{a}	7.50^{b}	6.83^{d}	1.00^{d}	7.33^{b}	11.93^{b}	8.33^{d}
SE(m)	0.17	0.35	0.11	0.32	0.17	0.157	0.156	0.32	0.13	0.189	0.339
SE(d)	0.24	0.50	0.16	0.45	0.241	0.222	0.221	0.45	0.19	0.267	0.479
CD	0.51	1.04	0.33	0.94	0.506	0.466	0.464	0.95	0.4	0.561	1.007

Unifoliate

Bifoliate

Plate 3. Leaf types of *Cattleya* hybrids

Unifoliate leaves of the varieties 1. Rlc.Taichung Beauty, 2. Rlc.Mahina Yahiro, 3. Rlc.Irene Dopkin, 4. Rlc.Petch Wangnam Khiew, 5. Rlc.Morning Stars, 6. Rlc. Haadyayi Delight x Mary Song, 7. Rlc.Memoria Anna Balmores 8. *C.* Tipo

Bifoliate leaves of the varieties 1. Rth. Burana Beauty,
2. *C.*Chocolate Drops Volcano Queen
3. *C.*Aurantiaca x Netrasiri Beauty

Plate 4. Variation in leaf characters

Table 4. Variability in floral quantitative characters of the *Cattleya* hybrids (Continued)

Geno type	DSL	DSW	LSL	LSW	PL	PW	LL	LW	CL	LFP	FLP	VF
B	2.83^{e}	2.00^{d}	2.50^{f}	1.50^{d}	2.70^{f}	1.76^{f}	3.06^{f}	1.93^{e}	1.13^{c}	4.16^{e}	13.33^{f}	3.66^{cd}
TB	5.66^{c}	1.66^{f}	6.26^{b}	1.56^{d}	6.16^{cd}	3.46^{de}	5.46^{cd}	3.93^{cd}	2.06^{b}	5.06^{cd}	15.66^{de}	3.00^{e}
MY	5.86^{c}	1.83^{e}	6.16^{bc}	1.46^{d}	6.06^{d}	3.36^{e}	5.40^{cd}	3.90^{cd}	1.93^{b}	5.00^{cd}	15.00^{e}	3.16^{de}
PW	7.23^{a}	3.96^{a}	6.70^{a}	3.30^{a}	6.90^{a}	6.73^{a}	7.70^{a}	6.86^{a}	2.36^{a}	5.93^{b}	24.00^{b}	6.00^{a}
ID	5.83^{c}	1.83^{e}	6.20^{b}	1.73^{c}	6.10^{cd}	3.60^{d}	5.63^{bc}	4.23^{bc}	2.10^{b}	5.20^{c}	15.66^{de}	3.00e
H X M	6.66^{b}	2.73^{b}	5.10^{d}	2.86^{b}	6.26^{c}	5.40^{b}	5.16^{de}	4.50^{b}	2.50^{a}	5.96^{b}	17.00^{d}	3.33^{de}
A X N	3.83^{d}	1.20^{h}	3.23^{e}	1.06^{e}	3.23^{e}	1.26^{g}	2.63^{g}	2.06^{e}	0.66^{d}	4.70^{d}	20.33^{c}	4.00^{c}
T	5.83^{c}	1.50^{g}	5.83^{c}	1.50d	6.50^{b}	3.50^{de}	5.00^{e}	3.73^{d}	2.00^{b}	5.66^{b}	10.33^{g}	2.83^{e}
MA	6.80^{b}	2.50^{c}	6.00^{bc}	2.93^{b}	6.63^{b}	5.00^{c}	6.00^{b}	4.43^{b}	2.43^{a}	7.43^{a}	26.33^{a}	5.17
MS	0.11	0.032	0.117	0.05	0.064	0.07	0.13	0.11	0.06	0.14	0.552	0.173
SE(d)	0.16	0.045	0.166	0.071	0.091	0.09	0.18	0.16	0.08	0.2	0.781	0.245
CD	0.33	0.094	0.349	0.15	0.191	0.19	0.37	0.34	0.17	0.41	1.641	0.514

Flowering was not observed in the genotypes T S and CD during the observation period

PHBT-plant height from base to tip of the flowering s hoot, P L- p seudo b ulb l ength a t fl owering (c m), PB - ps eudo bu lb br eadth at broadest part at flowering (cm), I N-inflorescence nu mber pe r ps eudobulb, IL -inflorescence len gth (cm), PSL - ped uncle she ath length (cm), PL- peduncle length (cm), FN- flower number per peduncle, FL-flower length from tip of dorsal sepal to tip of lip (cm), FW-flower width in front view (cm), DEOI- days from emergence to opening of the inflorescence, FLP-flower longevity on the plant, DSL- dorsal sepal length (cm), DSW-dorsal sepal width (cm), LSL-lateral sepal length(cm), LSW- lateral sepal width(cm), PL-petal length (cm), PW-petal width (cm),LL- lip length (cm), LW- lip width (cm), CL- column length (cm), LFP- length of flower pedicel (cm),VF- vase life of flowers (days)

For the characters, flower width in front view (12.76 cm), dorsal sepal length (7.23 cm), dorsal sepal width (3.96 cm), lateral sepal length (6.7 cm), lateral sepal width (3.3 cm) , petal length (6.9 cm), petal width (6.73 cm), lip length (7.7 cm), lip width (6.86 cm) and vase life of flowers (6 days), the highest value was observed for the genotype PW and lowest value was recorded for the genotype B for the characters flower width in front view (4.83 cm), dorsal sepal length (2.83 cm), lateral sepal length (2.5 cm), petal length (2.7cm) and lip width (1.93 cm) and for other characters, lowest was for the genotype A X N. Flower number per peduncle was highest (3.33 nos) and vase life (2.83 days) was lowest for the genotype T. The genotypes MS and MA (26.33 days) recorded the highest value for flower longevity on the plant and it was the lowest for the genotype T (10.33 days).

4.1.3. Qualitative characters

The qualitative characters of the ten genotypes in the experiment are given in Table 5.

The qualitative parameters like sepal dominant colour, sepal colour pattern inside, petal predominant colour, petal colour pattern, lip predominant colour, lip colour number and pattern, column colour number and pattern were observed for the ten genotypes in which flowering was observed.

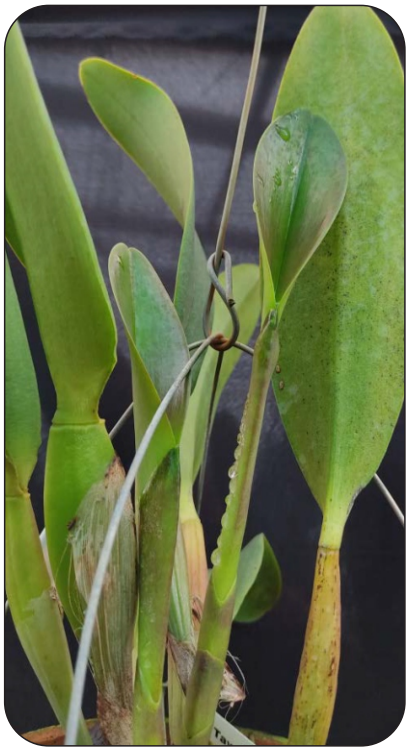
4.1.4. Pseudo qualitative characters

The pseudo qualitative characters of the ten genotypes in the experiment are presented in Table 6.

The pseudo qualitative characters were recorded for the ten flowered genotypes. The frequency distribution of all qualitative and pseudo qualitative characters were done and given in Table 7. 60% of the genotypes possessed lanceolate dorsal sepal shape, incurved dorsal sepal and lateral sepal curvature, oblong lateral sepal shape, and incurved petal curvature, according to the frequency distribution. Ovate and elliptic petal shapes were present in 50% of genotypes, respectively. 10% of the genotypes had an entire margin, while 90% had an undulating petal margin. In leaf shape, leaf colour pattern, dorsal sepal shape, dorsal sepal apex, dorsal sepal curvature, lateral sepal shape, lateral sepal apex, lateral sepal curvature, petal shape, petal curvature, petal margin, lip shape, lip lateral lobe shape, lip lateral lobe margin,

10 days after emergence

20 days after emergence



30 days after emergence

Matured leaf

Plate 5. Stages of leaf emergence

Table 5. Qualitative characters of the *Cattleya* hybrids

Variety	Sepal predominant colour	Sepal colour pattern inside	Petal predominant colour	Petal colour pattern inside	Lip predominant colour	Lip colour number and pattern	Column colour number and pattern
B	Yellow (RHS 13A)	spotted	Deep pink (RHS 52C)	mixed	Yellow (RHS 13A)	two, spotted	two, spotted
TB	light purplish pink (RHS 68D)	uniform	light purplish pink (RHS 68D)	uniform	light purplish pink (RHS 68D)	three, spotted	two, spotted
MY	moderate purplish pink (RHS 70D)	uniform	moderate purplish pink (RHS 70D)	uniform	moderate purplish red (RHS 70A)	three, spotted	two, shaded
PW	strong pink (RHS 47D)	uniform	strong pink (RHS 47D)	uniform	strong purple (RHS 55A)	two, shaded	three, shaded
ID	red purple (RHS 73A)	uniform	Red purple (RHS 73A)	uniform	strong purplish pink (RHS55B)	two, spotted	two, shaded
H X M	yellowgreen (RHS 150D)	spotted	strong purplish pink (RHS55B)	mixed	strong purplish pink (RHS55B)	three, shaded	two, shaded
A X N	yellow orange (RHS 23A)	uniform	Yellow orange (RHS 23A)	uniform	yellow orange (RHS 23A)	two, spotted	two, shaded
T	Light purple (RHS 80C)	uniform	Light purple (RHS 80C)	uniform	vivid purple (RHS 80A)	three, spotted	two, shaded
MA	White (RHS 155A)	striped	white(RHS 155A)	striped	red purple (RHS 66A)	three, spotted	two, shaded
MS	White (RHS 155A)	striped	white(RHS 155A)	striped	red purple (RHS 66A)	two, spotted	two, shaded

Table 6. Pseudo qualitative characters of the *Cattleya* hybrids

Variety	Nature of pseudo bulb(at flower-ing)	Leaf shape	Leaf colour pattern	Dorsal se-pal shape	Dor-sal sepal apex	Dorsal sepal curva-ture	Lateral sepal shape	Lat-eral sepal apex	Lateral sepal curva-ture	Petal shape	Petal curva-ture	Petal margin
* Rlc. Tawan Shine		narrow oblong	uniformly green on both sides									
Rth.Burana Beauty	clavate	narrow oblong	uniformly green on both sides	lanceolate	acute	incurved	lanceolate	acute	incurved	elliptic	incurved	undulate
Rlc.Taichung Beauty	clavate	narrow oblong	uniformly green on both sides	lanceolate	acute	incurved	lanceolate	acute	incurved	ovate	incurved	undulate
Rlc. Mahina Yahiro	clavate	narrow oblong	uniformly green on both sides	lanceolate	acute	incurved	lanceolate	acute	incurved	ovate	incurved	undulate
Rlc.Petch Wangnam Khiew	clavate	narrow oblong	uniformly green on both sides	oblong	acute	incurved	oblong	acute	incurved	elliptic	incurved	undulate
* *C.* Chocolate Drops Volcano Queen		narrow oblong	uniformly green on both sides									
Rlc. Irene Dopkin	clavate	narrow oblong	uniformly green on both sides	lanceolate	acute	incurved	lanceolate	acute	incurved	ovate	incurved	undulate
Rlc. Haadyayi Delight x Mary Song	clavate	narrow oblong	uniformly green on both sides	oblong	acute	straight	oblong	acute	straight	elliptic	straight	undulate
*C.*Aurantica x Netrasiri Beauty	clavate	narrow oblong	uniformly green on both sides	lanceolate	acute	straight	lanceolate	acute	straight	elliptic	straight	entire
C. Tipo	clavate	narrow oblong	uniformly green on both sides	lanceolate	acute	incurved	lanceolate	acute	incurved	elliptic	incurved	undulate
Rlc.Memoria Anna Balmores	clavate	narrow oblong	uniformly green on both sides	oblong	acute	straight	oblong	acute	straight	elliptic	straight	undulate
Rlc.Morning Stars	clavate	narrow oblong	uniformly green on both sides	oblong	acute	straight	oblong	acute	straight	ovate	straight	undulate

*** Not Flowered during the observation period**

Table 6. Pseudo qualitative characters of the *Cattleya* hybrids (Continued)

Variety	Lip shape	Lip lateral lobe shape	Lip lateral lobe margin	Lip mid lobe shape	Lip mid lobe margin	Lip surface inside	Flowering season
Rth.Burana Beauty	ovate	oblong	undulate	oblong	undulate crisped	glabrous	rainy
Rlc.Taichung Beauty	oblong	oblong	entire	oblong	undulate	glabrous	winter
Rlc. Mahina Yahiro	oblong	oblong	entire	oblong	undulate crisped	glabrous	winter
Rlc.Petch Wangnam Khiew	oblong	oblong	entire	oblong	undulate	glabrous	winter
Rlc. Irene Dopkin	oblong	oblong	entire	oblong	undulate	glabrous	rainy
Rlc. Haadyayi Delight x Mary Song	oblong	oblong	undulate	oblong	undulate	glabrous	winter
C. Aurantica x Netrasiri Beauty	oblong	round	entire	oblong	undulate	glabrous	winter
*C.*Tipo	oblong	round	entire	oblong	undulate crisped	glabrous	rainy and winter
Rlc.Memoria Anna Balmores	ovate	oblong	entire	oblong	undulate crisped	glabrous	winter
Rlc.Morning Stars	oblong	oblong	entire	oblong	undulate crisped	glabrous	winter

Table 7. Frequency distribution of the characters for the *Cattleya* hybrids

Sl.No.	Qualitative characters	Expression	Frequency (%)
1.	Dorsal sepal shape	lanceolate	60
		oblong	40
2.	Dorsal sepal curvature	incurved	60
		straight	40
3.	Lateral sepal shape	lanceolate	40
		oblong	60
4.	Lateral sepal curvature	incurved	60
		straight	40
5.	Petal shape	ovate	50
		elliptic	50
6	Petal curvature	incurved	60
		straight	40
7	Petal margin	Undulate	90
		entire	10
8	Lip shape	ovate	20
		oblong	80
9	Lip lateral lobe shape	oblong	80
		round	20
10	Lip lateral lobe margin	Undulate	20
		entire	80

Plate 6. Pseudobulb of *Cattleya* orchid

Sepals

Petals

Column

Lip

Floral parts

Plate 7. Floral parts of *Cattleya* flower

Table 7. Frequency distribution of the characters for the *Cattleya* hybrids (Continued)

Sl.No.	Qualitative characters	Expression	Frequency (%)
11	Lip mid lobe margin	Undulate	50
		Undulate crisped	50
12	Flowering season	winter	70
		rainy	30
13	Sepal dominant colour	purple	40
		yellow	30
		white	20
		pink	10
14	Sepal colour pattern inside	uniform	70
		striped	30
15	Petal predominant colour	Purple	50
		White	20
		Pink	20
		yellow	10
16	Petal colour pattern	Uniform	60
		Striped	20
		mixed	20
17	Lip predominant colour	purple	80
		yellow	20
18	Lip colour number and pattern	2,spotted	40
		3,spotted	40
		2,shaded	10
		3,shaded	10
19	Column colour number and pattern	2,shaded	70
		3, shaded	10
		2,spotted	20

lip mid lobe shape, lip mid lobe margin, lip surface inside and flowering season case of lip lateral lob margin, 80% had entire shape whereas in case of lip mid lob margin, 50% had undulate and 50% had undulate crisped margin. All the genotypes exhibited purple as the predominant colour for sepal, petal, and lip. Occurrence of yellow, pink and white were also observed. 70% of the genotypes flowered during winter season and while 30% of the genotypes in rainy season. Uniform sepal colour pattern was exhibited by 70% of the genotypes whereas 60 % of the genotypes had uniform petal colour. The variation in 'lip colour number' and pattern was observed from two coloured and spotted (40% of the genotypes) to three coloured and spotted (40% of the genotypes) whereas in column number and pattern, 70% had 2 shaded pattern.

4.1.5. Morphological description of the genotypes

Based on the results, the genotypes used for performance evaluation are morphologically described in accordance with the DUS criteria as follows:

***Rhyncattleanthe* Burana Beauty (B)**

The genotype Rth.Burana Beauty showed an average height of 7.73 cm at flowering. It was bifoliate with flower size of 4.83 cm. The genotype had deep pink (RHS 52C) petal colour with spotted yellow (RHS 13A) lip. It produced 1.66 flowers per plant per year with a vase life of 3.66 days.

***Rhyncholaeliocattleya* Taichung Beauty (TB)**

The genotype Rlc.Taichung Beauty showed an average height of 12.04 cm at flowering. It was unifoliate with flower size of 9.93 cm. The genotype had light purplish pink (RHS 68D) petal colour with spotted light purplish pink (RHS 68D) lip. It produced single flower per plant per year with a vase life of 3 days.

***Rhyncholaeliocattleya* Mahina Yahiro (MY)**

The genotype Rlc.Mahina Yahiro showed an average height of 8.08 cm at flowering. It was unifoliate with flower size of 10.46 cm. The genotype had moderate purplish pink (RHS 70D) petal colour with spotted moderate purplish red (RHS 70A) lip. It produced single flower per plant per year with a vase life of 3.16 days.

Plate 8. Inflorescence of *Cattleya* Tipo

***Rhyncholaeliocattleya* Petch Wangnam Khiew (PW)**

The genotype Rlc.Petch Wangnam Khiew showed an average height of 10 cm at flowering. It was unifoliate with flower size of 12.76 cm. The genotype had strong pink (RHS 47D) petal colour with shaded strong purple (RHS 55A) lip. It produced single flower per plant per year with a vase life of 6 days.

***Rhyncholaeliocattleya* Irene Dopkin (ID)**

The genotype Rlc.Irene Dopkin showed an average height of 7.77 cm at flowering. It was unifoliate with flower size of 10.5 cm. The genotype had red purple (RHS 73A) petal colour with spotted strong purplish pink (RHS55B) lip. It produced two flowers per plant per year with a vase life of 3 days.

***Rhyncholaeliocattleya* Haadyayi Delight x Mary Song (H X M)**

The genotype Rlc. Haadyayi Delight x Mary Song showed an average height of 8.78 cm at flowering. It was unifoliate with flower size of 9.66 cm. The genotype had strong purplish pink (RHS 55B) petal colour with shaded strong purplish pink (RHS 55B) lip. It produced two flowers per plant per year with a vase life of 3.33 days.

***Cattleya* Aurantiaca x Netrasiri Beauty (A X N)**

The genotype *C.*Aurantiaca x Netrasiri Beauty showed an average height of 8.36 cm at flowering. It was bifoliate with flower size of 5.66 cm. The genotype had yellow orange (RHS 23A) petal colour with spotted yellow orange (RHS 23A) lip. It produced 2.66 flowers per plant per year with a vase life of 4 days.

***Cattleya* Tipo (T)**

The genotype *C.*Tipo showed an average height of 9.74 cm at flowering. It was unifoliate with flower size of 9.33 cm. The genotype had light purple (RHS 80C) petal colour with spotted vivid purple (RHS 80A) lip. It produced 3.33 flowers per plant per year with a vase life of 2.83 days.

***Rhyncholaeliocattleya* Memoria Anna Balmores (MA)**

The genotype Rlc.Memoria Anna Balmores showed an average height of 10.02 cm at flowering. It was unifoliate with flower size of 11.66 cm. The genotype had

white (RHS 155A) petal colour with spotted red purple (RHS 66A) lip. It produced single flower per plant per year with a vase life of 5 days.

***Rhyncholaeliocattleya* Morning Stars (MS)**

The genotype Rlc.Morning Stars showed an average height of 10.42 cm at flowering. It was unifoliate with flower size of 11.93 cm. The genotype had white (RHS 155A) petal colour with spotted red purple (RHS 66A) lip. It produced single flower per plant per year with a vase life of 5.17 days.

4.1.6. Cluster analysis of morphological and floral, qualitative and quantitative characters

Cluster analysis of morphological qualitative and quantitative characters are given in Table 8 and 9.

The genotypes were subjected to cluster analysis using 'Average method' and 'Euclidean Distance measures'. Based on cluster analysis, variability was observed in qualitative characters like dorsal and lateral sepal shape, petal shape, lip shape, dorsal and lateral sepal curvature, petal curvature, petal and lip lobe margin, sepal, petal and lip predominant colour, sepal and petal colour pattern and flowering season. Dendrogram (Fig.1 and Fig.2.) based on morphological quantitative and qualitative characters grouped the genotypes into 3 different clusters. The cluster 1 consisted of 6 genotypes (Rth.Burana Beauty, Rlc.Taichung Beauty, Rlc.Mahina Yahiro, Rlc.Irene Dopkin, Rlc.Petch Wangnam Khiew, Rlc. Haadyayi Delight x Mary Song), cluster 2 consisted of the genotypes *C.*Aurantiaca x Netrasiri Beauty and *C.* Tipo and cluster 3 consisted of two genotypes (Rlc.Memoria Anna Balmores and Rlc.Morning Stars) based on morphological qualitative characters. With respect to morphological quantitative characters, cluster 1 consisted of 3 genotypes (Rth.Burana Beauty, Rlc. Haadyayi Delight x Mary Song, *C.*Chocolate Drops Volcano Queen), cluster 2 consisted of the genotypes Rlc.Taichung Beauty and Rlc.Morning Stars and cluster 3 consisted of seven genotypes (Rlc.Tawan Shine, Rlc.Mahina Yahiro, Rlc.Irene Dopkin, *C.*Aurantiaca x Netrasiri Beauty, Rlc.Petch Wangnam Khiew, Rlc.Memoria Anna Balmores and *C.* Tipo).

