EVALUATION OF ELITE CLOVE (*Syzigium aromaticum* (L) Merr. & Perry) ACCESSIONS AND STANDARDIZATION OF POLLINATION TECHNIQUES

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"EVALUATION OF ELITE CLOVE (*Syzigium aromaticum* (L) Merr. & Perry) ACCESSIONS AND STANDARDIZATION OF POLLINATION TECHNIQUES"** 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.

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LIST OF ABBREVIATIONS AND SYMBOLS

&	and
FAO	Food and Agriculture Organization
ha	Hectare
et al.	Co-authors/And others
m	Meter
cm	Centimeter
IUCN	International Union for Conservation of Nature
NBPGR	National Bureau of Plant Genetic Resources
IISR	Indian Institute of Spice Research
IIHR	Indian Institute of Horticultural Research
E-W	East-West
N-S	North-South
AICRP	All India Coordinated Research Project
kg/tree/year	Kg per tree per year
ICAR	Indian Council of Agricultural Research
m^2	Meter square
cm^2	Centimeter square
±	Plus or minus
g	Gram
mg	milligram
m^{-2}	Per meter square
mm	millimeter
gm^{-2}	Gram per meter square
kg	Kilo gram
ml	milliliter
Kg/tree	Kg per tree
Viz.	Namely

sp	Species
PCA	Principal component analysis
MDS	Multi-Dimensional Scaling
2D	2 Dimensional
3D	3 Dimensional
RAPD	Random Amplified Polymorphic DNA
WAT	West African Tall
BRT	Brazil Tall
RIT	Rennell Island Tall
UPGMA	Unweighted pair group method with arithmetic mean
h	hour
am	ante meridieum
pm	post meridieum
µm	Micro meter
SEM	Scanning electron microscope
FCR	Flurochromatic reaction
DAB	diaminobenzidine
ºC	Degree Celsius
%	Percentage
rpm	Rotation per minute
CRD	Completely Randomized Design
IKI	Iodine potassium iodide
SBWF	Single bud weight fresh
SBWD	Single bud weight dry
NOI	Number of inflorescence/m2
LL	Leaf length
LB	Leaf breadth
LA	Leaf area
NOB	Number of flower buds/inflorescence

MBL	Mature bud length
MBD	Mature bud diameter
LOF	Length of flower
BOF	Breadth of flower
LOS	Length of sepal
LOP	Length of petal
FBY	Fresh bud yield
DBY	Dry bud yield
FW	Fruit weight
RFS	Ratio of fruit to seed
SL	Seed length
SB	Seed breadth
SW	Seed weight
CD	Critical difference
DAS	Days after storage
Fig.	Figure
min	Minute
Rb+	Rubidium ion
PIP2	Phosphatidylinositol (4,5)- biphosphate
Ca2+	Calcium ion
miRNA	micro- Ribo Nucleic Acid
RH	Relative humidity
W	watt
ARF17	Auxin response factor17
MAPK	Mitogen-Activated Protein Kinase
CO	Cytochrome oxidase
MDH	Malate dehydrogenase

INTRODUCTION

1. INTRODUCTION

The clove of commerce comprises of unopened flower buds of the clove tree, *Syzygium aromaticum* (L.) Merr. & Perry belonging to the family Myrtaceae. The genus Syzygium encompasses a large number of species of which *Syzygium aromaticum* (L.) Merr. & Perry is the only commercialized aromatic spice. Native to the Moluccas island of Indonesia, clove is one of the most antique and precious spices cultivated mainly in Indonesia, Zanzibar, Madagascar, Pemba and Sri Lanka. The world production of clove was estimated to be 1,67,508 t (FAO, 2018) with Indonesia contributing 73.66 per cent of world production. Clove production in India was only 1,230 t from 2180 ha in 2018-19 and India imported 19,510 t during 2017-18 (Spices Board, 2020).

Clove has been employed for centuries as food preservative and as medicinal plant because of its antioxidant and antimicrobial activities. The major clove growing regions in India are Kanyakumari, Nilgiris of Tamilnadu, Calicut, Kottayam, Quilon and Trivandrum districts of Kerala and South Kanara of Karnataka (Balakrishnamoorthy and Kennedy, 1999). The requirement of clove in world market is very high and has a steady market but the production in India is decreasing. Research work in identification of improved cultivars of clove is also meagre.