The cluster means of the five morphological quantitative characters are presented in Table 10. Cluster-2 showed the highest cluster mean for number of shoots

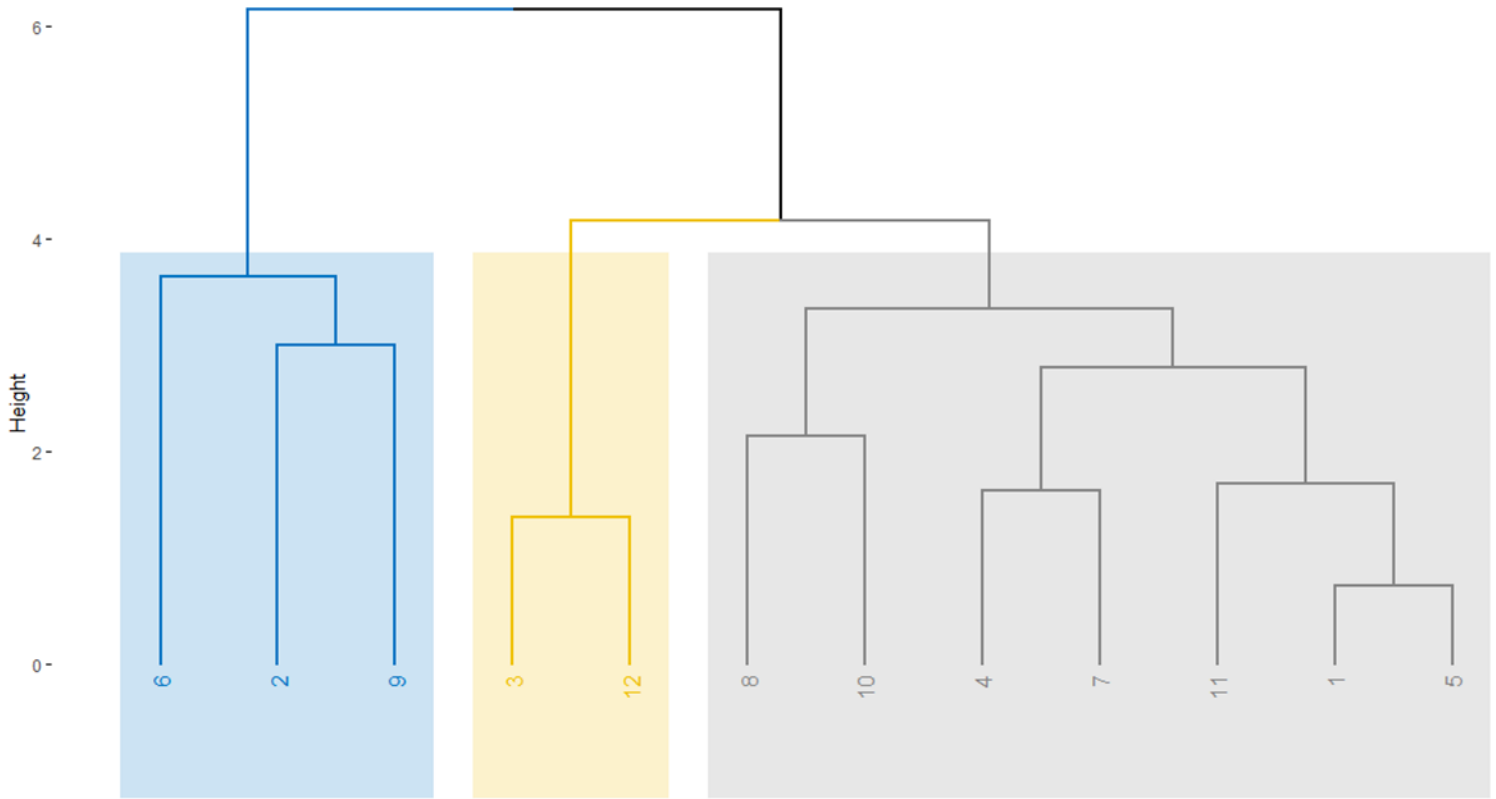


Figure 1. Dendrogram of morphological quantitative characters (UPGMA Method)

Plate 9. Stages of flower emergence

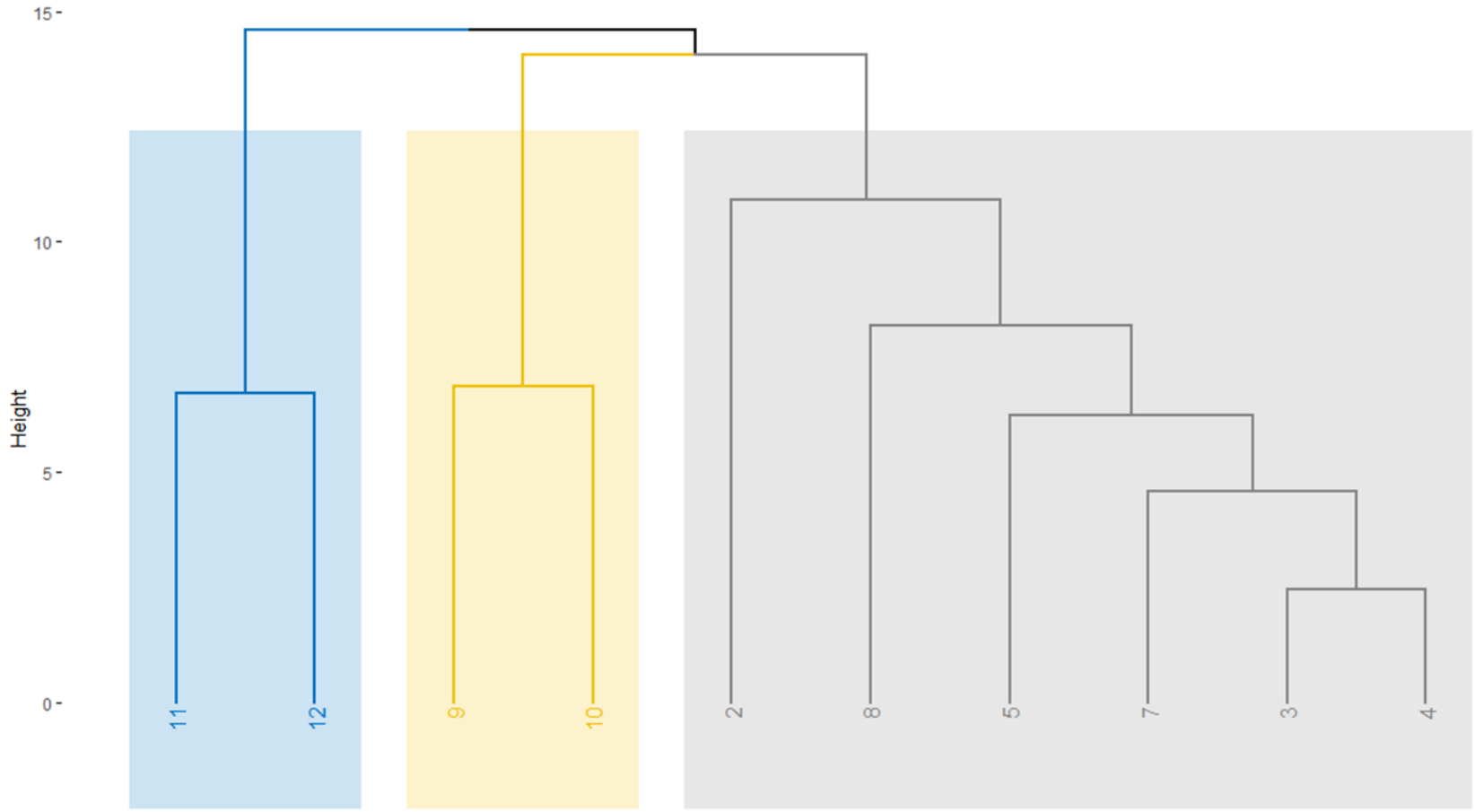


Figure 2. Dendrogram of qualitative characters (UPGMA Method)

Table 8. Clustering pattern of the genotypes based on morphological quantitative characters

Cluster number	Number of genotypes	Cluster members
Cluster 1	3	Rth.Burana Beauty, Rlc. Haadyayi Delight x Mary Song, *C*.Chocolate Drops Volcano Queen
Cluster 2	2	Rlc.Taichung Beauty, Rlc.Morning Stars
Cluster 3	**7**	Rlc.Tawan Shine, Rlc.Mahina Yahiro, Rlc.Irene Dopkin, *C*.Aurantiaca x Netrasiri Beauty, Rlc. Petch Wangnam Khiew, Rlc.Memoria Anna Balmores and *C.* Tipo

Table 9. Clustering pattern of the genotypes based on morphological qualitative characters

Cluster number	Number of genotypes	Cluster members
Cluster 1	6	Rth.Burana Beauty, Rlc.Taichung Beauty, Rlc. Mahina Yahiro, Rlc.Irene Dopkin, Rlc.Petch Wangnam Khiew, Rlc. Haadyayi Delight x Mary Song
Cluster 2	2	C.Aurantiaca x Netrasiri Beauty and C. Tipo
Cluster 3	2	Rlc.Memoria Anna Balmores and Rlc.Morning Stars

Table 10. Cluster means of the morphological characters of different clusters

Cluster number	Plant height(cm)	Number of shoots per plant	Length of the largest leaf(cm)	Width of the largest leaf(cm)	Days from emergence to maturity of leaves
Cluster 1	9.21	8.45	16.43	4.32	35.48
Cluster 2	7.87	9.37	12.04	3.05	39.33
Cluster 3	11.23	8.04	20.13	4.78	50.83

Table 11. Clustering pattern of the *Cattleya* hybrids based on floral characters

Cluster number	Number of genotypes	Cluster members
Cluster 1	4	Rlc.Petch Wangnam Khiew, Rlc.Memoria Anna Balmores, Rlc.Morning Stars and Rlc. Haadyayi Delight x Mary Song
Cluster 2	2	Rth.Burana Beauty and *C.*Aurantiaca x Netrasiri Beauty
Cluster 3	4	Rlc.Taichung Beauty, Rlc.Mahina Yahiro, Rlc.Irene Dopkin and *C.*Tipo

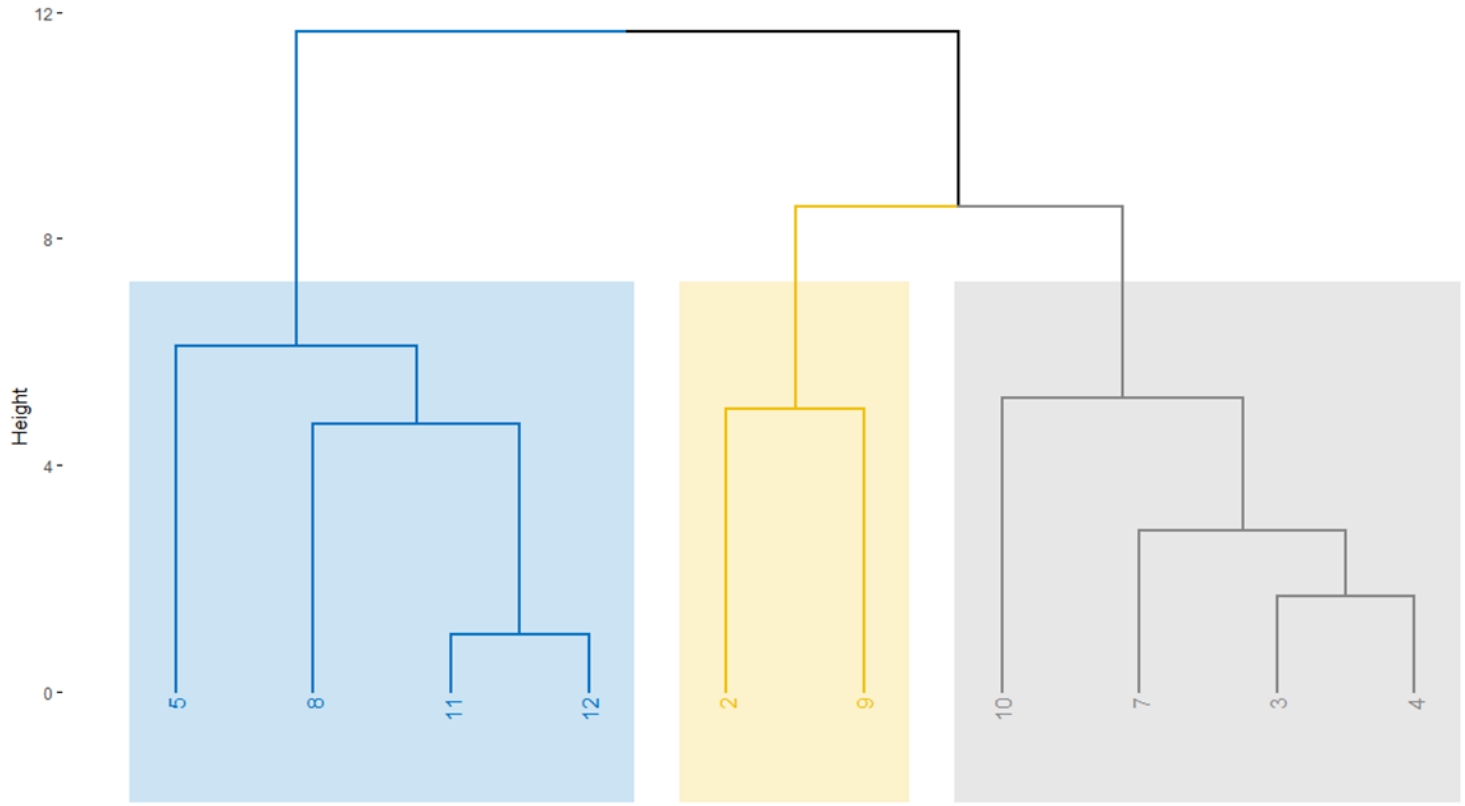


Figure 3. Dendrogram of floral quantitative characters (UPGMA Method)

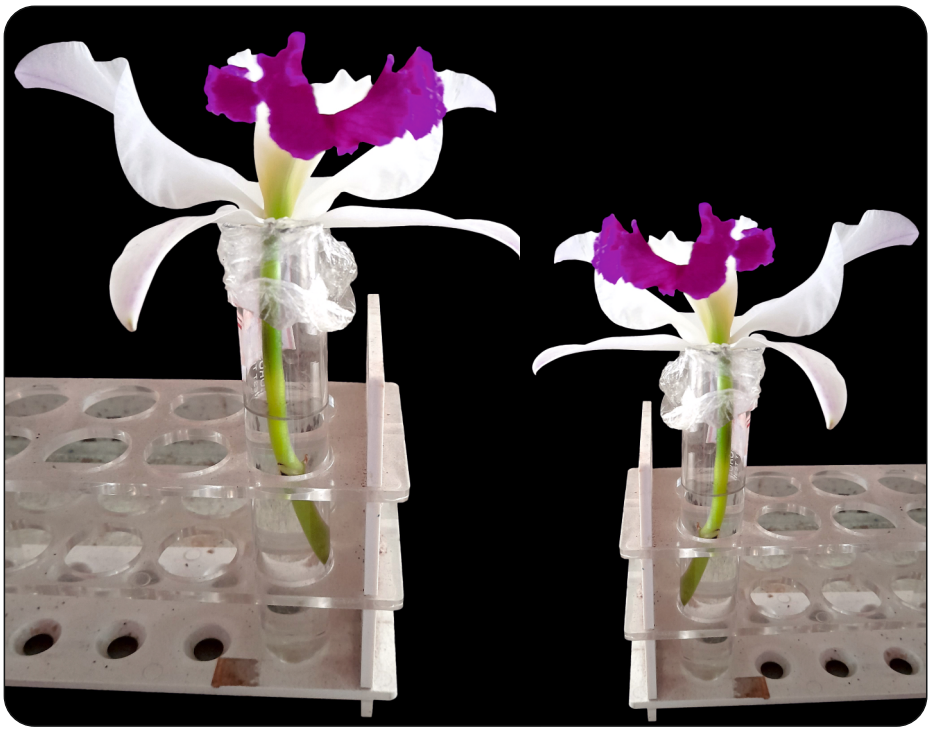


Plate 10. Vase life study

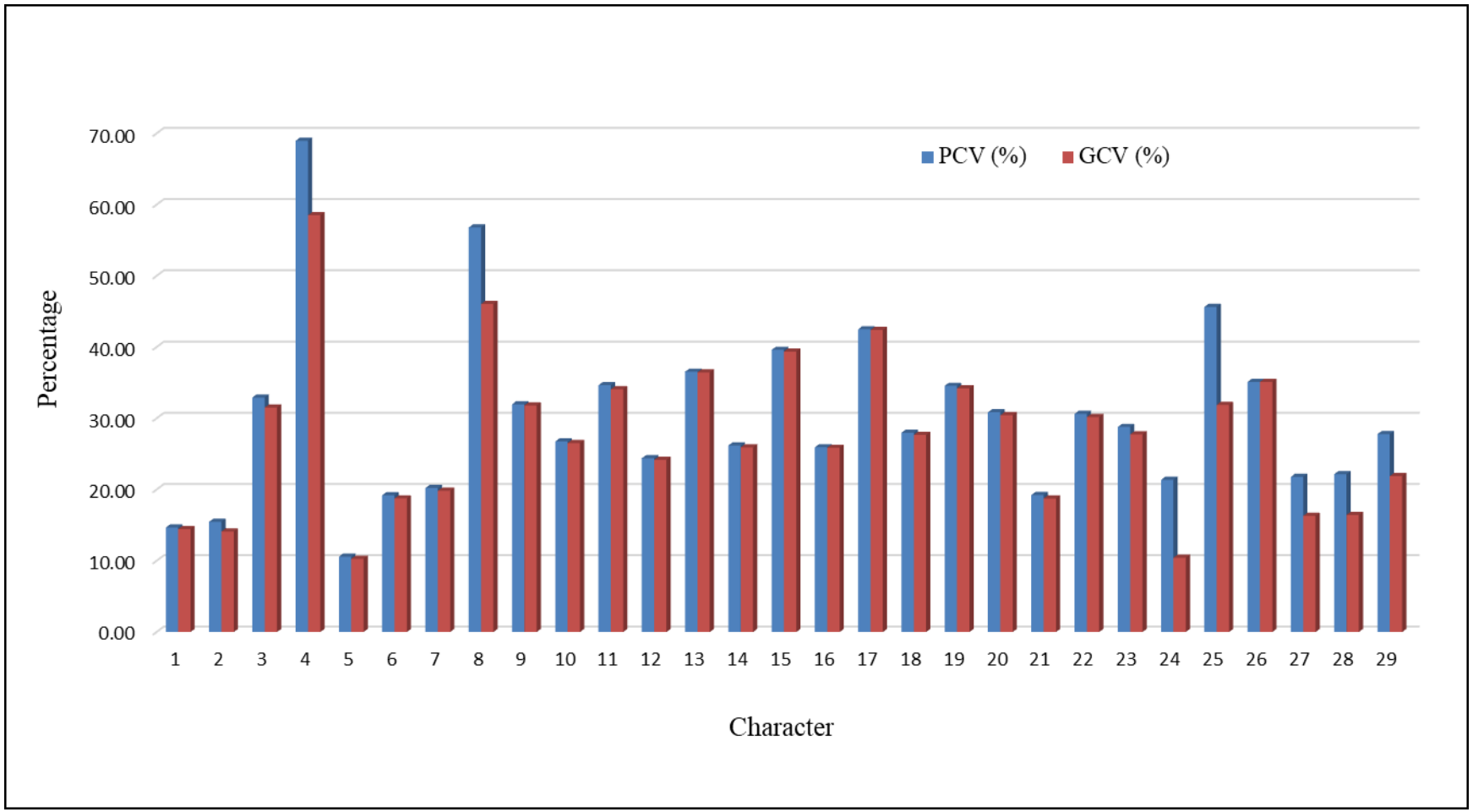


Figure 4. Phenotypic and genotypic coefficients of variation of various characters in *Cattleya* hybrids

per plant. The highest cluster mean for plant height, length and width of the largest leaf and number of days from emergence to maturity of leaves was recorded in C-3.

Dendrogram (Fig.3.) based on floral quantitative characters grouped the genotypes into 3 different clusters. The different clusters with the genotypes are given in Table 11. The cluster means of the 23 floral quantitative characters are presented in Table 12. Cluster-1 showed the highest cluster mean for inflorescence number per pseudobulb, peduncle length, flower number per peduncle and days from emergence to opening of inflorescence. The highest cluster mean for pseudobulb breadth at the broadest part, peduncle sheath length and lateral sepal length was recorded in C-2. C-3 recorded the highest mean for plant height from base to tip of the flowering shoot, pseudobulb length at flowering, inflorescence and flower length, dorsal sepal length, petal length, dorsal and lateral sepal width, lip and column length, petal and lip width, length of flower pedicel, flower longevity on the plant and vase life. The intra and inter cluster distance between the three clusters of the *Cattleya* genotypes are presented in Table 13. The intracluster distance recorded the lowest (1.38) for C-3 and the highest (3.64) for C-2. The intercluster distance value was recorded the highest between C-2 and C-3 (6.16) and the lowest between C-1and C-3 (4.17).

4.2. GENETIC PARAMETERS

4.2.1. Coefficient of variation

The phenotypic, genotypic and environmental variance and the phenotypic and genotypic coefficients of variation for all the characters studied are presented in Table 14 and Fig.4.

Phenotypic coefficient of variance (PCV) values ranged between 10.57 percent to 68.97 percent for the floral characters. The highest PCV was recorded for the inflorescence number per pseudobulb while lowest for inflorescence length. Genotypic coefficient of variance (GCV) values ranged between 10.28 percent to 58.51 percent. The highest genotypic coefficient of variation (GCV) was also recorded for the inflorescence number per pseudobulb and lowest for inflorescence length. The highest PCV and GCV was observed for number of shoots per plant (45.64 per cent and 31.88 per cent) and minimum for plant height (21.36 per cent and 10.43 per cent) among morphological characters**.** Maximum variability both at the phenotypic and

Table 12. Cluster means of the floral characters

Cluster number	Plant height from base to tip of the flowering shoot	Pseudo Bulb length at flowering	Pseudo bulb breadth at broadest part	Inflores-cence number per pseu-do bulb	Inflo-res-cence length	Peduncle sheath length	Peduncle length	Flower no per pedun-cle	Flower length	Flower width in front view	Days from emergence to opening of inflores-cence
Cluster 1	11.25	8.35	1.05	2.2	11.75	5.4	8.95	2.2	5.6	5.25	14.5
Cluster 2	10.725	8.7	2.325	1.675	11.275	7.025	5.675	1.825	6.925	10.05	6.575
Cluster 3	12.85	11	2.225	1	12.6	6.75	6.8	1.25	8.35	11.525	9.55

Table 12. Cluster means of the floral characters (Continued)

Cluster number	Dorsal sepal length	Dorsal sepal width	Lateral sepal length	Lateral sepal width	Petal length	Petal width	Lip length	Lip width	Column length	Lentgh of flower pedicel	Flower longev-ity on the plant	Vase life of flowers
Cluster 1	3.3	1.6	2.85	1.3	2.95	1.55	2.85	2	0.9	4.45	16.8	3.85
Cluster 2	5.8	1.7	6.125	1.575	6.225	3.5	5.375	3.925	2.025	5.25	14.175	3
Cluster 3	6.875	2.925	5.95	3.025	6.6	5.475	6.15	5.025	2.4	6.65	23.4	4.875

Table 13. Average intercluster and intracluster distances

	Cluster 1	Cluster 2	Cluster 3
Cluster 1	**3.345**	5.082	4.176
Cluster 2	5.08	**3.64**	6.16
Cluster 3	4.17	6.16	**1.38**

Bold figures in diagonals are the intra cluster distance

Table 14. Components of total variance for the characters in *Cattleya* hybrids

Characters	Genotypic variance	Phenotypic variance	Environmental variance	CV(%)	PCV (%)	GCV (%)
Plant heigh from base to tip of the flowering.shoot	2.84	2.93	0.09	2.56	14.67	14.45
Pseudobulb length at flowering	1.82	2.19	0.37	6.34	15.48	14.12
Pseudobulb breadth at broadest part cm	0.41	0.44	0.04	9.52	32.93	31.52
Inflorescence number per pseudobulb	0.77	1.07	0.30	36.52	68.97	58.51
Inflorescence length	1.50	1.59	0.09	2.48	10.57	10.28
Peduncle sheath length	1.53	1.61	0.07	4.12	19.21	18.76
Peduncle length	1.81	1.88	0.07	3.99	20.23	19.83
Flower number per peduncle	0.59	0.90	0.31	33.25	56.79	46.05
Flower length	5.28	5.34	0.05	3.19	31.97	31.81
Flower width in front view	6.59	6.70	0.11	3.38	26.74	26.53
Days from emergence to opening of inflorescence	10.19	10.54	0.34	6.26	34.66	34.09
Dorsal sepal length	1.92	1.96	0.04	3.36	24.40	24.17
Dorsal sepal width	0.63	0.63	0.00	2.52	36.54	36.45
Lateral sepal length	1.96	2.00	0.04	3.75	26.20	25.93
Lateral sepal width	0.68	0.69	0.01	4.27	39.61	39.38
Petal length	2.19	2.20	0.01	1.92	25.94	25.87
Petal width	2.73	2.74	0.01	2.93	42.50	42.40
Lip length	2.05	2.10	0.05	4.24	27.99	27.67
Lip width	1.87	1.91	0.04	4.88	34.56	34.21
Column length	0.35	0.36	0.01	5.12	30.86	30.43
Length of flower pedicel	1.12	1.18	0.06	4.23	19.22	18.75
Flower longevity on the plant	30.83	31.74	0.92	5.20	30.62	30.18
Vase life of flowers	1.18	1.27	0.09	7.66	28.79	27.75
Plant height	0.94	3.94	3.00	18.65	21.36	10.43
Number of shoots per plant	6.33	12.97	6.64	32.66	45.64	31.88
Number of leaves per Pseudobulb	0.18	0.18	0.00	0.00	35.13	35.13
Length of the largest leaf	7.05	12.59	5.55	14.46	21.79	16.30
Width of the largest leaf	0.46	0.83	0.37	14.86	22.16	16.44
Days from emergence to maturity of leaf	78.18	125.99	47.81	17.12	27.78	21.89

genotypic level was observed for the inflorescence number per pseudobulb followed by flower number per peduncle among floral characters.

4.2.2. Heritability and Genetic advance

The heritability and genetic advance as percentage of mean of all the characters studied were estimated and presented in Table 15 and Fig.5.

All the floral characters had high heritability in general and particularly in petal length, petal width, dorsal sepal length and flower length. Moderate heritability was exhibited in flower number per peduncle. Vegetative characters like number of shoots per plant, length and width of the largest leaf and days from emergence to maturity of leaves showed moderate heritability.

All the characters exhibited high genetic advance except plant height. The highest estimate of genetic advance was observed for inflorescence number per pseudobulb (102.26%) followed by petal width (87.14%).

High heritability combined with genetic advance was exhibited by petal width, flower length, sepal length and width and flower longevity on the plant.

4.3. CORRELATION STUDIES

The phenotypic, genotypic and environmental correlations among the various characters were estimated.

4.3.1. Phenotypic correlation

The phenotypic correlations among the various characters were estimated and presented in Table 16.

Highly significant positive correlation was observed for plant height from base to tip of the flowering shoot with flower longevity on the plant (0.679), pseudobulb length at flowering (0.532) and vase life of flowers (0.543). Pseudobulb length at flowering was recorded positively correlated with dorsal sepal length (0.693), dorsal sepal width (0.724), lateral sepal width (0.825), petal length (0.524), petal width (0.781), lip length (0.531), lip width (0.66), column length (0.633), length of flower pedicel (0.641), flower longevity on the plant (0.495), plant height from base to tip of the flowering shoot (0.532) and flower width in front view (0.556). Plant height had

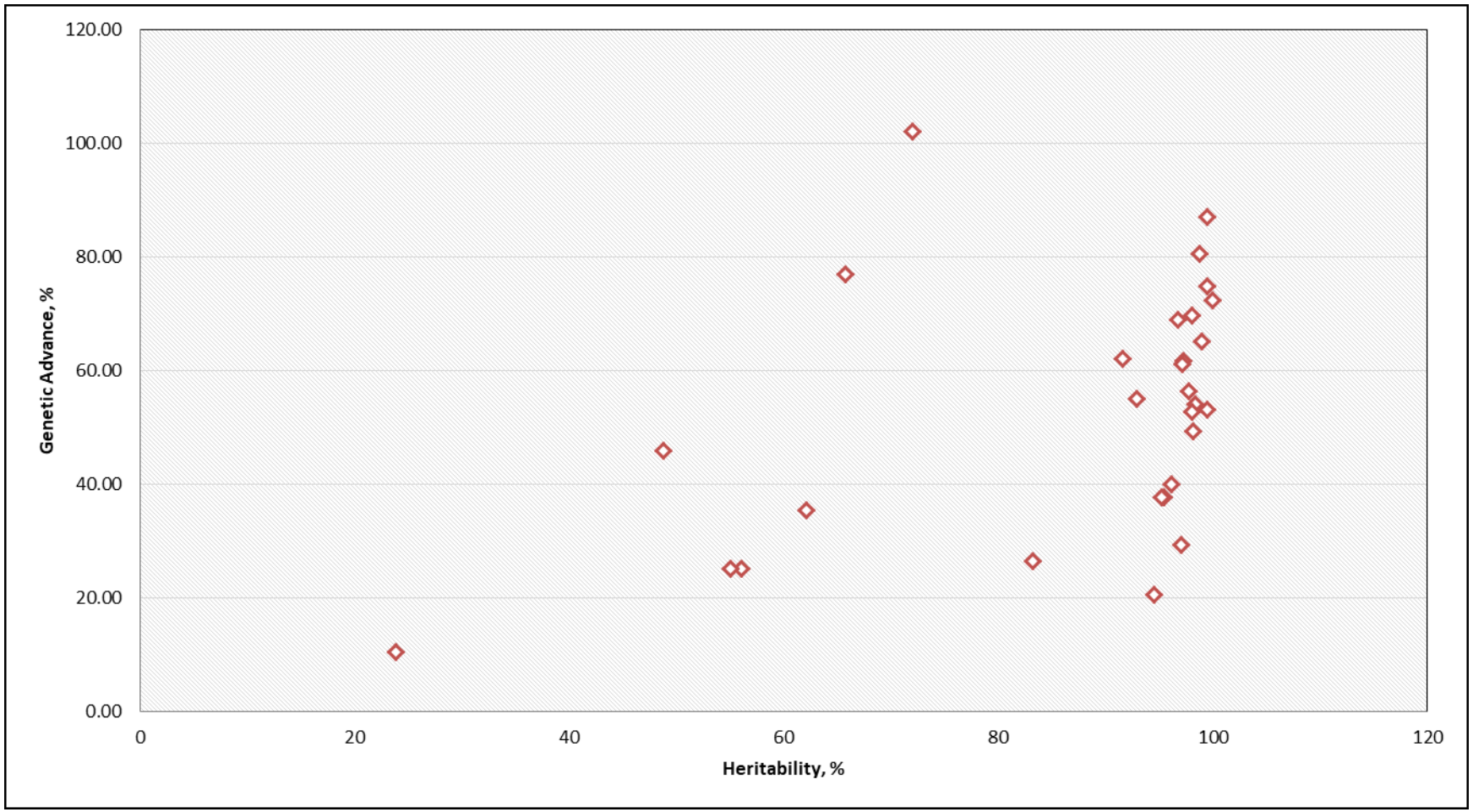


Figure 5. Heritability and Genetic advance of various characters in *Cattleya* hybrids

significant positive phenotypic correlation with width (0.641) and length of the largest leaf (0.749).

Pseudobulb breadth at broadest part had positive correlation with petal length (0.744), petal width (0.65), lip length (0.79), lip width (0.739), column length (0.715), lateral sepal length (0.807), lateral sepal width (0.392), dorsal sepal length (0.72), dorsal sepal width (0.465), flower length (0.493) and flower width in front view (0.769). Flower length recorded positive correlation with pseudobulb breadth at broadest part (0.493), flower width in front view (0.619), dorsal sepal length (0.517), dorsal sepal width (0.797), lateral sepal length (0.539), lateral sepal width (0.566), petal length (0.467), petal width (0.687), lip length (0.782), lip width (0.82) and width of the largest leaf (0.577). The length of flower had significant positive correlation with width of the flower.

Flower width in front view had positive correlation with pseudobulb length at flowering (0.556), pseudobulb breadth at broadest part (0.769), peduncle sheath length (0.534), flower length (0.619), dorsal sepal length (0.955), dorsal sepal width (0.606), lateral sepal length (0.939), lateral sepal width (0.708), petal length (0.943), petal width (0.864), lip length (0.95), lip width (0.893), column length (0.891), length of flower pedicel (0.729), flower longevity on the plant (0.498), vase life of flowers (0.413), length of the largest leaf (0.609) and width of the largest leaf (0.671).

Negative correlation was observed for flower number per peduncle with pseudobulb breadth at broadest part (0.545), peduncle sheath length (0.448), flower width in front view (0.471) and flower longevity on the plant (0.508). Inflorescence number per pseudobulb was negatively correlated with pseudobulb breadth at broadest part (0.581), flower width in front view (0.4745), dorsal sepal width (0.48), lateral sepal width (0.486) and column length (0.472).

Pseudobulb length at flowering had negative correlation with number of leaves per pseudobulb (0.436) and days from emergence to maturity of leaf (0.449). Pseudobulb breadth at broadest part was negatively correlated with inflorescence number per pseudobulb (0.581), peduncle length (0.619), flower number per peduncle (0.545) and number of leaves per pseudobulb (0.782).

Table 15. Heritability and Genetic advance as percentage of mean of 29 characters in *Cattleya* hybrids

Characters	Heritability (%)	Genetic advance (as % of mean)
Plant heigh from base to tip of the flowering. shoot	0.97	29.30
Pseudobulb length at flowering	0.83	26.53
Pseudobulb breadth at broadest part cm	0.92	62.15
Inflorescence number per pseudobulb	0.72	102.26
Inflorescence length	0.95	20.58
Peduncle sheath length	0.95	37.75
Peduncle length	0.96	40.05
Flower number per peduncle	0.66	76.91
Flower length	0.99	65.20
Flower width in front view	0.98	54.21
Days from emergence to opening of inflores-cence	0.97	69.06
Dorsal sepal length	0.98	49.31
Dorsal sepal width	1.00	74.91
Lateral sepal length	0.98	52.87
Lateral sepal width	0.99	80.66
Petal length	1.00	53.14
Petal width	1.00	87.14
Lip length	0.98	56.33
Lip width	0.98	69.77
Column length	0.97	61.82
Length of flower pedicel	0.95	37.69
Flower longevity on the plant	0.97	61.26
Vase life of flowers	0.93	55.11
Plant height	0.24	10.48
Number of shoots per plant	0.49	45.87
Number of leaves per Pseudobulb	1.00	72.36
Length of the largest leaf	0.56	25.12
Width of the largest leaf	0.55	25.12
Days from emergence to maturity of leaf	0.62	35.52

Table 16. Genotypic correlation coefficients among 29 characters in *Cattleya* hybrids

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	1**	0.6046	0.274	-0.1828	0.4296	-0.4643	0.3099	-0.2405	0.1946	0.3556	0.2213	0.4594	0.3749	0.1902
X2	0.6046	1**	0.3541	-0.3137	0.3396	-0.0919	-0.102	-0.1488	0.3831	0.5816	0.031	0.748*	0.7865**	0.3582
X3	0.274	0.3541	1**	-0.6196	-0.3487	0.282	-0.666*	-0.6151	0.5091	0.7891**	-0.6723*	0.7495*	0.4815	0.8318**
X4	-0.1828	-0.3137	-0.6196	1**	0.2971	-0.3773	0.2856	0.8661**	-0.3133	-0.5189	0.189	-0.4325	-0.5612	-0.3744
X5	0.4296	0.3396	-0.3487	0.2971	1**	0.0369	0.1953	0.1437	-0.1281	0.1962	0.0401	0.2748	0.0095	0.0442
X6	-0.4643	-0.0919	0.282	-0.3773	0.0369	1**	-0.5507	-0.4461	0.3505	0.5341	-0.6621*	0.3394	0.2075	0.5234
X7	0.3099	-0.102	-0.666*	0.2856	0.1953	-0.5507	1**	0.1603	0.0028	-0.5774	0.9139**	-0.5579	0.0114	-0.7192*
X8	-0.2405	-0.1488	-0.6151	0.8661**	0.1437	-0.4461	0.1603	1**	-0.4528	-0.5449	0.2351	-0.388	-0.5598	-0.3971
X9	0.1946	0.3831	0.5091	-0.3133	-0.1281	0.3505	0.0028	-0.4528	1**	0.622	-0.1756	0.5183	0.8011**	0.5395
X10	0.3556	0.5816	0.7891**	-0.5189	0.1962	0.5341	-0.5774	-0.5449	0.622	1**	-0.6871*	0.9617**	0.609	0.9422**
X11	0.2213	0.031	-0.6723*	0.189	0.0401	-0.6621*	0.9139**	0.2351	-0.1756	-0.6871*	1**	-0.5935	0.0361	-0.8439**
X12	0.4594	0.748*	0.7495*	-0.4325	0.2748	0.3394	-0.5579	-0.388	0.5183	0.9617**	-0.5935	1**	0.6177	0.8912**
X13	0.3749	0.7865**	0.4815	-0.5612	0.0095	0.2075	0.0114	-0.5598	0.8011**	0.609	0.0361	0.6177	1**	0.3884
X14	0.1902	0.3582	0.8318**	-0.3744	0.0442	0.5234	-0.7192*	-0.3971	0.5395	0.9422**	-0.8439**	0.8912**	0.3884	1**
X15	0.459	0.8973**	0.4102	-0.5615	0.3733	0.2902	-0.1049	-0.5357	0.5734	0.719*	-0.075	0.7685**	0.8932**	0.4665
X16	0.2514	0.5677	0.7731**	-0.3417	0.204	0.4654	-0.7165*	-0.3261	0.4718	0.9516**	-0.7766**	0.9657**	0.4685	0.9601**
X17	0.4074	0.8578**	0.6815*	-0.5282	0.1697	0.3252	-0.3635	-0.483	0.6918*	0.8741**	-0.3476	0.9168**	0.8727**	0.7299*
X18	0.2503	0.5636	0.8161**	-0.5397	-0.002	0.5373	-0.525	-0.5663	0.7922**	0.9552**	-0.6155	0.9019**	0.7357*	0.9081**
X19	0.3671	0.6972*	0.7592*	-0.482	0.004	0.3455	-0.3669	-0.4723	0.834**	0.897**	-0.4314	0.8934**	0.8279**	0.8181**
X20	0.1605	0.6611*	0.7369*	-0.5378	0.133	0.5267	-0.724*	-0.4519	0.4318	0.8947**	-0.6661*	0.9238**	0.607	0.8349**
X21	0.4755	0.6902*	0.2625	-0.2662	0.7645*	0.3791	-0.2497	-0.3161	0.2408	0.739*	-0.3317	0.7803**	0.477	0.5467
X22	0.6986*	0.554	0.1176	-0.4777	0.5928	0.136	0.3065	-0.5859	0.3895	0.5048	0.1577	0.4824	0.558	0.2421
X23	0.5883	0.5538	0.1033	-0.3707	0.4568	0.1626	0.4466	-0.5739	0.6768*	0.4462	0.2599	0.4016	0.759*	0.171
X24	0.2559	0.0664	0.5124	-0.2806	0.5049	0.4241	-0.2617	-0.6977*	0.4567	0.7228*	-0.5565	0.7249*	0.3064	0.7748**
X25	0.1051	0.3944	0.2168	0.4641	0.0678	-0.2971	-0.3085	0.4965	-0.0149	0.0922	-0.1532	0.3208	0.2141	0.1145
X26	-0.1303	-0.4779	-0.8167**	0.4003	-0.0703	-0.5037	0.8377**	0.3434	-0.3768	-0.9089**	0.8474**	-0.9129**	-0.3798	-0.9535**
X27	0.5716	0.3284	0.6709*	-0.3034	0.4453	0.2274	-0.3281	-0.4813	0.3943	0.8289**	-0.5411	0.8392**	0.3351	0.831**
X28	0.0163	0.3885	0.6321*	-0.4104	0.2184	0.7967**	-0.4337	-0.5165	0.8204**	0.934**	-0.6402*	0.8483**	0.6668*	0.8969**
X29	-0.4867	-0.4645	-0.2323	0.1878	0.1155	0.4135	0.0554	-0.1092	-0.0323	-0.2222	-0.0689	-0.3412	-0.152	-0.2012

Table 16. Genotypic correlation coefficients among 29 characters in *Cattleya* hybrids (Continued)