Variability in clove is limited due to narrow genetic base and self pollination. Survey and characterization of clove accessions conducted from the clove growing states in Southern part of Kerala and Kanyakumari by the Department of Plantation Crops and Spices, College of Agriculture, Vellayani during the period 2016-2018 has identified twenty clove accessions with variable characters. Variability in tree shape, bud combinations and yield were noticed in the accessions. Twelve elite accessions were identified from these accessions based on growth and yield parameters. These elite accessions need to be evaluated continuously for years to identify the regular bearers. But for creating variability and broadening the genetic base breeding methods such as hybridization need to be attempted. For standardizing hybridization technique observation of floral phenology, biology, method of emasculation, pollen collection and pollen storage need to be standardized. Hence a study was proposed to evaluate the identified elite accessions along with standardization of pollination techniques for hybridization.

In this background the present study entitled "Evaluation of elite clove (*Syzigium aromaticum* (L) Merr. & Perry) accessions and standardization of pollination techniques" was taken up exclusively with the objective to characterize and evaluate elite clove accessions and to standardize pollination techniques for hybridization.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Syzygium aromaticum (L.) Merr & Perry. (Myrtaceae), native of the Moluccas island of Indonesia, is widely grown as an under storey forest crop on the mountain valleys. The tree generally grows to a height of about 3.6 m to 6 m whereas in some places it grows even upto 12-15 m tall. The clove of trade or the commercial clove is the dried, aromatic fully grown unopened flower bud. The clove growing regions in India is limited to parts of Tamil Nadu, Karnataka, Kerala and Andaman and Nicobar Islands. The world production of clove is estimated to be 1,67,508 t (FAO, 2018) with Indonesia contributing 73.66 per cent of world production. Clove production in India is only 1,230 tonnes from 2180 ha in 2018-19 and India has imported 19,510 t during 2017-18 (Spices Board, 2020).

In spite of the clove tree's importance to several countries of the world, little has been published about the yielding behaviour of individual trees. Irregular bearing, staggered flowering and difficulty in harvesting the physiologically mature buds are the limiting factors in clove breeding partly because clove trees do not yield regularly each year, necessitating the collection of data over several years and partly because of the problems of picking an entire crop from a tree which, when mature, is usually more than 12 m high. Cloves develop from the terminal buds of twigs and most of these are produced on the periphery of the tree's canopy, cloves in the top part of tall trees are therefore particularly difficult to harvest, even if ladders are used. All the cloves on a tree seldom mature at the same time and, to harvest the entire crop, trees must be picked several times during the harvesting season (Martin *et al*., 1986).

Survey conducted by the Department of Plantation Crops and Spices, College of Agriculture, Vellayani in different clove growing locations of Southern Kerala and Kanyakumari districts of Kerala from 2016 to 2018 has identified 20 accessions which showed high yield and special characters (Avinash, 2018). In this chapter, the relevant literature on quantitative characters and floral phenology and floral biology studies on clove is reviewed under various captions. Since literature on clove is very limited, relevant research works on other perennial trees, belonging to same genus and tree spices of other genus were also reviewed.

2.1 Characterization and variability in clove

Introductions of clove were few to the country, hence genetic base of the clove was restricted (Ravindran, 1999). Variability in clove is very limited in India due to narrow genetic base. Genus *Syzygium* contains more than 500 species in which clove is one of its species. The important species of *Syzygium* that occur in the Indian subcontinent are *S. aromaticum, S. cumini, S. fruiticosum, S. jambos and S. zeylanicum.* The cultivable *Syzygium aromaticum* is not very close to any of these species (Sasikumar *et al.*, 1999). The East India Company introduced present day clove populations in India. Moreover clove is self-pollinated that resulted in lack of variability. There are no true varieties of clove in India, only local types are under cultivation (Nybe *et al*., 2006).