	X15	X16	X17	X18	X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X29
X1	0.459	0.2514	0.4074	0.2503	0.3671	0.1605	0.4755	0.6986*	0.5883	0.2559	0.1051	-0.1303	0.5716	0.0163	-0.4867
X2	0.8973**	0.5677	0.8578**	0.5636	0.6972*	0.6611*	0.6902*	0.554	0.5538	0.0664	0.3944	-0.4779	0.3284	0.3885	-0.4645
X3	0.4102	0.7731**	0.6815*	0.8161**	0.7592*	0.7369*	0.2625	0.1176	0.1033	0.5124	0.2168	-0.8167**	0.6709*	0.6321*	-0.2323
X4	-0.5615	-0.3417	-0.5282	-0.5397	-0.482	-0.5378	-0.2662	-0.4777	-0.3707	-0.2806	0.4641	0.4003	-0.3034	-0.4104	0.1878
X5	0.3733	0.204	0.1697	-0.002	0.004	0.133	0.7645*	0.5928	0.4568	0.5049	0.0678	-0.0703	0.4453	0.2184	0.1155
X6	0.2902	0.4654	0.3252	0.5373	0.3455	0.5267	0.3791	0.136	0.1626	0.4241	-0.2971	-0.5037	0.2274	0.7967**	0.4135
X7	-0.1049	-0.7165*	-0.3635	-0.525	-0.3669	-0.724*	-0.2497	0.3065	0.4466	-0.2617	-0.3085	0.8377**	-0.3281	-0.4337	0.0554
X8	-0.5357	-0.3261	-0.483	-0.5663	-0.4723	-0.4519	-0.3161	-0.5859	-0.5739	-0.6977*	0.4965	0.3434	-0.4813	-0.5165	-0.1092
X9	0.5734	0.4718	0.6918*	0.7922**	0.834**	0.4318	0.2408	0.3895	0.6768*	0.4567	-0.0149	-0.3768	0.3943	0.8204**	-0.0323
X10	0.719*	0.9516**	0.8741**	0.9552**	0.897**	0.8947**	0.739*	0.5048	0.4462	0.7228*	0.0922	-0.9089**	0.8289**	0.934**	-0.2222
X11	-0.075	-0.7766**	-0.3476	-0.6155	-0.4314	-0.6661*	-0.3317	0.1577	0.2599	-0.5565	-0.1532	0.8474**	-0.5411	-0.6402*	-0.0689
X12	0.7685**	0.9657**	0.9168**	0.9019**	0.8934**	0.9238**	0.7803**	0.4824	0.4016	0.7249*	0.3208	-0.9129**	0.8392**	0.8483**	-0.3412
X13	0.8932**	0.4685	0.8727**	0.7357*	0.8279**	0.607	0.477	0.558	0.759*	0.3064	0.2141	-0.3798	0.3351	0.6668*	-0.152
X14	0.4665	0.9601**	0.7299*	0.9081**	0.8181**	0.8349**	0.5467	0.2421	0.171	0.7748**	0.1145	-0.9535**	0.831**	0.8969**	-0.2012
X15	1**	0.6115	0.9161**	0.7257*	0.778**	0.7589*	0.8043**	0.7252*	0.7531*	0.4802	0.2167	-0.5178	0.5168	0.6988*	-0.1703
X16	0.6115	1**	0.8278**	0.8984**	0.8358**	0.9316**	0.6975*	0.2839	0.2054	0.7536*	0.3567	-0.9809**	0.8337**	0.8759**	-0.2065
X17	0.9161**	0.8278**	1**	0.9103**	0.9505**	0.8942**	0.7105*	0.5177	0.5695	0.6066	0.3945	-0.7588*	0.6691*	0.8639**	-0.2263
X18	0.7257*	0.8984**	0.9103**	1**	0.9704**	0.8694**	0.5853	0.4049	0.4638	0.6987*	0.1752	-0.8555**	0.7416*	1.008**	-0.1418
X19	0.778**	0.8358**	0.9505**	0.9704**	1**	0.8146**	0.5535	0.4412	0.537	0.5763	0.2404	-0.7689**	0.6868*	0.9127**	-0.2731
X20	0.7589*	0.9316**	0.8942**	0.8694**	0.8146**	1**	0.7192*	0.3033	0.2389	0.6761*	0.4107	-0.9341**	0.7076*	0.8763**	-0.0942
X21	0.8043**	0.6975*	0.7105*	0.5853	0.5535	0.7192*	1**	0.7379*	0.6025	0.7346*	0.1184	-0.6039	0.7188*	0.6818*	-0.0508
X22	0.7252*	0.2839	0.5177	0.4049	0.4412	0.3033	0.7379*	1**	0.9054**	0.475	-0.4109	-0.1487	0.5592	0.4396	-0.2339
X23	0.7531*	0.2054	0.5695	0.4638	0.537	0.2389	0.6025	0.9054**	1**	0.3575	-0.2324	-0.0404	0.3735	0.4828	-0.0288
X24	0.4802	0.7536*	0.6066	0.6987*	0.5763	0.6761*	0.7346*	0.475	0.3575	1**	0.2877	-0.6808*	1.0808**	0.7722**	0.5227
X25	0.2167	0.3567	0.3945	0.1752	0.2404	0.4107	0.1184	-0.4109	-0.2324	0.2877	1**	-0.3541	0.1777	0.1784	0.4323
X26	-0.5178	-0.9809**	-0.7588*	-0.8555**	-0.7689**	-0.9341**	-0.6039	-0.1487	-0.0404	-0.6808*	-0.3541	1**	-0.7587*	-0.8196**	0.1947
X27	0.5168	0.8337**	0.6691*	0.7416*	0.6868*	0.7076*	0.7188*	0.5592	0.3735	1.0808**	0.1777	-0.7587*	1**	0.7017*	0.0865
X28	0.6988*	0.8759**	0.8639**	1.008**	0.9127**	0.8763**	0.6818*	0.4396	0.4828	0.7722**	0.1784	-0.8196**	0.7017*	1**	0.2558
X29	-0.1703	-0.2065	-0.2263	-0.1418	-0.2731	-0.0942	-0.0508	-0.2339	-0.0288	0.5227	0.4323	0.1947	0.0865	0.2558	1**

X1-Plant height from base to tip of the flowering shoot, X2-Pseudobulb length at flowering, X3-Pseudobulb breadth at broadest part, X4-Inflorescence number per pseudobulb, X5-Inflorescence length, X6-Peduncle sheath length, X7-Peduncle length, X8-Flower number per peduncle, X9-Flower length, X10-Flower width in front view, X11-Days from emergence to opening of inflorescence, X12-Dorsal sepal length, X13-Dorsal sepal width, X14-Lateral sepal length, X15-Lateral sepal width, X16-Petal length, X17-Petal width, X18-Lip length, X19-Lip width, X20-Column length, X21-Length of flower pedicel, X22-Flower longevity on the plant, X23-Vase life of flowers, X24-Plant Height, X25-Number of shoots per plant, X26-Number of leaves per pseudobulb, X27-Length of the largest leaf, X28-Width of the largest leaf, X29-Days from emergence to maturity of leaf.

Table 17. Phenotypic correlation coefficients among 29 characters in *Cattleya* hybrids

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	1**	0.5322**	0.2755	-0.1757	0.3957*	-0.4444*	0.2932	-0.2103	0.1924	0.3471	0.2213	0.4512*	0.3673*	0.1905
X2	0.5322**	1**	0.3077	-0.2903	0.3302	-0.0624	-0.075	-0.1397	0.3524	0.5558**	0.0564	0.6931**	0.7245**	0.3514
X3	0.2755	0.3077	1**	-0.5812**	-0.3229	0.295	-0.6195**	-0.5451**	0.4933**	0.7694**	-0.615**	0.72**	0.4646**	0.8066**
X4	-0.1757	-0.2903	-0.5812**	1**	0.2746	-0.3914*	0.2793	0.8886**	-0.2955	-0.4748**	0.1533	-0.3864*	-0.479**	-0.3706*
X5	0.3957*	0.3302	-0.3229	0.2746	1**	0.0373	0.216	0.1432	-0.1275	0.2031	0.0562	0.2816	0.0114	0.0446
X6	-0.4444*	-0.0624	0.295	-0.3914*	0.0373	1**	-0.5183**	-0.4478*	0.3592	0.5338**	-0.6425**	0.3442	0.2072	0.5221**
X7	0.2932	-0.075	-0.6195**	0.2793	0.216	-0.5183**	1**	0.1732	0.006	-0.5541**	0.8875**	-0.5283**	0.0154	-0.6964**
X8	-0.2103	-0.1397	-0.5451**	0.8886**	0.1432	-0.4478*	0.1732	1**	-0.4042*	-0.4708**	0.2037	-0.3418	-0.4564*	-0.3666*
X9	0.1924	0.3524	0.4933**	-0.2955	-0.1275	0.3592	0.006	-0.4042*	1**	0.6186**	-0.1776	0.5174**	0.7965**	0.5386**
X10	0.3471	0.5558**	0.7694**	-0.4748**	0.2031	0.5338**	-0.5541**	-0.4708**	0.6186**	1**	-0.6572**	0.9548**	0.6063**	0.9394**
X11	0.2213	0.0564	-0.615**	0.1533	0.0562	-0.6425**	0.8875**	0.2037	-0.1776	-0.6572**	1**	-0.5705**	0.037	-0.811**
X12	0.4512*	0.6931**	0.72**	-0.3864*	0.2816	0.3442	-0.5283**	-0.3418	0.5174**	0.9548**	-0.5705**	1**	0.6134**	0.8841**
X13	0.3673*	0.7245**	0.4646**	-0.479**	0.0114	0.2072	0.0154	-0.4564*	0.7965**	0.6063**	0.037	0.6134**	1**	0.3867*
X14	0.1905	0.3514	0.8066**	-0.3706*	0.0446	0.5221**	-0.6964**	-0.3666*	0.5386**	0.9394**	-0.811**	0.8841**	0.3867*	1**
X15	0.457*	0.8249**	0.3918*	-0.4862**	0.352	0.2825	-0.1112	-0.4436*	0.5664**	0.7084**	-0.0746	0.7547**	0.8856**	0.4594*
X16	0.2509	0.5237**	0.7443**	-0.3074	0.1958	0.4568*	-0.707**	-0.2824	0.4672**	0.9435**	-0.7597**	0.9535**	0.4657**	0.9484**
X17	0.3966*	0.7811**	0.6502**	-0.4385*	0.1684	0.3198	-0.3543	-0.3892*	0.6868**	0.8644**	-0.3451	0.9048**	0.869**	0.7153**
X18	0.2332	0.5313**	0.7901**	-0.4523*	0.0233	0.533**	-0.4887**	-0.4515*	0.7824**	0.9501**	-0.5883**	0.8918**	0.7306**	0.8937**
X19	0.3598	0.6605**	0.7392**	-0.4225*	0.0104	0.3379	-0.351	-0.3758*	0.8201**	0.893**	-0.4032*	0.8772**	0.8222**	0.8132**
X20	0.1497	0.633**	0.7153**	-0.4722**	0.1493	0.5245**	-0.6889**	-0.381*	0.426*	0.8912**	-0.6343**	0.9102**	0.6039**	0.8234**
X21	0.4569*	0.6414**	0.2713	-0.3047	0.7349**	0.3978*	-0.2421	-0.3579	0.2467	0.7297**	-0.3235	0.7702**	0.4663**	0.5411**
X22	0.6798**	0.4947**	0.1317	-0.4403*	0.5575**	0.1495	0.2971	-0.5083**	0.3872*	0.4976**	0.1476	0.4719**	0.5499**	0.2414
X23	0.5431**	0.4915**	0.0612	-0.227	0.4296*	0.1265	0.4182*	-0.3695*	0.641**	0.4132*	0.2383	0.3661*	0.7243**	0.145
X24	0.1378	0.1039	0.1834	0.0028	0.2461	0.1182	-0.1378	-0.087	0.1701	0.3297	-0.2271	0.3086	0.1223	0.341
X25	0.0504	0.381*	0.1968	0.1573	0.0589	-0.1852	-0.2091	0.1983	-0.0026	0.1016	-0.0828	0.2306	0.1535	0.1291
X26	-0.1283	-0.436*	-0.7817**	0.3396	-0.0683	-0.492**	0.8212**	0.2784	-0.3749*	-0.9016**	0.8335**	-0.9043**	-0.3789*	-0.9436**
X27	0.43*	0.285	0.4412*	-0.179	0.3181	0.1453	-0.2492	-0.2716	0.2851	0.6088**	-0.3895*	0.6225**	0.2313	0.6117**
X28	0.0297	0.2836	0.3933*	-0.2367	0.1244	0.5264**	-0.3688*	-0.2724	0.5771**	0.6708**	-0.4561*	0.6002**	0.4852**	0.6458**
X29	-0.3697*	-0.4493*	-0.2053	0.2497	0.0679	0.2856	0.0486	0.0244	-0.038	-0.21	-0.0601	-0.2642	-0.122	-0.1991

Table 17. Phenotypic correlation coefficients among 29 characters in *Cattleya* hybrids (Continued)

	X15	X16	X17	X18	X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X29
X1	0.457*	0.2509	0.3966*	0.2332	0.3598	0.1497	0.4569*	0.6798**	0.5431**	0.1378	0.0504	-0.1283	0.43*	0.0297	-0.3697*
X2	0.8249**	0.5237**	0.7811**	0.5313**	0.6605**	0.633**	0.6414**	0.4947**	0.4915**	0.1039	0.381*	-0.436*	0.285	0.2836	-0.4493*
X3	0.3918*	0.7443**	0.6502**	0.7901**	0.7392**	0.7153**	0.2713	0.1317	0.0612	0.1834	0.1968	-0.7817**	0.4412*	0.3933*	-0.2053
X4	-0.4862**	-0.3074	-0.4385*	-0.4523*	-0.4225*	-0.4722**	-0.3047	-0.4403*	-0.227	0.0028	0.1573	0.3396	-0.179	-0.2367	0.2497
X5	0.352	0.1958	0.1684	0.0233	0.0104	0.1493	0.7349**	0.5575**	0.4296*	0.2461	0.0589	-0.0683	0.3181	0.1244	0.0679
X6	0.2825	0.4568*	0.3198	0.533**	0.3379	0.5245**	0.3978*	0.1495	0.1265	0.1182	-0.1852	-0.492**	0.1453	0.5264**	0.2856
X7	-0.1112	-0.707**	-0.3543	-0.4887**	-0.351	-0.6889**	-0.2421	0.2971	0.4182*	-0.1378	-0.2091	0.8212**	-0.2492	-0.3688*	0.0486
X8	-0.4436*	-0.2824	-0.3892*	-0.4515*	-0.3758*	-0.381*	-0.3579	-0.5083**	-0.3695*	-0.087	0.1983	0.2784	-0.2716	-0.2724	0.0244
X9	0.5664**	0.4672**	0.6868**	0.7824**	0.8201**	0.426*	0.2467	0.3872*	0.641**	0.1701	-0.0026	-0.3749*	0.2851	0.5771**	-0.038
X10	0.7084**	0.9435**	0.8644**	0.9501**	0.893**	0.8912**	0.7297**	0.4976**	0.4132*	0.3297	0.1016	-0.9016**	0.6088**	0.6708**	-0.21
X11	-0.0746	-0.7597**	-0.3451	-0.5883**	-0.4032*	-0.6343**	-0.3235	0.1476	0.2383	-0.2271	-0.0828	0.8335**	-0.3895*	-0.4561*	-0.0601
X12	0.7547**	0.9535**	0.9048**	0.8918**	0.8772**	0.9102**	0.7702**	0.4719**	0.3661*	0.3086	0.2306	-0.9043**	0.6225**	0.6002**	-0.2642
X13	0.8856**	0.4657**	0.869**	0.7306**	0.8222**	0.6039**	0.4663**	0.5499**	0.7243**	0.1223	0.1535	-0.3789*	0.2313	0.4852**	-0.122
X14	0.4594*	0.9484**	0.7153**	0.8937**	0.8132**	0.8234**	0.5411**	0.2414	0.145	0.341	0.1291	-0.9436**	0.6117**	0.6458**	-0.1991
X15	1**	0.6125**	0.9102**	0.7071**	0.7684**	0.7459**	0.7854**	0.7121**	0.7255**	0.2546	0.1443	-0.515**	0.3966*	0.548**	-0.1426
X16	0.6125**	1**	0.8249**	0.8841**	0.8277**	0.9202**	0.6863**	0.2834	0.194	0.3852*	0.2473	-0.9781**	0.6263**	0.6636**	-0.1692
X17	0.9102**	0.8249**	1**	0.9015**	0.9364**	0.8843**	0.6963**	0.5073**	0.5542**	0.2924	0.262	-0.757**	0.4978**	0.6403**	-0.1768
X18	0.7071**	0.8841**	0.9015**	1**	0.9596**	0.8676**	0.5738**	0.3946*	0.4387*	0.313	0.1414	-0.8458**	0.5343**	0.7063**	-0.1306
X19	0.7684**	0.8277**	0.9364**	0.9596**	1**	0.8105**	0.5332**	0.434*	0.5066**	0.2964	0.1963	-0.7612**	0.4973**	0.6738**	-0.2498
X20	0.7459**	0.9202**	0.8843**	0.8676**	0.8105**	1**	0.7084**	0.2959	0.2215	0.3113	0.2924	-0.921**	0.4985**	0.6331**	-0.1111
X21	0.7854**	0.6863**	0.6963**	0.5738**	0.5332**	0.7084**	1**	0.7192**	0.5462**	0.2871	0.1218	-0.5891**	0.5289**	0.47**	-0.0839
X22	0.7121**	0.2834	0.5073**	0.3946*	0.434*	0.2959	0.7192**	1**	0.8364**	0.2212	-0.2646	-0.1466	0.387*	0.286	-0.1789
X23	0.7255**	0.194	0.5542**	0.4387*	0.5066**	0.2215	0.5462**	0.8364**	1**	0.1995	-0.1438	-0.039	0.2848	0.3839*	-0.0367
X24	0.2546	0.3852*	0.2924	0.313	0.2964	0.3113	0.2871	0.2212	0.1995	1**	0.0879	-0.3323	0.7498**	0.6414**	0.2053
X25	0.1443	0.2473	0.262	0.1414	0.1963	0.2924	0.1218	-0.2646	-0.1438	0.0879	1**	-0.2473	0.2046	0.0731	0.0232
X26	-0.515**	-0.9781**	-0.757**	-0.8458**	-0.7612**	-0.921**	-0.5891**	-0.1466	-0.039	-0.3323	-0.2473	1**	-0.5675**	-0.608**	0.1534
X27	0.3966*	0.6263**	0.4978**	0.5343**	0.4973**	0.4985**	0.5289**	0.387*	0.2848	0.7498**	0.2046	-0.5675**	1**	0.6855**	-0.015
X28	0.548**	0.6636**	0.6403**	0.7063**	0.6738**	0.6331**	0.47**	0.286	0.3839*	0.6414**	0.0731	-0.608**	0.6855**	1**	0.147
X29	-0.1426	-0.1692	-0.1768	-0.1306	-0.2498	-0.1111	-0.0839	-0.1789	-0.0367	0.2053	0.0232	0.1534	-0.015	0.147	1**

X1-Plant height from base to tip of the flowering shoot, X2-Pseudobulb length at flowering, X3-Pseudobulb breadth at broadest part, X4-Inflorescence number per pseudobulb, X5-Inflorescence length, X6-Peduncle sheath length, X7-Peduncle length, X8-Flower number per peduncle, X9-Flower length, X10-Flower width in front view, X11-Days from emergence to opening of inflorescence, X12-Dorsal sepal length, X13-Dorsal sepal width, X14-Lateral sepal length, X15-Lateral sepal width, X16-Petal length, X17-Petal width, X18-Lip length, X19-Lip width, X20-Column length, X21-Length of flower pedicel, X22-Flower longevity on the plant, X23-Vase life of flowers, X24-Plant Height, X25-Number of shoots per plant, X26-Number of leaves per pseudobulb, X27-Length of the largest leaf, X28-Width of the largest leaf, X29-Days from emergence to maturity of leaf.

4.3.3. Environmental correlation

Environmental correlation coefficient among different characters are given in Table 18.

Highly significant positive correlation was observed for flower width in front view with lateral sepal length (0.795) and had positive correlation with lip width (0.681) and column length (0.764). Length of the largest leaf recorded positive correlation with width of the largest leaf (0.665). Length of flower pedicel exhibited significant negative correlation with inflorescence number per pseudobulb (0.725). Inflorescence number per pseudobulb had negative correlation with peduncle sheath length (0.694), lateral sepal length (0.74) and length of flower pedicel (0.725).

4.4. SCORING OF THE CHARACTERS BASED ON DUS GUIDELINES

Scoring of the characters studied based on DUS guidelines were done and presented in Table 19.

Four hybrids *viz*., Rlc.Morning Stars, Rlc. Haadyayi Delight x Mary Song, Rlc.Memoria Anna Balmores and *C.* Tipo recorded high scores based on scoring given by DUS guidelines.

4.5. MOLECULAR CHARACTERIZATION USING ISSR PRIMERS

Molecular characterization of twelve genotypes was carried out using ten ISSR primers. Total genomic DNA yield varied from 0.776 ng/µl to 27 ng/µl as shown in Table 20.

Primer associated total number of amplicons and polymorphic bands were done and presented in Table 21. Representation of banding pattern of DNA of twelve *Cattleya* hybrids using ten primers are shown from Table 22. to Table 31. A total of 14 scorable bands were obtained by the primer 818. Lower number of bands was produced by the primer 862. Total number of amplicons obtained was 97. Of these 96 were polymorphic and one was monomorphic with percentage of polymorphic loci 98.97%.

Table 18. Environmental correlation coefficients among 29 characters in *Cattleya* hybrids

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	1	-0.153	0.342	-0.249	-0.381	0.059	-0.175	-0.179	0.1	-0.013	0.222	0.132	-0.079	0.208
X2	-0.153	1	-0.012	-0.219	0.302	0.222	0.202	-0.124	0.115	0.568	0.386	0.304	0.306	0.477
X3	0.342	-0.012	1	-0.509	0.023	0.505	0.094	-0.4	0.292	0.551	0.341	0.238	0.246	0.45
X4	-0.249	-0.219	-0.509	1	0.238	-0.694*	0.4	0.945**	-0.588	-0.569	-0.046	-0.317	-0.111	-0.74*
X5	-0.381	0.302	0.023	0.238	1	0.045	0.647*	0.218	-0.151	0.467	0.421	0.526	0.135	0.062
X6	0.059	0.222	0.505	-0.694*	0.045	1	0.212	-0.754**	0.868**	0.603	-0.167	0.535	0.338	0.526
X7	-0.175	0.202	0.094	0.4	0.647*	0.212	1	0.397	0.163	0.3	0.177	0.497	0.313	0.05
X8	-0.179	-0.124	-0.4	0.945**	0.218	-0.754**	0.397	1	-0.667*	-0.441	0.154	-0.375	-0.091	-0.571
X9	0.1	0.115	0.292	-0.588	-0.151	0.868**	0.163	-0.667*	1	0.377	-0.319	0.474	0.189	0.514
X10	-0.013	0.568	0.551	-0.569	0.467	0.603	0.3	-0.441	0.377	1	0.574	0.569	0.423	0.795**
X11	0.222	0.386	0.341	-0.046	0.421	-0.167	0.177	0.154	-0.319	0.574	1	0.309	0.127	0.402
X12	0.132	0.304	0.238	-0.317	0.526	0.535	0.497	-0.375	0.474	0.569	0.309	1	0.317	0.529
X13	-0.079	0.306	0.246	-0.111	0.135	0.338	0.313	-0.091	0.189	0.423	0.127	0.317	1	0.324
X14	0.208	0.477	0.45	-0.75**	0.062	0.526	0.05	-0.571	0.514	0.795**	0.402	0.529	0.324	1
X15	0.412	0.251	0.044	-0.225	-0.361	0.025	-0.431	-0.191	-0.088	-0.069	-0.065	-0.154	-0.07	0.021
X16	0.308	0.238	0.292	-0.463	-0.114	0.216	-0.443	-0.43	-0.124	0.228	0.148	-0.036	-0.067	0.081
X17	-0.31	0.013	-0.032	0.238	0.238	0.196	0.096	0.036	0.008	-0.089	-0.329	-0.13	0.087	-0.548
X18	-0.399	0.372	0.411	0.005	0.715	0.44	0.678*	0.026	0.208	0.7	0.372	0.419	0.485	0.246
X19	0.079	0.531	0.483	-0.237	0.197	0.124	0.181	0.038	-0.099	0.681*	0.657*	0.063	0.472	0.574
X20	-0.21	0.561	0.412	-0.252	0.56	0.483	0.333	-0.203	0.14	0.764**	0.386	0.351	0.588	0.365
X21	0.003	0.302	0.412	-0.725*	0.192	0.777**	-0.076	-0.838	0.59	0.524	-0.132	0.538	0.139	0.422
X22	0.065	-0.049	0.423	-0.456	-0.262	0.512	0.028	-0.405	0.313	0.192	-0.168	0.046	0.11	0.217
X23	-0.332	0.041	-0.442	0.542	0.023	-0.469	-0.073	0.507	-0.307	-0.401	-0.168	-0.476	-0.305	-0.475
X24	0.097	0.208	-0.221	0.258	0.032	-0.449	-0.073	0.37	-0.592	-0.183	0.254	-0.349	-0.445	-0.264
X25	-0.176	0.442	0.25	-0.311	0.076	0.114	0.015	-0.198	0.108	0.417	0.174	0.088	0.088	0.485
X26	0.078	0.224	-0.203	0.038	-0.037	-0.147	-0.066	0.052	-0.127	-0.075	0.072	0.008	-0.409	-0.036
X27	0.152	0.075	-0.285	0.061	-0.21	-0.354	-0.404	0.097	-0.424	-0.194	0.091	-0.251	-0.179	-0.131
X28	0.073	-0.458	-0.169	0.381	-0.142	-0.247	0.048	0.261	-0.206	-0.467	-0.06	0.024	-0.059	-0.478
X29	1	-0.153	0.342	-0.249	-0.381	0.059	-0.175	-0.179	0.1	-0.013	0.222	0.132	-0.079	0.208

Table 18. Environmental correlation coefficients among 29 characters in *Cattleya* hybrids (Continued)

	X15	X16	X17	X18	X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X29
X1	0.412	0.308	-0.31	-0.399	0.079	-0.21	0.003	0.065	-0.332	0.097	-0.176	0.078	0.152	0.073	0.412
X2	0.251	0.238	0.013	0.372	0.531	0.561	0.302	-0.049	0.041	0.208	0.442	0.224	0.075	-0.458	0.251
X3	0.044	0.292	-0.032	0.411	0.483	0.412	0.412	0.423	-0.442	-0.221	0.25	-0.203	-0.285	-0.169	0.044
X4	-0.225	-0.463	0.238	0.005	-0.237	-0.252	-0.725*	-0.456	0.542	0.258	-0.311	0.038	0.061	0.381	-0.225
X5	-0.361	-0.114	0.238	0.715*	0.197	0.56	0.192	-0.262	0.023	0.032	0.076	-0.037	-0.21	-0.142	-0.361
X6	0.025	0.216	0.196	0.44	0.124	0.483	0.777**	0.512	-0.469	-0.449	0.114	-0.147	-0.354	-0.247	0.025
X7	-0.431	-0.443	0.096	0.678*	0.181	0.333	-0.076	0.028	-0.073	-0.073	0.015	-0.066	-0.404	0.048	-0.431
X8	-0.191	-0.43	0.036	0.026	0.038	-0.203	-0.838	-0.405	0.507	0.37	-0.198	0.052	0.097	0.261	-0.191
X9	-0.088	-0.124	0.008	0.208	-0.099	0.14	0.59	0.313	-0.307	-0.592	0.108	-0.127	-0.424	-0.206	-0.088
X10	-0.069	0.228	-0.089	0.7	0.681*	0.764**	0.524	0.192	-0.401	-0.183	0.417	-0.075	-0.194	-0.467	-0.069
X11	-0.065	0.148	-0.329	0.372	0.657*	0.386	-0.132	-0.168	-0.168	0.254	0.174	0.072	0.091	-0.06	-0.065
X12	-0.154	-0.036	-0.13	0.419	0.063	0.351	0.538	0.046	-0.476	-0.349	0.088	0.008	-0.251	0.024	-0.154
X13	-0.07	-0.067	0.087	0.485	0.472	0.588	0.139	0.11	-0.305	-0.445	0.088	-0.409	-0.179	-0.059	-0.07
X14	0.021	0.081	-0.548	0.246	0.574	0.365	0.422	0.217	-0.475	-0.264	0.485	-0.036	-0.131	-0.478	0.021
X15	1	0.779**	0.177	-0.401	0.171	0.105	0.22	0.08	0.127	0.234	-0.083	0.175	0.459	-0.143	1
X16	0.779**	1	0.26	-0.137	0.248	0.337	0.471	0.343	-0.178	0.283	-0.021	0.089	0.313	-0.152	0.779**
X17	0.177	0.26	1	0.359	-0.236	0.415	0.32	-0.155	0.355	-0.049	-0.265	-0.035	0.019	0.026	0.177
X18	-0.401	-0.137	0.359	1	0.469	0.801	0.281	0.006	-0.084	-0.183	0.189	-0.142	-0.326	-0.217	-0.401
X19	0.171	0.248	-0.236	0.469	1	0.652*	-0.04	0.15	-0.156	0.145	0.297	-0.12	0.037	-0.422	0.171
X20	0.105	0.337	0.415	0.801**	0.652*	1	0.454	0.04	-0.126	-0.097	0.08	-0.211	-0.07	-0.369	0.105
X21	0.22	0.471	0.32	0.281	-0.04	0.454	1	0.262	-0.349	-0.326	0.262	0.03	-0.159	-0.331	0.22
X22	0.08	0.343	-0.155	0.006	0.15	0.04	0.262	1	-0.527	-0.049	0.15	-0.224	-0.311	0.026	0.08
X23	0.127	-0.178	0.355	-0.084	-0.156	-0.126	-0.349	-0.527	1	0.135	0.066	0.088	0.216	-0.091	0.127
X24	0.234	0.283	-0.049	-0.183	0.145	-0.097	-0.326	-0.049	0.135	1	-0.016	0.613	0.618	0.008	0.234
X25	-0.083	-0.021	-0.265	0.189	0.297	0.08	0.262	0.15	0.066	-0.016	1	0.235	-0.04	-0.487	-0.083
X26	0.175	0.089	-0.035	-0.142	-0.12	-0.211	0.03	-0.224	0.088	0.613	0.235	1	0.665*	-0.161	0.175
X27	0.459	0.313	0.019	-0.326	0.037	-0.07	-0.159	-0.311	0.216	0.618	-0.04	0.665*	1	-0.006	0.459
X28	-0.143	-0.152	0.026	-0.217	-0.422	-0.369	-0.331	0.026	-0.091	0.008	-0.487	-0.161	-0.006	1	-0.143
X29	0.412	0.308	-0.31	-0.399	0.079	-0.21	0.003	0.065	-0.332	0.097	-0.176	0.078	0.152	0.073	0.412

X1-Plant height from base to tip of the flowering shoot, X2-Pseudobulb length at flowering, X3-Pseudobulb breadth at broadest part, X4-Inflorescence number per pseudobulb, X5-Inflorescence length, X6-Peduncle sheath length, X7-Peduncle length, X8-Flower number per peduncle, X9-Flower length, X10-Flower width in front view, X11-Days from emergence to opening of inflorescence, X12-Dorsal sepal length, X13-Dorsal sepal width, X14-Lateral sepal length, X15-Lateral sepal width, X16-Petal length, X17-Petal width, X18-Lip length, X19-Lip width, X20-Column length, X21-Length of flower pedicel, X22-Flower longevity on the plant, X23-Vase life of flowers, X24-Plant Height, X25-Number of shoots per plant, X26-Number of leaves per pseudobulb, X27-Length of the largest leaf, X28-Width of the largest leaf, X29-Days from emergence to maturity of leaf.

Table 19. Scoring of the characters of *Cattleya* hybrids based on DUS guidelines

Characters	Rth. Burana Beauty	Rlc. Taichung Beauty	Rlc. Mahina Yahiro	Rlc.Petch Wangnam Khiew	Rlc. Irene Dopkin	Rlc. Haadyayi Delight x Mary Song	*C*.Aurantiaca x Netrasiri Beauty	*C*. Tipo	Rlc.Memoria Anna Balmores	Rlc. Morning Stars
Inflorescence number per peduncle	1	1	1	1	1	1	5	5	1	1
Peduncle sheath length	7	7	7	7	7	5	5	7	7	7
Flower no per peduncle	3	3	3	3	3	3	3	5	3	3
Flower length	3	3	3	5	3	3	3	3	3	3
Flower width in front view	5	7	7	7	7	7	5	7	7	7
Dorsal sepal length	3	5	5	5	5	5	3	5	5	5
Dorsal sepal width	5	3	3	5	3	5	3	3	5	5
Lateral sepal length	3	5	5	5	5	5	3	5	5	5
Lateral sepal width	3	3	3	5	3	5	3	3	5	5
Petal length	3	5	5	5	5	5	3	5	5	5
Petal width	3	5	5	7	5	5	3	5	5	5
Lip length	3	5	5	7	5	5	3	5	5	5
Lip width	3	3	3	5	5	5	3	3	5	5
Column length	1	5	1	5	5	5	1	5	5	5
Length of flower pedicel	1	5	5	5	5	5	1	5	5	5
Flower longevity on the plant	3	5	5	5	5	5	5	3	5	3
Dorsal sepal shape	3	3	3	1	3	1	3	3	1	1
Dorsal sepal curvature	1	1	1	1	1	3	3	1	3	3
Lateral sepal shape	3	3	3	1	3	1	3	3	1	1
Lateral sepal curvature	1	1	1	1	1	3	3	1	3	3

Table 19. Scoring of the characters of *Cattleya* hybrids based on DUS guidelines (Continued)

Characters	Rth. Burana Beauty	Rlc.Taichung Beauty	Rlc. Mahina Yahiro	Rlc.Petch Wangnam Khiew	Rlc. Irene Dopkin	Rlc. Haady-ayi Delight x Mary Song	*C*.Au-ranti-aca x Netrasiri Beauty	*C*. Tipo	Rlc. Memoria Anna Balmores	Rlc.Morn-ing Stars
Petal shape	5	7	7	5	7	5	5	5	5	7
Petal curvature	1	1	1	1	1	3	3	1	3	3
Petal margin	3	3	3	3	3	3	1	3	3	3
Lip shape	7	1	1	1	1	1	1	1	7	1
Lip lateral lobe shape	1	1	1	1	1	1	9	9	1	1
Lip lateral lobe margin	3	1	1	1	1	3	1	1	1	1
Lip mid lobe margin	5	3	5	3	3	3	3	5	5	5
Flowering season	5	1	1	1	5	5	1	1	5	1
Sepal dominant colour	4	6	6	3	6	4	4	6	2	2
Sepal colour pattern inside	3	1	1	1	1	3	1	1	5	5
Petal predominant colour	4	6	6	4	6	6	3	6	2	2
Petal colour pattern	3	1	1	1	1	3	1	1	7	7
Lip predominant colour	3	6	6	6	6	6	3	6	6	6
Lip colour number and pattern	8	10	10	8	8	10	8	10	10	8
Column colour number and pattern	12	12	16	16	16	16	16	16	16	16
Total score	125	138	140	141	146	154	124	154	162	150

Table 20. Concetration of DNA obtained from the samples

Sample No.	Concentration in ng/µl
1	0.895
2	27.0
3	4.98
4	22.3
5	11.0
6	0.776
7	4.0
8	16.0
9	1.2
10	19.0
11	18.0
12	24.0

Table 21. Primer associated total number of amplicons and polymorphic bands

Sl.No.	Primers	Number of amplicons	Number of polymorphic bands
1	810	11	11
2	812	13	13
3	814	9	9
4	818	14	14
5	836	11	11
6	840	9	9
7	842	9	9
8	843	8	8
9	862	5	4
10	885	8	8
Total		97	96

Percentage of polymorphic loci = 98.97%

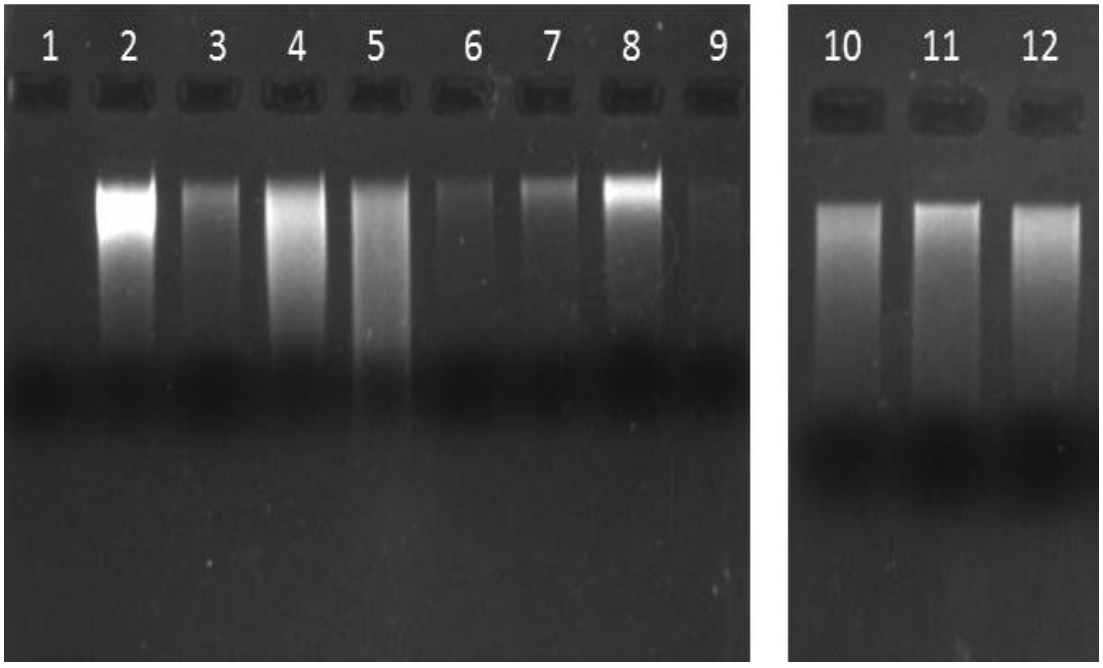


Plate 11. DNA bands obtained by Agarose Gel electrophoresis

Table 22. Representation of banding pattern of the DNA of twelve *Cattleya* hybrids using the primer 810

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
0	0	0	0	0	0	0	0	0	0	1	0
1	0	0	1	0	0	1	1	0	0	0	0
1	1	0	1	1	0	1	0	0	1	1	0
0	0	0	0	0	0	0	1	0	0	1	1
1	1	0	1	1	0	1	1	0	0	0	1
1	1	0	1	1	0	1	0	0	0	1	0
0	0	0	1	0	1	0	1	1	0	1	1
0	1	0	1	0	1	0	1	1	0	1	1
0	0	0	0	0	0	0	1	0	0	0	0
0	1	1	0	0	0	1	0	0	0	0	1
0	0	0	1	1	0	0	0	0	0	0	0

1 = presence of band 0 = absence of band

V1- Rlc.Tawan Shine

V2- Rth.Burana Beauty

V3- Rlc.Taichung Beauty

V4- Rlc.Mahina Yahiro,

V5- Rlc.Petch Wangnam Khiew,

V6- CH. Chocolate Drops 'Volcano Queen'

V7- Irene dopkin

V8- Rlc. Haadyayi Delight x Mary Song,

V9 -CH.Aurantica x Netrasiri Beauty

V10 - *Cattleya labiate* 'Tipo'

V11 - Rlc.Memoria Anna Balmores

V12 - Rlc.Morning Stars

Table 23. Representation of banding pattern of the DNA of twelve *Cattleya* hybrids using the primer 812

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
0	0	1	1	1	0	0	0	0	0	1	1
0	0	0	0	0	1	0	0	0	0	0	0
1	1	1	1	1	1	0	1	1	1	1	1
0	0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0	1
0	0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	1	0	1	0	0	0	0
1	1	1	0	1	0	1	1	0	1	1	1
0	1	0	0	0	1	0	0	1	0	0	0
0	1	0	0	0	1	0	0	1	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	1	1	0	0
0	0	0	0	1	1	0	1	1	0	1	1

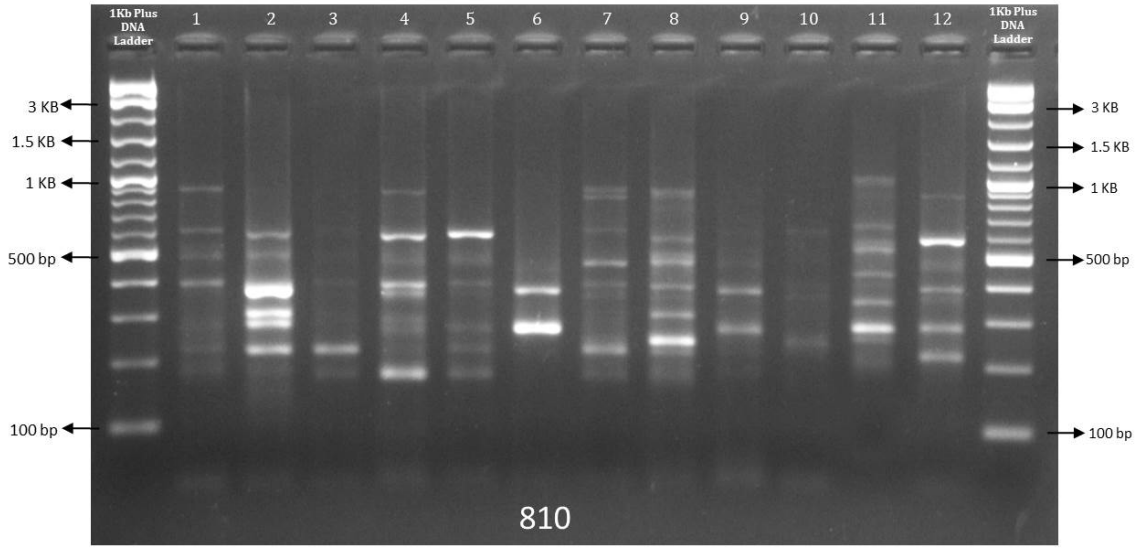


Plate 12. Amplification profiles of the DNA of twelve *Cattleya* hybrids using ISSR primer 810

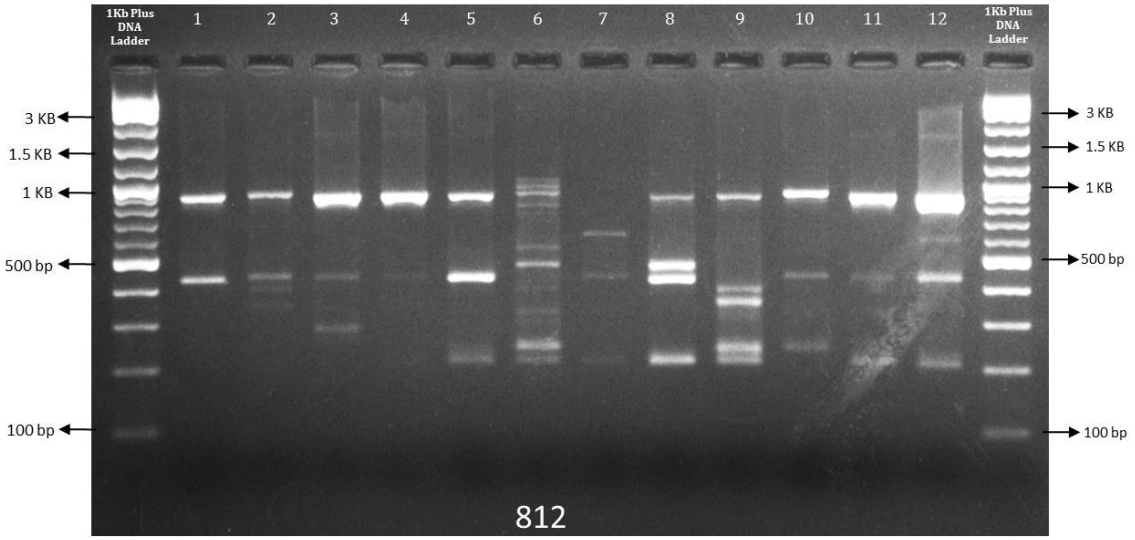


Plate 13. Amplification profiles of the DNA of twelve *Cattleya* hybrids using ISSR primer 812