2.1.1 Tree characteristics

Syzygium pycnanthum is a evergreen tree with 120 cm diameter which grows to a height of 15-20 m (Backer and Brink, 1963). The clove tree, although related to the eucalyptus and some other large trees, is relatively small, 3.65 to 6.09 m or, rarely, to 12.20 m tall. The stem is often forked with two or three main trunks (Wit, 1969). According to Purseglove *et al*. (1981), clove tree was little to medium evergreen tree which developed to a stature of 12-15 m, cone shaped fit when young and later getting generally round and hollow. The clove tree is evergreen which starts to bloom in around 7 years and keeps on giving economic yield for more than seven decades (Pruthi, 2001). *Syzygium occidentale* (Bourd.) Ghandhi (Myrtaceae) enlisted as " vulnerable" in IUCN 2016 , endemic to Western Ghats of Kerala and Karnataka, is a small tree measuring upto 5-7 m tall (Varghese and Sreekala, 2017).

Syzygium cuminii is a lasting and evergreen tree accomplishing a stature up to 25-30 m. The trunk is thick and grayish white. The trunk has 3 to 4 m circumference with a semi spreading crown up to 10 m in distance across. The branches are regularly wide spreading and hanging at the closures (Mishra *et al*., 1975).

Balakrishnamoorthy and Kennedy (1999) identified twelve high yielding clove types from the survey based on five year study conducted from a private estate at Nagercoil. A clove accession was registered with NBPGR, New Delhi in 2004 by IISR for its dwarf stature with

accession number INGR-04112. The morphological features of this dwarf clove reported was 52.62 cm plant height at 8 th year with a canopy of 50 cm having bushy shape (IISR, 2009).

Variability in clove for shape of trees, cropping season, bearing habits, yield, shape, colour, and dimension has been reported. Tree girth and leaf area were the two important morphological traits for assessing the productivity (Balakrishnan *et al.*, 1998; Kennedy and Nageswari, 2000).

Ponnuswamy *et al*. (1982) reported wide variability in number of peeler shoots per plant, plant height, plant girth and spread in 101 open pollinated accessions of cinnamon.

Fourteen genotypes of *Syzygium cumini* that were fifteen years old maintained in the field gene bank of IIHR, Bengaluru were characterized for the tree morphological characters such as height, trunk girth, canopy spread, leaf length, width and petiole length. Variability was observed for tree characters like tree height, plant canopy spread in E-W and N-S direction among genotypes (Anushma and Sane, 2018).

Four promising genotypes of clove were selected at Dapoli among the germplasm that were planted during 1996-97. Among the accessions the plant height ranged from 5.89 to 7.15 m, girth varied from 35-40 cm and canopy spread varied from 2.50 m to 3.05 m (AICRP, 2019).

Among the 24 accessions of clove maintained at Pechiparai, SA-1 recorded the highest tree height of 11.78 m, followed by SA-3 (11.63 m) compared with local check (9.31 m). The accession SA-13 was significantly superior to other accessions and recorded the highest stem girth (49.59 cm) compared with local check (40.57 cm). The accession SA-3 recorded the highest number of branches (16) and dry bud yield (1.52 kg/tree/year) (AICRP, 2019).

A wild relative *Syzygium claviflorum* collected from Nicobar islands was obtained from ICAR-NBPGR, Thrissur and added to the germplasm of IISR (IISR, 2019).

Whistler and Elevitch (2006) observed that malay apple (*Syzygium malaccense*) tree grows up to height of 16 m or more in height and it grows to a height of 5-12 m.

Patil *et al*. (2009) reported that in jamun crown area was significantly higher in Krishnagiri-1(43.40 m^2/plant) followed by Krishnagiri-4 (41.93 m^2/plant), Krishnagiri-2 (40.5 m^2/plant) and Krishnagiri-3 (34.30 m^2/plant) as compared to local check (26.80 m^2/plant).

In a study conducted by Avinash (2018) on survey, characterization and evaluation of clove accessions concluded that plant height ranged from 5.15 m to 15.25 m and the highest plant height among the accessions was for BLC-18. The girth at 45 cm varied from 44.10 cm to 138.10 cm and it was the highest for BRC-3. The canopy spread N-S ranged from 3.1 m to 7.42 m and it was the highest for MRC-7 and canopy spread E-W ranged from 2.95 m to 7.9 m and was the highest for AMC-11. The number of branches ranged from 26 to 55, AMC-11 showed maximum number of branches.

2.1.2 Leaf characteristics

Clove leaves are simple, opposite, coriaceous, exstipulate, hairless and fragrant. The stalk is slender and about 2-3 cm long, swollen and pink at the bottom showing 7-13 cm length and 3 cm breadth. The shape of the leaf pinnacle is shortly or broadly obtusely and acuminate. New leaves appeared within the flushes were shining pink in colour and mature leaves were dark green (Purseglove *et al*., 1981). The leaves were obovate, oblong to elliptic, opposite and possess bounty of oil glands on the lower surface (Ravindran, 2006).