Table 24. Representation of banding pattern of the DNA of twelve *Cattleya* hybrids using the primer 814

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
0	0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	1	1	0	0	0
1	0	1	1	0	1	1	1	1	1	1	1
1	1	1	1	1	1	0	1	1	0	0	1
1	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	0	0	1	1	1
0	0	1	1	1	1	0	1	1	1	1	1
0	0	0	0	0	1	0	0	0	0	0	1
0	0	0	1	0	1	1	1	1	0	1	1

Table 25. Representation of banding pattern of the DNA of twelve C*attleya* hybrids using the primer 818

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
0	0	0	0	0	0	0	1	0	0	0	1
0	0	0	0	0	1	0	0	0	0	0	0
0	1	0	0	0	0	1	1	0	0	0	0
1	0	0	0	0	0	0	1	0	1	1	0
0	0	0	1	0	0	0	1	0	0	0	0
1	0	1	1	1	1	1	1	1	0	0	1
1	0	0	1	1	0	0	0	0	0	0	1
1	0	1	0	1	0	1	0	0	0	0	0
1	0	1	1	1	1	1	1	0	1	0	1
0	0	1	0	0	0	0	0	0	0	0	0
1	1	1	1	1	0	0	1	0	1	0	1
1	1	1	0	1	1	1	1	0	1	1	0
0	0	0	0	0	0	0	1	0	1	0	0
0	0	0	0	0	0	0	0	0	1	0	1

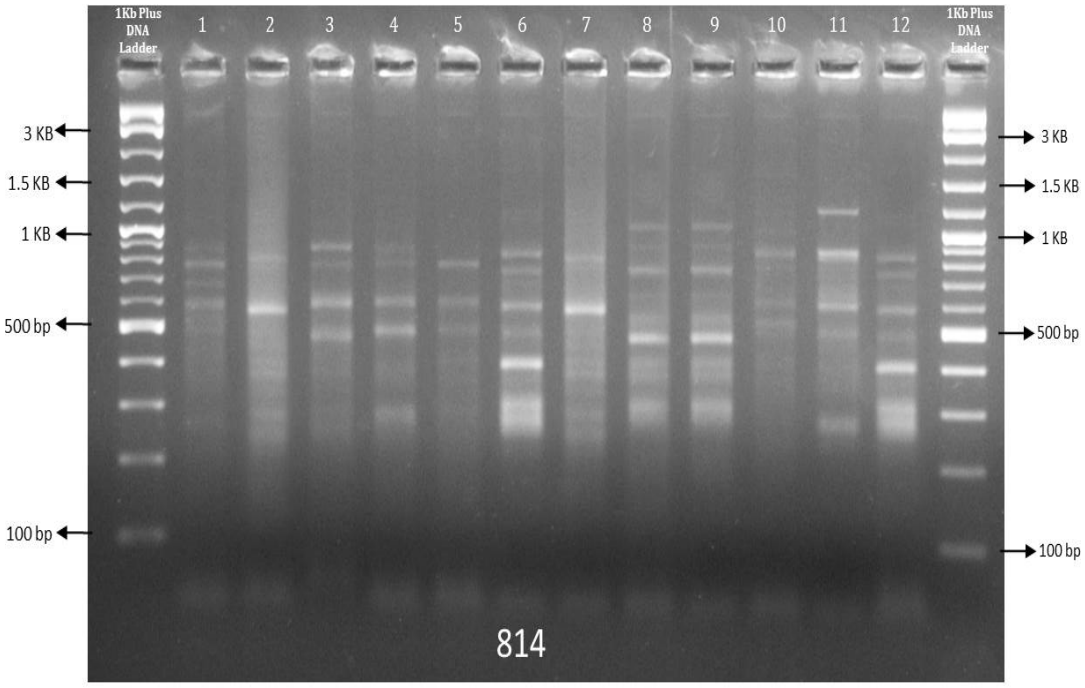


Plate 14. Amplification profiles of the DNA of twelve *Cattleya* hybrids using ISSR primer 814

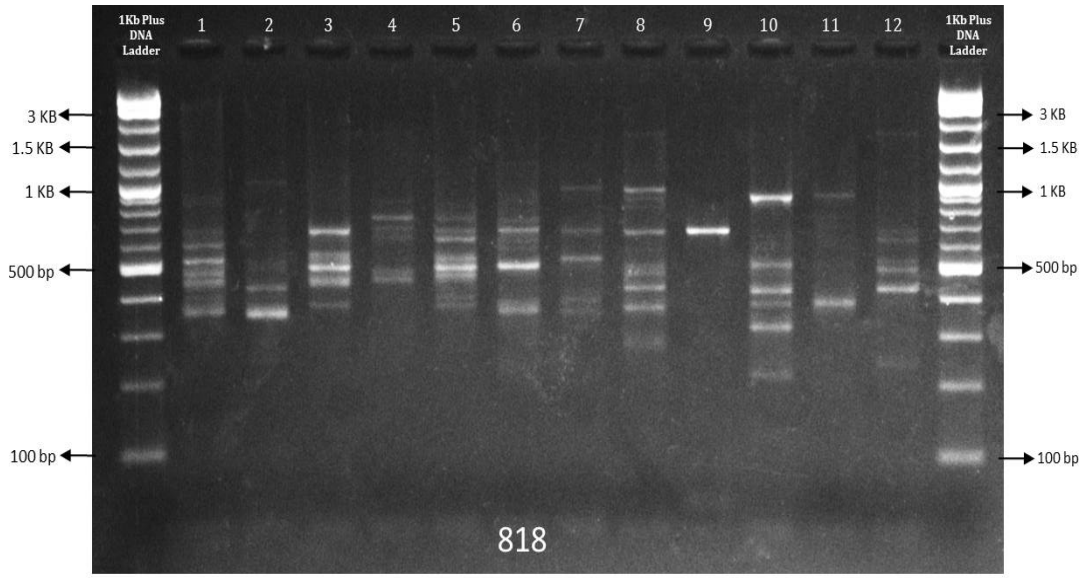


Plate 15. Amplification profiles of the DNA of twelve *Cattleya* hybrids using ISSR primer 818

Table 26. Representation of banding pattern of the DNA of twelve C*attleya* hybrids using the primer 836

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
0	0	0	0	0	1	1	1	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	1	1	1	0	0	0	0
0	0	0	0	0	1	0	0	0	1	0	0
0	1	0	1	1	1	0	1	0	0	0	0
1	0	1	1	1	0	0	1	1	1	1	1
0	0	0	0	0	1	1	0	0	0	0	0
0	0	0	1	1	1	1	1	1	1	1	0
0	0	0	1	0	1	0	0	1	0	0	0
0	1	0	0	0	1	0	0	1	0	0	1
1	0	1	1	1	0	1	1	1	1	1	1

Table 27. Representation of banding pattern of the DNA of twelve *Cattleya* hybrids using the primer 840

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
1	1	1	1	1	0	1	1	1	1	0	1
1	1	1	1	1	0	1	1	1	1	1	1
0	1	0	1	0	0	0	0	0	0	1	0
0	0	0	0	0	1	0	0	1	0	0	1
0	0	0	0	0	1	0	0	1	0	0	0
1	0	0	1	0	0	0	1	1	1	0	1
0	1	0	0	0	0	0	0	0	0	1	0
1	0	1	0	0	0	1	1	0	0	0	0
0	1	1	1	0	1	1	0	0	0	0	0

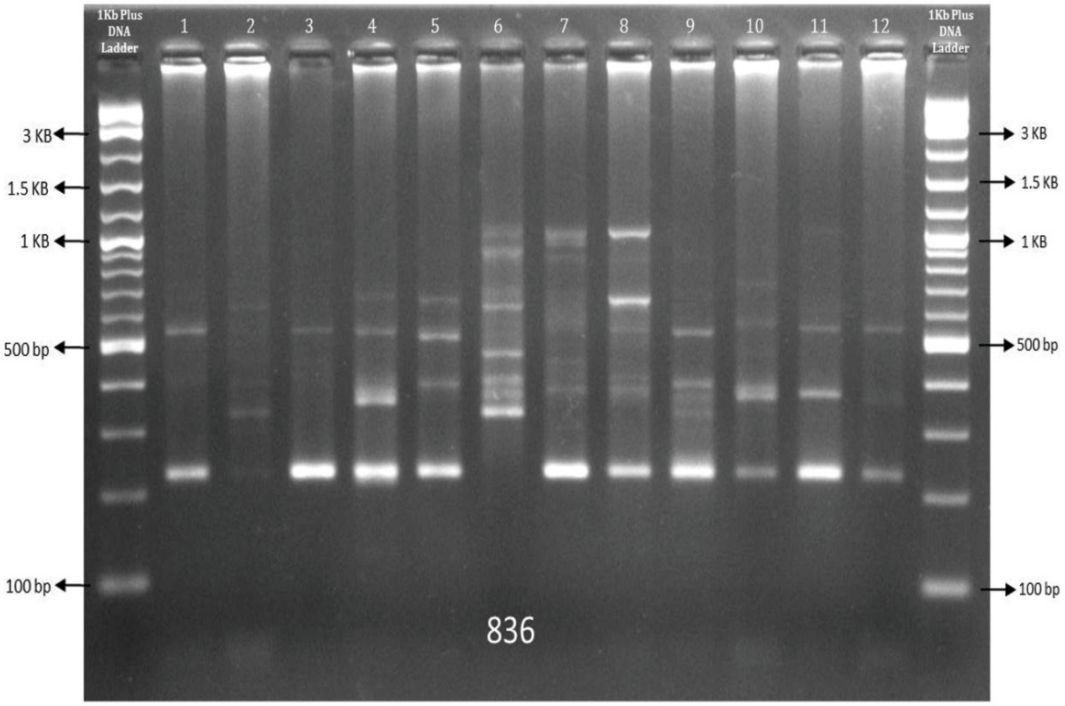


Plate 16. Amplification profiles of the DNA of twelve *Cattleya* hybrids using ISSR primer 836

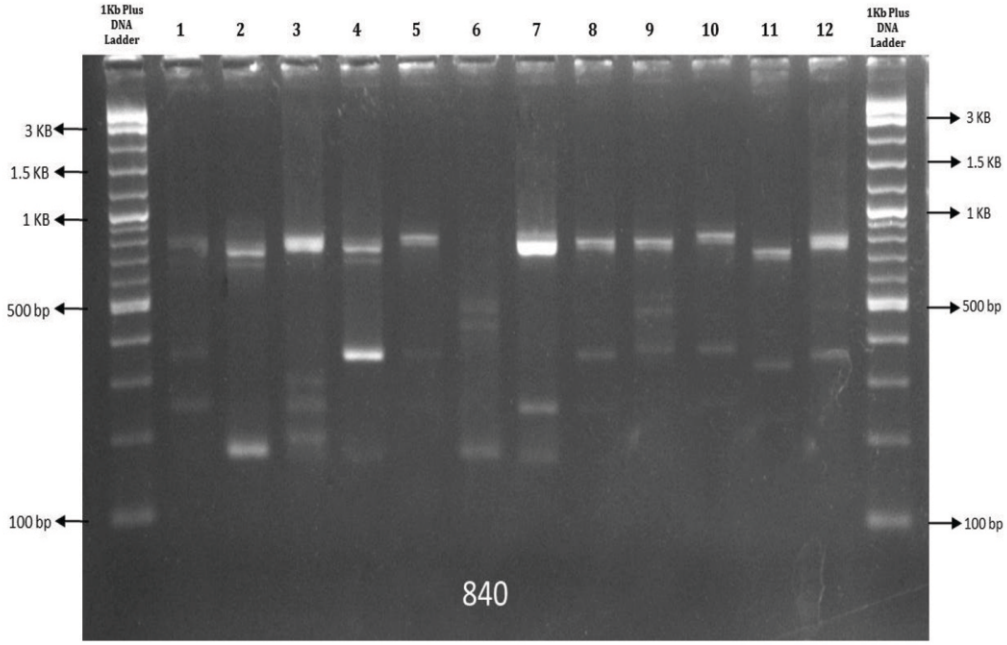


Plate17. Amplification profiles of the DNA of twelve *Cattleya* hybrids using ISSR primer 840

Table 28. Representation of banding pattern of the DNA of twelve *Cattleya* hybrids using the primer 842

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
0	0	0	0	0	1	1	1	1	1	0	0
0	0	0	0	0	1	1	0	1	0	0	0
0	0	0	0	0	1	1	1	1	1	0	0
0	1	1	1	1	1	1	1	1	1	0	0
1	1	1	1	1	0	0	1	1	1	1	1
0	0	0	0	0	1	1	0	0	0	0	0
0	0	0	1	1	1	1	1	1	1	1	0
0	1	0	1	0	1	0	0	1	0	0	0
1	1	1	1	1	0	1	1	1	1	1	1

Table 29. Representation of banding pattern of the DNA of twelve C*attleya* hybrids using the primer 843

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
0	0	0	0	0	0	1	0	0	0	1	0
0	0	0	0	0	0	0	0	0	1	0	0
1	1	1	1	0	1	1	1	0	1	1	1
1	1	1	1	1	1	0	1	1	1	1	1
1	1	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	1	0
1	1	0	1	0	1	0	0	0	1	0	1

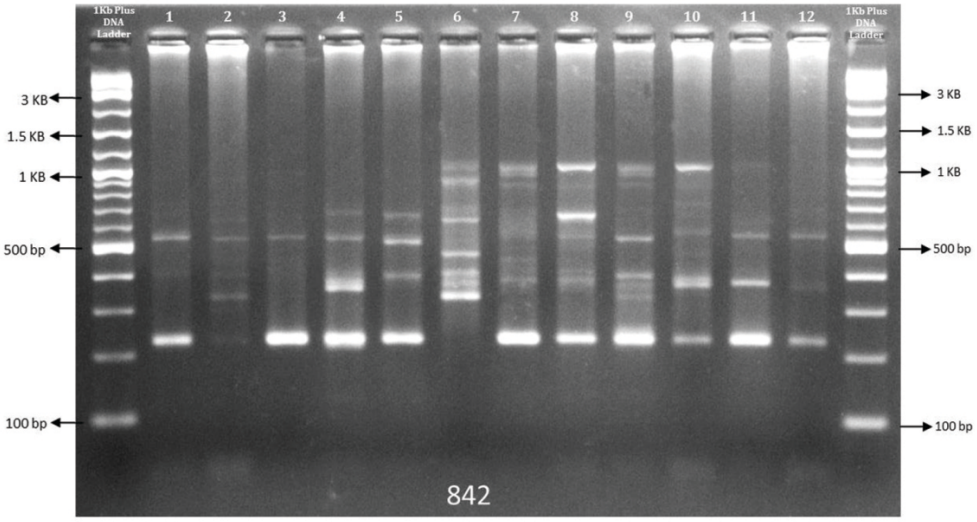


Plate18. Amplification profiles of the DNA of twelve *Cattleya* hybrids using ISSR primer 842

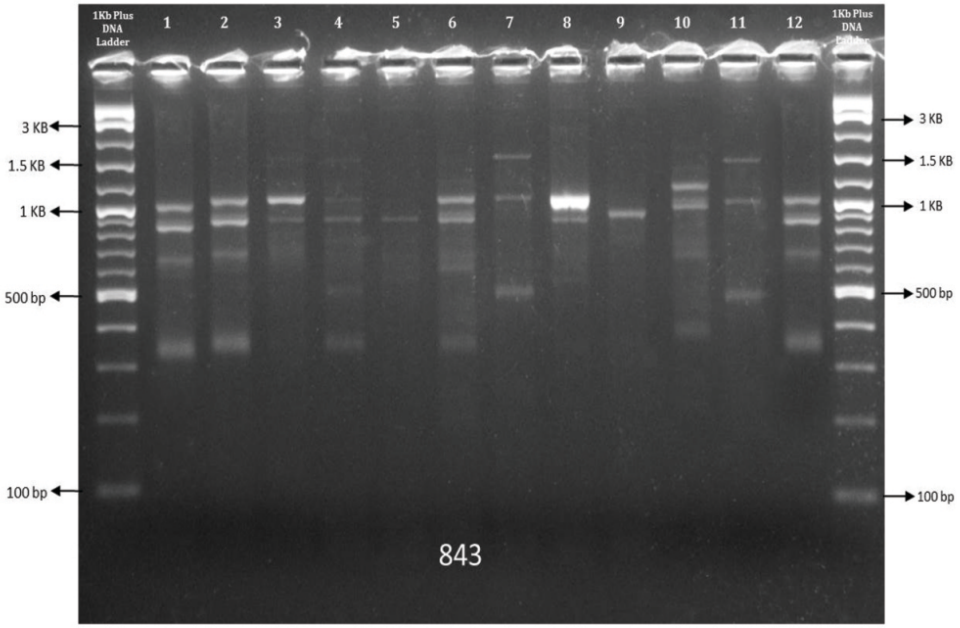


Plate 19. Amplification profiles of the DNA of twelve *Cattleya* hybrids using ISSR primer 843

Table 30. Representation of banding pattern of the DNA of twelve C*attleya* hybrids using the primer 862

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
0	1	1	1	1	1	1	1	1	1	1	1
0	1	0	0	0	0	0	0	0	1	1	1
0	1	1	1	0	1	1	1	1	1	1	1
0	1	0	0	0	1	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1

Table 31. Representation of banding pattern of the DNA of twelve C*attleya* hybrids using the primer 885

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
1	0	0	1	0	0	0	1	0	0	0	0
0	1	0	0	1	0	1	0	1	0	0	0
1	0	1	1	1	0	0	1	0	0	0	0
0	0	0	0	0	0	0	1	0	0	1	1
1	0	1	1	1	1	1	1	0	1	1	1
0	1	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	1	0	1	1	0	0
0	0	0	0	0	0	1	0	1	1	0	0

4.5.1. Cluster analysis and genetic relationship of *Cattleya* genotypes based on ISSR primers

The similarity matrices was developed using the data obtained by ISSR primers in twelve *Cattleya* genotypes using "SIMQUAL" sub- programme ofzsoftware NTSYS-pc. The allelic diversity was used to produce a dendrogram using 'cluster tree analysis' sub programme of same software which revealed the genetic relationship between the *Cattleya* genotypes.

Dendrogram obtained from 10 ISSR primers (polymorphic) data using "SIMQUAL" sub- programme of software NTSYS-pc showed 4 major clusters from the 12 genotypes at similarity coefficient 0.65 as shown in Table 32. and Fig 6. The similarity coefficient ranged from 0.57 to 0.90. The cluster 1 consists of 2 genotypes (*C.* Chocolate Drops Volcano Queen and *C.* Aurantiaca x Netrasiri Beauty), cluster 2 consists of the genotype Rlc. Irene dopkin, cluster 3 consists of the genotypes Rth.Burana Beauty and cluster 4 consists of 8 genotypes (Rlc.Tawan Shine, Rlc.Taichung Beauty, Rlc.Mahina Yahiro, Rlc.Petch Wangnam Khiew, Rlc.Morning Stars, Rlc. Haadyayi Delight x Mary Song, Rlc.Memoria Anna Balmores and *C.* Tipo).

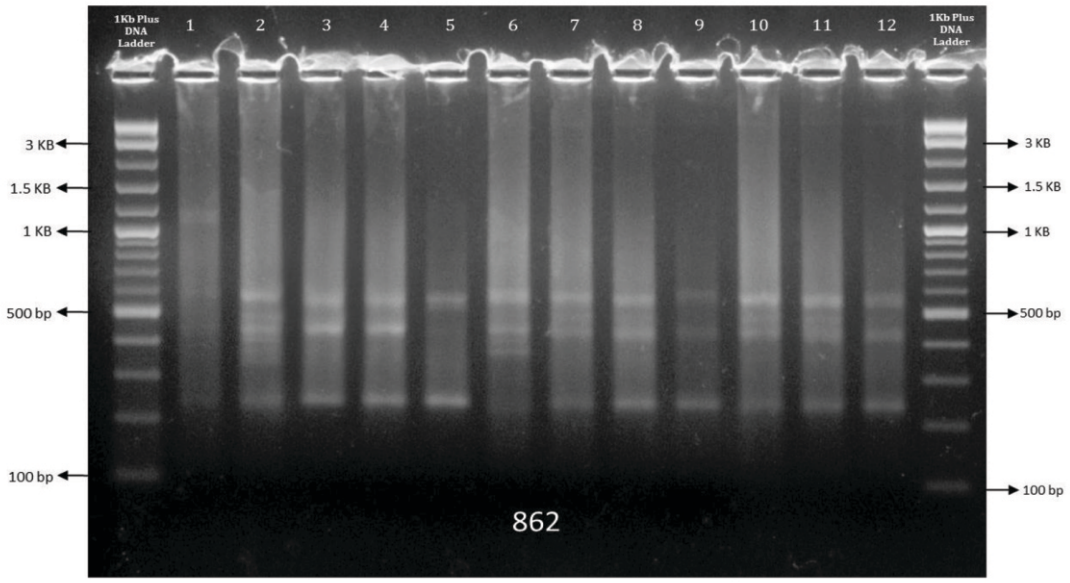


Plate 20. Amplification profiles of the DNA of twelve *Cattleya* hybrids using ISSR primer 862

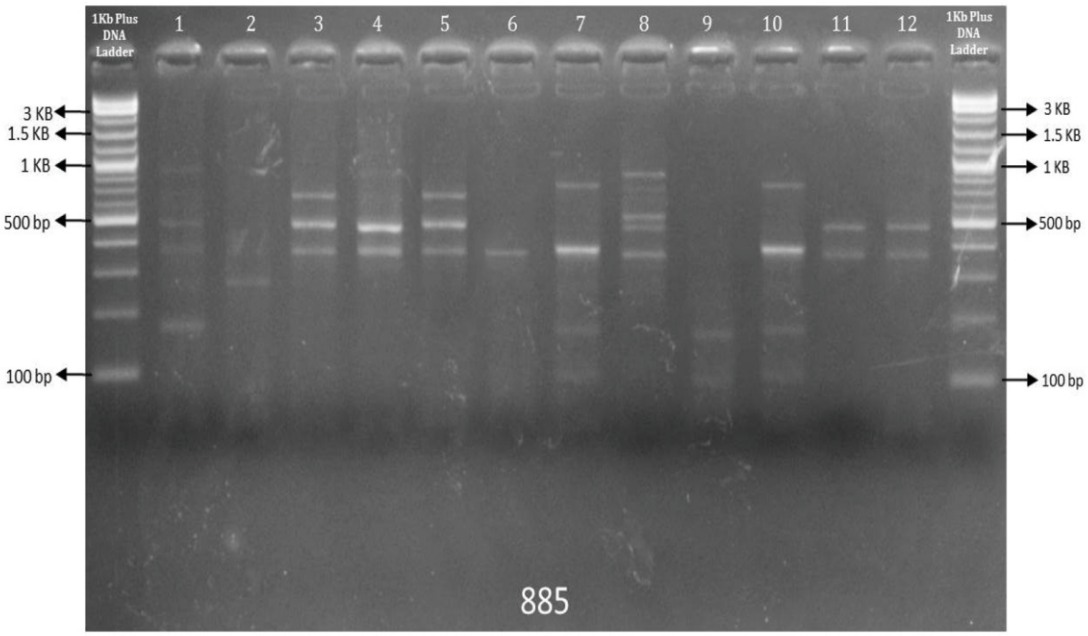


Plate 21. Amplification profiles of the DNA of twelve *Cattleya* hybrids using ISSR primer 885

Table 32. Clustering pattern of the genotypes based on molecular characterization

Major clusters	Genotypes	No. of genotypes
Cluster I	*C*. Chocolate Drops Volcano Queen and *C*.Aurantiaca x Netrasiri Beauty	2
Cluster II	Rlc. Irene dopkin	1
Cluster III	Rth.Burana Beauty	1
Cluster IV	Rlc.Tawan Shine, Rlc.Taichung Beauty, Rlc. Mahina Yahiro, Rlc.Petch Wangnam Khiew, Rlc.Morning Stars, Rlc.Haadyayi Delight x Mary Song, Rlc.Memoria Anna Balmores and *C.* Tipo	8

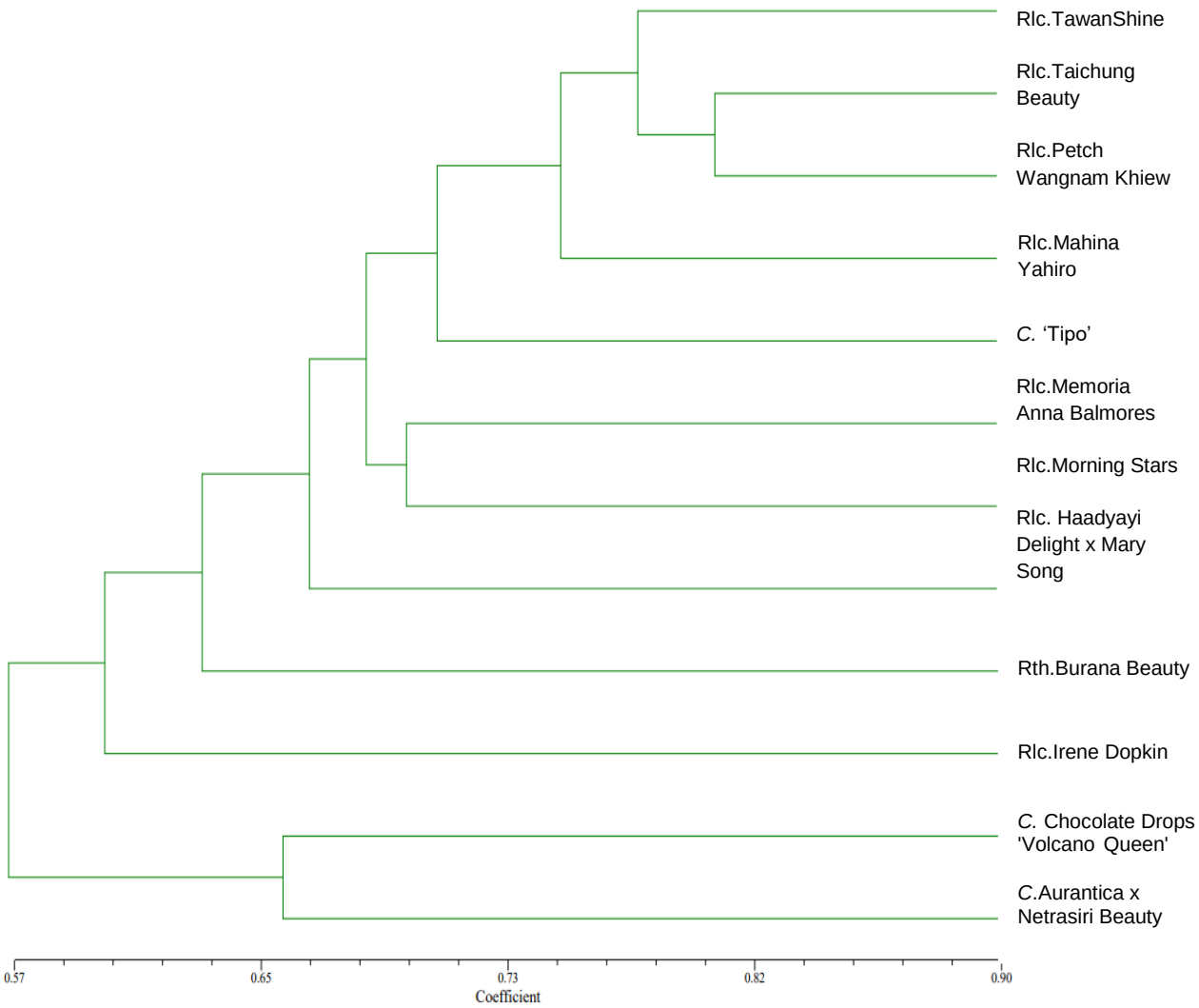


Figure 6. Dendrogram showing relationship among twelve genotypes based on ISSR markers

DISCUSSION

5. DISCUSSION

The present study was carried out to evaluate the morphological, floral and molecular variation among twelve *Cattleya* hybrids and assess their suitability in Kerala conditions.

5.1. EVALUATION OF THE HYBRIDS AND MORPHOLOGICAL CHARACTERIZATION

Orchids are produced all over the world as cut flowers and potted plants because of their great diversity in size, shape, colour, and decoration. They are among the top ten cut flowers in the world. Regarding their development patterns and floral characteristics, they show a great deal of natural diversity. Orchids of the genus *Cattleya* are the most notable members of the Orchidaceae family. Acquisition of new germplasm and their evaluation is essential in crop improvement programmes. The success of any breeding programme depends on the effective use of the genetic variability present for various traits in the germplasm. When choosing the parents for a successful hybridization programme, it is crucial to have a thorough awareness of the vegetative and floral characteristics. This will help to better comprehend the diversity found in *Cattleya*. When choosing parents for a hybridization programme, general health and excellent vegetative qualities are crucial considerations. Mc Donald (1991) reported that robust hybrids produce bigger, better blooms and are more floriferous with more substantial flowers, highlighting the significance of vegetative vigour in orchids.

Differential responses were exhibited for the vegetative parameters among the *Cattleya* hybrids studied except for plant height. The genotype Chocolate Drops Volcano Queen (15.7 cm) recorded maximum plant height while the genotype Rlc. Memoria Anna Balmores (4.5 cm) recorded the lowest value. The highest value for length of the largest leaf was noticed in the genotype Rlc.Taichung Beauty (20.29 cm) while the lowest was reported in Rth.Burana Beauty (10.07 cm). For width of the largest leaf, the highest mean value was observed for the genotype Rlc.Petch Wangnam Khiew (5.23 cm) whereas the lowest value was exhibited by the genotype *C.* Aurantica x Netrasiri Beauty (2.81cm). Rlc.Petch Wangnam Khiew had the widest leaf, which in turn showed good floral characteristics. Sobhana (2000) and Lekharani (2002) noted significant variation in leaf length and width in a study of variability

using *Dendrobium* orchids. Roychowdhury *et al*. (2004) also observed variation in the vegetative characteristics of orchids.

A wide range of variation was observed among the genotypes for characters such as the number of shoots per plant, number of leaves per pseudobulb, length and width of the largest leaf, and number of days from leaf emergence to maturity. This was in accordance with the findings of Anand *et al.* (2013). He also reported that vegetative growth characters in terms of plant height, number of leaves, leaf length, leaf width and habitat play a key role in ultimately deciding crop yield. The fact that the genotypes included in the study are higher order monogeneric, bigeneric, or multigeneric hybrids may be the source of the vast range of variation. This was in line with research findings of Rahi (2017).

Hurst (1898) reported that higher order multigeneric hybrids in orchids displayed a broader range of character variation than lower order primary hybrids. Less variance was detected between genotypes for traits like plant height and the number of leaves per plant. This might be attributed to the fact that the majority of the parental genotypes employed in the study are elite hybrids with standardized vegetative characters but improved floral traits in accordance with consumer preferences.

Cattleyas are characterized by flowers with three sepals and three well-defined petals, one of them modified, known as the labellum. A clear understanding on the floral biology is of great importance in orchid breeding due to its structural and functional complications. The important floral characters like flower size, pedicel size, flowering period, number of spikes per plant and spike/stalk length, flower longevity and vase life exhibited significant variation which was in conformity with the reports of Anand *et al.* (2013) and De *et al.* (2019).

Out of the twelve *Cattleya* hybrids, flowering was observed in ten hybrids such as Rlc. Irene Dopkin, Rth.Burana Beauty, Rlc.Memoria Anna Balmores, *C.* Tipo and Rlc. Haadyayi Delight x Mary Song, *C.*Aurantica x Netrasiri Beauty, Rlc.Taichung Beauty, Rlc.Petch Wangnam Khiew, Rlc.Mahina Yahiro and Rlc.Morning Stars during the experimental period. This might be due to the result of the prolonged juvenile stage, which lasts between three and seven years, or due to

seasonal effects. *Cattleya* hybrids were reported to bloom only once a year as reported by Cardoso *et al*. (2016). In the present study, *C*. Tipo flowered thrice a year (25 flowers per plant per year) compared to the other hybrids. Hence, hybrids that flower more than once a year can be utilized for the crop improvement programmes of *Cattleya*.

For floral characteristics including the number of flowers per inflorescence and the length and width of blooms, high variability was seen. In Rlc.Memoria Anna Balmores, Rlc.Petch Wangnam Khiew, Rlc.Mahina Yahiro and Rlc.Morning Stars flower were single type whereas, flowers were produced in inflorescence in *C.* Tipo, *C.*Aurantica x Netrasiri Beauty, Rth.Burana Beauty, Haadyayi Delight x Mary Song and Rlc. Irene Dopkin. The number of flowers per inflorescence, the length and width of blooms are important floral attributes in orchid breeding as suggested by Cardoso *et al*.(2016).

For the characters, peduncle length and days from emergence to opening of inflorescence, the highest value was observed for the genotype *C.*Aurantiaca x Netrasiri Beauty (9.66 cm and 15.33 days respectively) and lowest value for the first character was noticed in the genotypes Rth.Burana Beauty (8.83 cm) and others were in Rlc.Mahina Yahiro (5.43 cm and 6 days) respectively. Plant height is a genetic character influenced by the environment. The highest value for pseudobulb breadth at flowering, was observed for the genotype Rlc.Mahina Yahiro (2.96 cm) and lowest value was exhibited by the genotype *C.*Aurantica x Netrasiri Beauty (1 cm).This was in accordance with the study of Neto and Vieira (2011) and Anand *et al*.(2013).

The characters, inflorescence number per pseudobulb (3.66 numbers) and flower number per peduncle (3.33 numbers) was highest for the genotype *C.* Tipo and the lowest value (one) was obtained for all other genotypes except in *C.*Aurantica x Netrasiri Beauty and Rth.Burana Beauty. High variation in flower count was found among Dendrobium genotypes, according to Sobhana (2000) and Lekharani (2002).

Length of flower pedicel (7.43 cm), flower length (7.33 cm) and inflorescence length (13.83 cm) was observed highest for the genotype Rlc.Morning Stars and lowest value was noticed in the genotypes Rth.Burana Beauty (4.16 cm), *C.*Aurantica x Netrasiri Beauty (5 cm) and Rlc.Mahina Yahiro 10.5 cm). The length and width of

the flower is an important character of display value. For the characters, flower width in front view (12.76 cm), petal length (6.9 cm), petal width (6.73 cm), lip length (7.7 cm), lip width (6.86 cm) and vase life of flowers (6 days), the highest value was observed for the genotype Rlc.Petch Wangnam Khiew and lowest value was recorded for the genotype Rth.Burana Beauty for the characters flower width in front view (4.83 cm), petal length (2.7cm) and lip width (1.93 cm) and for other characters, lowest was for the genotype *C.*Aurantica x Netrasiri Beauty. Flower number per peduncle was the highest (3.33 numbers) and vase life (2.83 days) was lowest for the genotype *C.* Tipo. The genotypes Rlc.Morning Stars and Rlc.Memoria Anna Balmores (26.33 days) recorded the highest value for flower longevity on the plant and it was lowest for the genotype *C.* Tipo (10.33 days). Large and showy flowers are attractive features of cattleya. Longest longevity of the flowers in plant is an ideal character for orchids. Being a potted orchid, cultivars with maximum longevity will get more customer preference in the market. Neto and Vieira (2011) found that in wild and bred *C. intermedia* accessions, the genetic variability including flower shape and number, precocity, texture and the thickness of floral parts as the result of plant domestication and the use of superior genotypes in the crop improvement programme. This was in accordance with the study of Pachuau *et al*.(2021) in *Dendrobium.*

5.2. QUALITATIVE AND PSEUDO QUALITATIVE CHARACTERS

The genotypes used in the present study were evaluated with respect to qualitative and pseudo qualitative characters. The pseudo qualitative characters like nature of pseudo bulb, leaf shape and leaf colour pattern were found similar in all the genotypes. Other parameters differed significantly among the ten *Cattleya* hybrids flowered. It was reported by De *et al*. (2014) that *Cattleya* hybrids were assessed using common descriptors like quantitative, qualitative and pseudo qualitative characters in order to create Distinctiveness, Uniformity and Stability (DUS) test guidelines, which were then used to categorize hybrids. In the present study it was observed that different genotypes of *Cattleya* had different blooming seasons, while some, like *C.* Tipo, produced blooms thrice a year. This was in accordance with the study of Cardoso *et al*. (2016) which described two *Cattleya* flowering types, one of

which produced flowers once a year and the other of which produced flowers multiple times a year.

5.3. CLUSTER ANALYSIS OF MORPHOLOGICAL AND FLORAL, QUALITATIVE AND QUANTITATIVE CHARACTERS

Cluster analysis is a biometrical tool used in grouping of genotypes in a manner that genotypes which belong to the same group are much more similarly compared to those of other groups or clusters. This helps to reveal the relationship between genotypes from varied origins and consequently extremely thorough identification of genotypes with valuable traits in the various clusters for hybridization.

The genotypes were subjected to cluster analysis using Average method and Euclidean Distance measures and clustered into three groups based on morphological quantitative characters. The cluster 1 consisted of three genotypes (Rth.Burana Beauty, Rlc. Haadyayi Delight x Mary Song and *C*.Chocolate Drops Volcano Queen), cluster 2 consisted of the genotypes Rlc.Taichung Beauty and Rlc.Morning Stars and cluster 3 consisted of seven genotypes (Rlc.Tawan Shine, Rlc.Mahina Yahiro, Rlc.Irene Dopkin, *C*.Aurantiaca x Netrasiri Beauty, Rlc.Petch Wangnam Khiew, Rlc.Memoria Anna Balmores and *C.* Tipo). Cluster-2 showed the highest cluster mean for number of shoots per plant. The highest cluster mean for plant height, length and width of the largest leaf and number of days from emergence to maturity of leaves was registered by C-3.

The genotypes were clustered into three groups based on floral quantitative characters. The cluster 1 consisted of four genotypes (Rlc.Petch Wangnam Khiew, Rlc.Memoria Anna Balmores, Rlc.Morning Stars and Rlc. Haadyayi Delight x Mary Song), cluster 2 consisted of the genotypes Rth.Burana Beauty and *C*.Aurantiaca x Netrasiri Beauty and cluster 3 consisted of four genotypes (Rlc.Taichung Beauty, Rlc.Mahina Yahiro, Rlc.Irene Dopkin and *C*.Tipo). Cluster-1 showed the highest cluster mean for inflorescence number per pseudobulb, peduncle length, flower number per peduncle and days from emergence to opening of inflorescence. The highest cluster mean for pseudobulb breadth at the broadest part, peduncle sheath length and lateral sepal length was registered by C-2. C-3 recorded the highest mean

for plant height from base to tip of the flowering shoot, pseudobulb length at flowering, inflorescence and flower length, dorsal sepal length, petal length, dorsal and lateral sepal width, lip and column length, petal and lip width, length of flower pedicel, flower longevity on the plant and vase life. Rlc.Mahina Yahiro, Rlc.Irene Dopkin and *C.*Tipo were the genotypes grouped in cluster C-3 based on both morphological and floral quantitative characters.

The genotype divergence increases in proportion to the distance between the clusters. Genotypes are less divergent inside a cluster than they are between clusters. In the genotypes, the cluster means revealed that C-3 was potential contributor of plant height from base to tip of the flowering shoot, pseudobulb length at flowering, inflorescence and flower length, dorsal sepal length, petal length, dorsal and lateral sepal width, lip and column length, petal and lip width, length of flower pedicel, flower longevity on the plant and vase life. This cluster can be used for further selection for improvement in quality and quantity of flower yield in *Cattleya.*

The intracluster distance was lowest for C-3 (1.38 for Rlc.Taichung Beauty, Rlc.Mahina Yahiro, Rlc.Irene Dopkin and *C.*Tipo) and largest for C-2 (3.64 for Rth.Burana Beauty and *C.*Aurantiaca x Netrasiri Beauty). C-2 and C-3 had the maximum intercluster distance (6.16), whereas C-1 and C-3 had the lowest value (4.17). Choosing parents from diverse clusters will be very effective in hybridization. Maximum divergence was observed for C-2 and C-3. So selection of parents from C-2 (Rth.Burana Beauty and *C.*Aurantiaca x Netrasiri Beauty) and C-3 (Rlc.Taichung Beauty, Rlc.Mahina Yahiro, Rlc.Irene Dopkin and *C.*Tipo) will be effective in crop improvement programme of *Cattleya*.

5.4. COEFFICIENT OF VARIATION

The phenotypic, genotypic and environmental variance and the phenotypic and genotypic coefficients of variation for all the characters were studied.

Phenotypic coefficient of variation (PCV) values ranged between 10.57 per cent to 68.97 per cent for the floral characters. The highest PCV was recorded for inflorescence number per pseudobulb while lowest for inflorescence length. Genotypic coefficient of variation (GCV) values ranged between 10.27 per cent to

58.51 per cent. The highest genotypic coefficient of variation (GCV) was also recorded for inflorescence number per pseudobulb and lowest for inflorescence length. The highest PCV and GCV was observed for number of shoots per plant (45.64 per cent and 31.88 per cent) and minimum for plant height (21.36 per cent and 10.43 per cent) among morphological characters**.**

Phenotypic coefficient of variation was higher than genotypic coefficient of variation for all the characters indicating the influence of environment. Maximum variability both at the phenotypic and genotypic level was observed for the inflorescence number per pseudobulb followed by flower number per peduncle among floral characters. High GCV obtained for this character is important in orchids since it determines the market value of the spike. This was in accordance with Sobhana (2000).

In line with the findings of Rahi (2017) and De *et al*.(2019), the length of the blossom shown a considerable variation as well as high genotypic and phenotypic coefficients of variation among the genotypes employed in the study.

5.5. HERITABILITY AND GENETIC ADVANCE

Allard (1960) has pointed out that genotypic coefficient along with heritability estimates gives better idea about the amount of genetic advance in the next generation. Estimation of GCV alone is not enough to determine the amount of heritable variation. However, high heritability does not necessarily mean a high genetic advance for a particular character. Johnson *et al*. (1955) reported that heritable variation could be estimated with greater degree of accuracy, if heritability is coupled with genetic advance. The assessment of the heritability along with genetic advance was suggested to aid effective selection based on phenotypic performance.

In the present study heritability is of low to high magnitude. All the characters exhibited high genetic advance except for plant height. The highest estimate of genetic advance was observed for inflorescence number per pseudobulb (102.26%) followed by petal width (87.14%). All the floral characters had high heritability especially petal length, petal width, dorsal sepal length and flower length.The importance of floret size and its high heritability suggest that there is scope for improvement through selection.

A high heritability estimate for bloom size was also reported by Rehman *et al*. (1993) and Sobhana (2000).