Balakrishnan *et al*. (1998) reported that measurement of leaf area was one of the important methods for assessing the variability and productivity in clove. Twenty four clove accessions maintained at Horticulture Research Station, Pechiparai revealed that the accession SA-3 recorded the highest leaf length of 16.5 cm and leaf breadth of 6.2 cm (AICRP, 2015).

Among the nutmeg accessions conserved at Pechiparai MF 4 recorded maximum leaf length (20.72 cm), leaf breadth (9.66 cm) (AICRP, 2019). Among the 24 accessions of clove that maintained at Pechiparai, the accession SA-3 recorded the highest leaf length (12.47 cm), leaf breadth (7.46 cm), number of branches (16) and dry bud yield (1.52 kg/tree/year) (AICRP, 2019).

Investigation on the genetic variability among 234 accessions of cinnamon maintained at the Aromatic and Medicinal Plants Research Station, Odakali revealed that 14 per cent had deep flushes, 72 per cent medium coloured flushes and 14 per cent light coloured or green flushes. Similarly, the leaves size was small to medium in 46 per cent, medium to large in 22 per cent, and small to large in 32 per cent of the accessions (Joy *et al*., 1998).

A study by Wijesinghe and Gunarathna (2001) showed correlation between leaf size and shape with yield in seven different types of true cinnamon. According to them trees with large round leaves and big leaves had high bark yield. Moreover bark oil (percentage of cinnamaldehyde) quality was higher in the variety of inwardly curved leaves and high quality leaf oil was obtained from the small round leaves.

Among the characterization and evaluation of *Syzygium sp. Syzigium malaccense* had the highest leaf length (34.45 cm) followed by *Syzygium samarangense* (white) (21.25 cm), *Syzigium jambos* (19.81 cm) and *Syzygium samarangense* (red) (17.25 cm). With regard to leaf breadth of *Syzygium sp. Syzigium malaccense* had the highest leaf breadth (9.38 cm) followed by *Syzygium samarangense* (red) (6.39 cm), *Syzygium samarangense* (white) (5.63 cm) and *Syzigium jambos* (3.09 cm). With regard to leaf area of *Syzygium sp.* the highest leaf area was observed in *Syzigium malaccense* group (320.35 cm^2) and the lowest leaf area was observed in *Syzigium jambos* (62.09 cm^2). The genotypes *Syzygium samarangenese* (white) and *Syzygium samarangenese* (red) had a leaf area of 173.45 cm^2 and 111.30 cm^2, respectively (Jayasree, 2019).

Avinash (2018) concluded that leaf length in clove varied from 9.66 cm in AMC-20 to 13.93 cm in MRC-5, leaf breadth extended from 3.55 cm in ANC-20 to 4.72 cm in AMC-11 and leaf area varied from 23.07 cm^2 in ANC-20 to 42.93 cm^2 in AMC-11.

2.1.3 Bud, flower, fruit and seed characteristics

The clove tree inflorescence is a terminal branching cyme of 3 to 20 hermaphrodite florets, the whole about 1 l/2 inch long. Each pale yellow floret consists of a cylindrical thick ovary, one-quarter inch long. Above the ovary are four fleshy ovate sepals, and above these are the four tiny petals, numerous slender white 3/8-inch filaments, and a slender central style.

There are two flowering seasons a year, July to October and November to January. Few flowers develop into fruit. The fruit, called mother of cloves, contains one seed or rarely two seeds. The ovary and sepals constitute the specific part marketed as cloves (Purseglove, 1981). The inflorescence in clove is terminal, trichotomous panicle, corymbose, shortly pedunculate and branched from the base and is highly variable in the number of flowers. It varies from 3-20 flowers per panicle (Purseglove *et al*., 1981; Ravindran, 2006).

The flowers in clove were produced at the terminal end of the four to five cm long clusters. Botanically, they are paniculate cymes and consisted of three to ten groups of three flowers each (Wit, 1969).