High heritability along with high genetic advance as percentage of mean in characters suggested that the genotypic variations for such characters are probably due to high additive genetic effects whereas environmental effects had least effect on such characters (Johnson *et al*., 1955). High heritability combined with genetic advance was exhibited by petal width, flower length, sepal length and width and flower longevity on the plant. Thus it may be inferred that selection based on these characters would be more effective and are generally controlled by additive gene action and hence amenable to genetic improvement through selection (Panse, 1957).

5.6. CORRELATION STUDIES

The correlation studies measure the degree of association and reciprocal link between the numerous qualities under study. A positive genotypic correlation between pairs of characters indicated that an improvement in one character will improve the other character also. That will enable the breeder to select characters responsive to selection.

In this study, plant height had significant positive phenotypic correlation with length of the largest leaf, petal length and width. Flower number per peduncle showed a significant negative correlation with flower length and width. The length of flower had significant positive correlation with width of the flower, which is an important attribute of cut flower selection. This was reported by Sobhana (2000) in her findings. Number of leaves per pseudobulb showed negative correlation with good floral attributes. So plants with unifoliate leaves produced larger flowers and took minimum days from emergence to maturity of inflorescence (De *et al*., 2014)**.**

Important floral attributes like flower width, petal length, sepal length, lip length and width were influenced by the length and width of the largest leaf. So the selection of plants with higher length and width of leaves can result in larger inflorescences. It was in accordance with the findings of Sobhana (2000) that there was positive correlation with flowers per spike and number of leaves in orchids.

5.7. MOLECULAR CHARACTERIZATION USING ISSR MARKERS

The study of genetic diversity and variability among various plant species is thought to benefit greatly from the use of molecular markers. In order to identify prospective genotypes for the creation of novel and superior hybrids, molecular markers offer an alternative approach. Molecular markers are used in plant breeding programs as they can rapidly assess population variability without environment interference, with advantages over morphological characters, in terms of discrimination, confidence and costs (Assis *et al*., 2003).

Molecular characterization of twelve *Cattleya* hybrids were carried out using ten ISSR primers. DNA yield varied from 0.776 ng/µl to 27 ng/µl. Total number of amplicons obtained were 97 of which 96 were polymorphic and one was monomorphic.

Dendrogram obtained from the ten ISSR primers showed four major clusters from the 12 genotypes at similarity coefficient of 0.65. The similarity coefficient ranged from 0.57 to 0.90. The cluster 1 consisted of two genotypes (*C.* Chocolate Drops Volcano Queen and *C.* Aurantiaca x Netrasiri Beauty), cluster 2 consisted of the genotype Rlc. Irene dopkin, cluster 3 consisted of the genotype Rth.Burana Beauty and cluster 4 consisted of eight genotypes (Rlc.Tawan Shine, Rlc.Taichung Beauty, Rlc.Mahina Yahiro, Rlc.Petch Wangnam Khiew, Rlc.Morning Stars, Rlc.Haadyayi Delight x Mary Song, Rlc.Memoria Anna Balmores and *C*. Tipo). There are a number of variables that can affect this genetic diversity variance, with the breeding system having a particularly large impact (Nybom and Bartish, 2000). *Cattleya* is a crucial genus employed in the creation of artificial hybrids, as evidenced by the short genetic distances found in its genome.

The clusters formed by morphological and molecular characterization grouped the genotypes *C.* Chocolate Drops Volcano Queen and *C.*Aurantiaca x Netrasiri Beauty in the same cluster. All genotypes, with the exception of two, were part of another similar cluster. Both clusters showed very slight differences (Neto and Vieira, 2011).

Morphological characterization of the twelve *Cattleya* hybrids revealed that *Rhyncholaeliocattleya* Memoria Anna Balmores, *Rhyncholaeliocattleya* Morning Stars and *Rhyncholaeliocattleya* Petch Wangnam Khiew were good potted orchids in terms of flower size and flower longevity and vase life. *Cattleya* Tipo and *Cattleya* Aurantiaca x Netrasiri Beauty were found to be good bloomers based on the number of flowers produced per plant per year. The variability exhibited by the hybrids in the morphological and molecular characterization can be exploited for further crop improvement programme to produce novel *Cattleya* varieties suitable for Kerala.

SUMMARY

6. SUMMARY

The research programme entitled 'Morphological and molecular characterization of *Cattleya* hybrids' was carried out in the Department of Floriculture and Landscaping, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2021-2022 to evaluate the performance of twelve *Cattleya* hybrids based on growth and flowering attributes and to characterize them using morphological descriptors and molecular markers.

The analysis of variance revealed significant differences in 12 genotypes for all of the studied morphological characters, with the exception of plant height, such as the number of shoots per plant, number of leaves per pseudobulb, length and width of the largest leaf, and number of days from leaf emergence to maturity.

Out of the 12 *Cattleya* hybrids, flowering was observed in ten hybrids *viz*., Rth.Burana Beauty, Rlc.Taichung Beauty, Rlc.Mahina Yahiro, Rlc.Irene Dopkin, *C*.Aurantiaca x Netrasiri Beauty, Rlc.Petch Wangnam Khiew, Rlc.Morning Stars, Rlc. Haadyayi Delight x Mary Song, Rlc.Memoria Anna Balmores and *C*.Tipo during the experiment.

Significant varietal differences were observed among the ten genotypes with respect to the floral characters studied.

Variance component analysis revealed that highest PCV and GCV was observed for number of shoots per plant and minimum for plant height among morphological characters.

Maximum variability both at the phenotypic and genotypic level was observed for the inflorescence number per pseudobulb followed by flower number per peduncle among floral characters

High heritability values were recorded for the floral characters *viz.*, length and width of petal and dorsal sepal width followed by flower length.

High heritability with genetic advance was observed for petal and sepal width followed by inflorescence number per pseudobulb.

Flower width, length and width of petal, sepal and lip had significant positive genotypic correlation with length and width of the largest leaf**.** Flower number per peduncle exhibited positive correlation with plant height and number of leaves per pseudobulb showed negative correlation with flower width, petal, sepal and lip length and width**.**

Plant height had significant positive phenotypic correlation with length and width of the petal and length of the largest leaf. Flower number per peduncle showed a significant negative correlation with flower length and width. The length of flower had significant positive correlation with width of the flower, which is an important attribute of cut flower selection.

Low values of environmental correlation coefficient due to environmental effect were estimated for most of the characters studied.

The genetic diversity among the genotypes was studied using 'Average method' and 'Euclidean Distance measures'. The genotypes were clustered into three groups based on morphological and floral quantitative characters.

Maximum inter cluster distance was observed between the clusters C2 and C3 and minimum distance between C1 and C3. So selection of parents from the clusters C2 and C3 will be very effective in hybridization.

Four hybrids viz., Rlc.Morning Stars, Rlc. Haadyayi Delight x Mary Song, Rlc.Memoria Anna Balmores and *C.* Tipo recorded high scores based on scoring given by DUS guidelines.

Molecular characterization of 12 *Cattleya* genotypes were carried out using ISSR primers.

DNA yield varied from 0.776 ng/µl to 27 ng/µl. Out of the 97 amplicons obtained, 96 were polymorphic and one was monomorphic.The twelve genotypes were divided into four clusters at similarity coefficient of 0.65.

The clusters formed by morphological and molecular characterization grouped the genotypes *C***.** Chocolate Drops Volcano Queen and *C.*Aurantica x Netrasiri Beauty

in the same cluster. The genotypes in the various clusters were nearly identical in terms of morphology and genetics.

High petal and sepal width was observed for the genotype Rlc.Petch Wangnam Khiew. *C*. Tipo was floriferous (25 flowers per plant per year) followed by *C*. Aurantiaca x Netrasiri Beauty (20 flowers per plant per year) compared to the other hybrids. All other hybrids exhibited seasonal variation in flower production. So Rlc.Petch Wangnam Khiew, *C*. Tipo and *C*. Aurantiaca x Netrasiri Beauty may be selected as parental genotypes with good floral attributes.

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7. REFERENCES

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ABSTRACT

"MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *CATTLEYA* HYBRIDS"

By

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ABSTRACT

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ABSTRACT

The research programme entitled 'Morphological and molecular characterization of *Cattleya* hybrids' was carried out in the Department of Floriculture and Landscaping, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2021-2022 to evaluate the performance of twelve *Cattleya* hybrids based on growth and flowering attributes and to characterize them using morphological descriptors and molecular markers.

The analysis of variance revealed significant differences in 12 genotypes for all of the studied morphological characters, with the exception of plant height, such as the number of shoots per plant, number of leaves per pseudobulb, length and width of the largest leaf, and number of days from leaf emergence to maturity.

Out of the 12 *Cattleya* hybrids, flowering was observed in ten hybrids *viz*., Rth.Burana Beauty, Rlc.Taichung Beauty, Rlc.Mahina Yahiro, Rlc.Irene Dopkin, *C.*Aurantiaca x Netrasiri Beauty, Rlc.Petch Wangnam Khiew, Rlc.Morning Stars, Rlc. Haadyayi Delight x Mary Song, Rlc.Memoria Anna Balmores and *C.*Tipo during the experiment.

Significant varietal differences were observed among the ten genotypes with respect to the floral characters studied.

Variance component analysis revealed that highest PCV and GCV was observed for number of shoots per plant and minimum for plant height among morphological characters.

Maximum variability both at the phenotypic and genotypic level was observed for the inflorescence number per pseudobulb followed by flower number per peduncle among floral characters

High heritability values were recorded for the floral characters *viz.*, length and width of petal and dorsal sepal width followed by flower length.

High heritability with genetic advance was observed for petal and sepal width followed by inflorescence number per pseudobulb.

Flower width, length and width of petal, sepal and lip had significant positive genotypic correlation with length and width of the largest leaf**.** Flower number per peduncle exhibited positive correlation with plant height and number of leaves per pseudobulb showed negative correlation with flower width, petal, sepal and lip length and width**.**

Plant height had significant positive phenotypic correlation with length and width of the petal and length of the largest leaf. Flower number per peduncle showed a significant negative correlation with flower length and width. The length of flower had significant positive correlation with width of the flower, which is an important attribute of cut flower selection.

Low values of environmental correlation coefficient due to environmental effect were estimated for most of the characters studied.

The genetic diversity among the genotypes was studied using 'Average method' and 'Euclidean Distance measures'. The genotypes were clustered into three groups based on morphological and floral quantitative characters.

Maximum inter cluster distance was observed between the clusters C2 and C3 and minimum distance between C1 and C3. So selection of parents from the clusters C2 and C3 will be very effective in hybridization.

Four hybrids viz., Rlc.Morning Stars, Rlc. Haadyayi Delight x Mary Song, Rlc.Memoria Anna Balmores and *C.* Tipo recorded high scores based on scoring given by DUS guidelines.

Molecular characterization of 12 *Cattleya* genotypes were carried out using ISSR primers.

DNA yield varied from 0.776 ng/µl to 27 ng/µl. Out of the 97 amplicons obtained, 96 were polymorphic and one was monomorphic.The twelve genotypes were divided into four clusters at similarity coefficient of 0.65.

The clusters formed by morphological and molecular characterization grouped the genotypes *C***.** Chocolate Drops Volcano Queen and *C.*Aurantiaca x Netrasiri

Beauty in the same cluster. The genotypes in the various clusters were nearly identical in terms of morphology and genetics.

High petal and sepal width which are good floral attributes was observed for the genotype Rlc.Petch Wangnam Khiew. *C.* Tipo was floriferous (25 flowers per plant per year) followed by *C.* Aurantiaca x Netrasiri Beauty (20 flowers per plant per year) compared to the other hybrids. All other hybrids exhibited seasonal variation in flower production. So Rlc.Petch Wangnam Khiew, *C.* Tipo and *C.* Aurantiaca x Netrasiri Beauty may be selected as parental genotypes with good floral attributes.

സംഗ്രഹം

‘കാറ്റ്ലിയ സങ്കരയിനം ഓർക്കിഡുകളിലെ രൂപശാസ്ത്രപരവും ജനിതകവുമായ വൈവിധ്യപഠനം’ എന്ന ഗവേഷണ പരിപാടി വെള്ളായണി കാർഷിക കോളേജിലെ ഫ്ളോറികൾച്ചർ വിഭാഗത്തിൽ 2021-2022 കാലയളവിൽ നടത്തുകയുണ്ടായി. കാറ്റ്ലിയ സങ്കരയിനങ്ങളിലെ രൂപശാസ്ത്രപരമായ വൈവിധ്യത്തിന്റെ അടിസ്ഥാനത്തിൽ പ്രകടനം വിലയിരുത്തുക, തന്മാത്ര പ്രൈമറുകൾ ഉപയോഗിച്ച് ജനിതക വ്യതിയാനം വിലയിരുത്തുക എന്നിവയായിരുന്നു പഠനത്തിന്റെ ലക്ഷ്യങ്ങൾ.

പഠനത്തിന്റെ ആദ്യഘട്ടത്തിൽ ചെടികൾ തമ്മിൽ രൂപശാസ്ത്രപരവും പുഷ്പപരവുമായ സ്വഭാവങ്ങളിൽ വ്യത്യാസങ്ങൾ കാണിച്ചു. വേരിയൻസ് ഘടക വിശകലനം നടത്തിയതിൽ ചെടിയിലെ ചിനപ്പൊട്ടലിന്റെ എണ്ണത്തിന ഏറ്റവും ഉയർന്ന PCV യും GCV യും, ചെടികളുടെ ഉയരത്തിന ഏറ്റവും കുറഞ്ഞ PCV യും GCV യും രേഖപ്പെടുത്തി. ഫിനാടൈപ്പിക്ക് തലത്തിലും ജനിതക തലത്തിലും പരമാവധി വൈവിധ്യം രേഖപ്പെടുത്തിയത് കപടകാണ്ഡത്തിലെ പൂങ്കുലകളുടെ എണ്ണത്തിലും, പൂക്കളുടെ എണ്ണത്തിലുമാണ്. ദളത്തിന്റെ നീളം, വീതി, മുകളിലത്തെ വിദളങ്ങളുടെ വീതി തുടർന്ന് പൂവിന്റെ നീളം എന്നിവയിലാണ ഉയർന്ന ജനിതക മുന്നേറ്റം കണ്ടെത്തിയത്. ഓരോ കപടകാണ്ഡത്തിലെ പൂങ്കുലകളുടെ എണ്ണത്തിലും തുടർന്ന് ഇതളുകൾ, വിദളങ്ങൾ എന്നിവയുടെ വീതിയിലും ഉയർന്ന ജനിതക മുന്നേറ്റം രേഖപ്പെടുത്തി. ഓരോ കപട കാണ്ഡത്തിലെയും പൂങ്കുലകളുടെ എണ്ണത്തിലും ദളങ്ങളുടെയും വിദളങ്ങളുടെയും വീതിയിലും ജനിതക മുന്നേറ്റത്തോട് കൂടിയ ഉയർന്ന പാരമ്പര്യം രേഖപ്പെടുത്തി.

പൂക്കളുടെ വീതി, നീളം, ദളങ്ങൾ, ലിപ്പുകൾ എന്നിവയും, ഏറ്റവും വലിയ ഇലയുടെ നീളവും വീതിയും തമ്മിൽ ഉയർന്ന പോസിറ്റീവ് ജനിതകബന്ധം ഉണ്ടായിരുന്നു. ഒരു പൂങ്കുലത്തണ്ടിലെ പൂക്കളുടെ എണ്ണവും ചെടിയുടെ ഉയരവുമായി പോസിറ്റീവ് ബന്ധവും ഒരു കപട കാണ്ഡത്തിലെ ഇലകളുടെ എണ്ണവും പൂക്കളുടെ വീതി, ദളങ്ങൾ, വിദളങ്ങൾ, ലിപ്പിന്റെ നീളം, വീതി എന്നിവയുമായി നെഗറ്റീവ് ബന്ധവും പ്രകടമായി. ചെടിയുടെ ഉയരത്തിന ദളത്തിന്റെ നീളവും വീതിയും, ഏറ്റവും വലിയ ഇലയുടെ നീളവും തമ്മിൽ ഉയർന്ന പോസിറ്റീവ് ഫിനോടൈപ്പിക്ക് ബന്ധമുണ്ട്. ഓരോ പുങ്കുലത്തണ്ടിലുമുള്ള പൂക്കളുടെ എണ്ണം, പൂവിന്റെ നീളവും വീതിയുമായി ഉയർന്ന നെഗറ്റീവ് ബന്ധം കാണിച്ചു. പുഷ്പത്തിന്റെ നീളത്തിന പുഷ്പത്തിന്റെ വീതിയുമായി ഉയർന്ന പോസിറ്റീവ് ബന്ധം കാണപ്പെട്ടു. ഇത് മുറിച്ച പൂക്കളുടെ തിരഞ്ഞെടുപ്പിന ഒരു പ്രധാന ഘടകമാണ്.

പത്ത് ISSR പ്രൈമറുകൾ ഉപയോഗിച്ചാണ് പന്ത്രണ്ട് കാറ്റലിയ സങ്കരയിനങ്ങളുടെ തന്മാത്രാ സ്വഭാവം വിലയിരുത്തിയത്. DNA യുടെ അളവ് 0.776 ng/µl മുതൽ 27ng/µl വരെ വ്യത്യാസപ്പെടുന്നു. ലഭിച്ച 97 ആംപ്ലിക്കോണുകളിൽ 36 എണ്ണം പോളിമോർഫിക്കും ഒന്ന് മോണോമോർഫിക്കും ആയിരുന്നു. രൂപശാസ്ത്രപരമായ പ്രതീകങ്ങളും തന്മാത്രാ പ്രൈമറുകളും അടിസ്ഥാനമാക്കിയുള്ള ക്ളസ്റ്ററിങ് നടത്തി, പന്ത്രണ്ട് കാറ്റലിയ സങ്കരയിന

ങ്ങളെ യഥാക്രമം മുന്ന്, നാല് ക്ളസ്റ്ററുകളായി തിരിച്ചിരിക്കുന്നു. രൂപശാസ്ത്രപരമായ ക്ള സ്റ്ററുകളിൽ പരമാവധി ജനികരുപങ്ങൾ C-3 യിലും കുറഞ്ഞത് C-2 വിലും ആയിരുന്നു. C-2, C-3 എന്നീ ക്ളസ്റ്ററുകൾക്കിടയിൽ പരമാവധി ക്ളസ്റ്റർ ദുരവും C-1, C-3 എന്നിവയ്ക്കിടയിൽ ഏറ്റവും കുറഞ്ഞ ദൂരവും നിരീക്ഷിക്കപ്പെട്ടു.

ISSR പ്രൈമർ വിശകലനത്തെ അടിസ്ഥാനമാക്കിയുള്ള ക്ളസ്റ്ററിംഗ് ജനിതകരൂപങ്ങ ളെ നാല് ക്ളസ്റ്ററുകളായി തരംതിരിച്ചു. കാറ്റ്ലിയ ചോകളേറ്റ് ഡ്രോപ്സ് വോൾക്കാനോ ക്വീൻ, കാറ്റ്ലിയ ഔറാന്റിക്ക x നെട്രാസിരിബ്യൂട്ടി എന്നീ ഇനങ്ങൾ രൂപശാസ്ത്രപരവും ജനി തകപരവുമായി ഒരേ ക്ളസ്റ്ററിൽ കാണപ്പെട്ടു.

പന്ത്രണ്ട് കാറ്റ്ലിയ സങ്കരയിനങ്ങളെ രൂപശാസ്ത്രപരമായ പ്രതീകങ്ങളെ ഉപയോ ഗിച്ച് ക്ളസ്റ്ററിംഗ് നടത്തി. Rth. ബുറാനാ ബ്യൂട്ടി, Rlc. തായ്ച്യുംഗ് ബ്യൂട്ടി, Rlc. മഹിന യാഹിരോ, Rlc. ഐറിൻ ഡോപകീൻ, കാറ്റ്ലിയ ഓറന്റിക്കാ x നെട്രാസിരി ബ്യൂട്ടി, Rlc. പെച്ച് വാൻഗ്നം ക്യൂ, Rlc. മോണിംഗ് സ്റ്റാർസ്, Rlc. ഹാഡിയായ് ഡിലൈറ്റ് x മേരി സോംഗ, Rlc. മെമ്മോറിയ അന്ന ബാൽമോർസ്, കാറ്റ്ലിയ ടിപ്പോ എന്നിങ്ങനെ പത്ത് സങ്കരയിനങ്ങ ളിൽ പരീക്ഷണവേളയിൽ പൂക്കളുണ്ടായി. മറ്റ് സങ്കരയിനങ്ങളെ അപേക്ഷിച്ച് കാറ്റ്ലിയ ടിപ്പോ (ഒരു ചെടിയിൽ നിന്ന് 25 പൂക്കൾ), കാറ്റ്ലിയ ഓറന്റിക്കാ x നെട്രാസിരി ബ്യൂട്ടി, (20 പൂക്കൾ) എന്നിവ കൂടുതൽ പൂക്കൾ ഉത്പാദിപ്പിച്ചു. മറ്റെല്ലാ സങ്കരയിനങ്ങളും പൂക്കളുടെ ഉൽപാദനത്തിൽ കാലാനുസൃതമായ വ്യതിയാനം പ്രകടമായി. രൂപഘടനയിലും തന്മാത്രാ സ്വഭാവത്തിലും സങ്കരയിനങ്ങൾ പ്രകടിപ്പിക്കുന്ന വ്യതിയാനം, കേരളത്തിന്റെ കാലാവ സ്ഥയ്ക്ക് അനുയോജ്യമായ കൂടുതൽ ഉത്പാദനക്ഷമതയുള്ള ഇനങ്ങൾ വികസിപ്പിക്കുന്നതി നായി ഉപയോഗിക്കാം.