In *Cinnamomum sulphuratum*, flowers are hermaphrodite on axillary panicles. The mean number of flowers per inflorescence reported was 62.48 ± 7.01. Flowers had greenish white coloured peduncles (Shivaprasad *et al*., 2015)

Avinash (2018) on survey, characterization and evaluation of clove accessions concluded that the number of inflorescence /m^2 was the highest in AMC-12 (155.5) and the lowest in ANC-20 (36.25). The number of flower buds /inflorescence ranged from 6.08 to 18.21 in MRC-6. The single bud (fresh and dry) weight, was the highest for BRC-3. The single bud dry weight ranged from 66.5 to 128.5 g.

In the same study, it was also observed that the mature bud length varied from 14.94 mm to 19.06 mm while the mature bud diameter ranged from 4.9 mm in AMC-9 to 6.41 mm in BRC-3. The pooled mean of bud yield (fresh) per tree varied from 2.7 kg to 40.25 kg. However BRC-1, MRC-5, MRC-6, MRC-8, AMC-10 and MMC-15 were good yielders yielding more than 30 kg/tree as revealed from pooled mean. The bud weight (dry) per tree was the highest for MRC-6 (12.25 kg/tree) followed by MRC-5 (12.2 kg/tree). Most of the accessions showed biennial nature, while the yield gap was less in accessions like AMC-12 and BLC-16.

Avinash (2018) on Survey, characterization and evaluation of clove accessions concluded that the length of flower ranged from 16.36 mm to 21.84 mm and the breadth 9.85 mm to 14.82 mm respectively. The length of sepal ranged from 2.49 mm to 3.15 mm. The

fruit weight ranged from 1.2 g in ANC-20 to 3.53 g in BRC-3. The ratio of fruit to seed ranged from 2.41 to 3.64. The highest seed length corresponded to highest fruit length and was noted in BRC-3. The seed length varied from 13.2 to 19.31 mm and seed breadth from 6.11 to 9.19 mm in the selected accessions. The seed weight was the highest for BRC-3 (1.18 g) followed by 1.1 g in AMC-11.

The flower in clove is hermaphrodite and consists of a fleshy hypanthium which is surmounted by four fleshy sepals. The four fleshy sepals are triangular, slightly incurved and3-4 mm long. The four sepals are imbricate, red tinged, rounded, 6 mm diameter and looks like a hemispherical calyptra (Purseglove *et al*., 1981).

Balakrishnamoorthy and Kennedy (1999) reported three different types of clove flower buds *viz.*, small, medium and big. The King cloves had a length of 1.6 to 1.8 cm, breadth of 1.2 to 1.6 cm while that of medium sized clove had length of 1 to 1.2 cm with a breadth of 0.6 to 0.8 cm and for mini clove, the length and breadth ranged from 0.75 to 0.8 cm and 0.4 to 0.5 cm, respectively.

Krishnamoorthy and Rema (1992) reported that age, number of flower buds per inflorescence, number of inflorescences per branch and size of the flower bud determines the yield in clove.

Clove fruit is single seeded drupe and popularly known as mother of clove. The colour of the fruit is reddish purple, 2.5-3.5 cm long and 1.2-1.5 cm in diameter. It is usually tapering at each end and surmounted by the four enlarged fleshy calyx lobes. Fruit contains fleshy pericarp about 2-3 mm thick. The seed is oblong in shape and rounded at both ends, about 2 cm long, purplish colour testa, with two large cotyledons and no endosperm (Purseglove *et al*., 1981).

Seed characters of progenies of 14 elite clove trees revealed no appreciable variation in 100 fruit weight, 100 seed weight, fruit breadth, fruit length, seed breadth and seed length (Krishnamoorthy and Rema, 1994). Ravindran *et al*. (2006) reported that clove fruit contains one oblong shaped fruit of about 1.5 cm length.

According to Pursglove *et al*. (1981), it was occasional to get good yield sequentially due to physical shock of picking. Clove trees flower from fourth year onwards under good management conditions and full bearing stage is reached only after 15 years (Kumar *et al*., 1993). Flowering season varies from September-October in plains to December-January at high altitude (Thangaselvabai *et al*., 2010).

Morton (1987) reported that 3 to 30 flower buds were seen in *Syzygium samarangense* at the branch tips or in smaller clusters in the axils of fallen leaves. Orwa *et al*. (2009) reported 4-5 buds/inflorescence in *Syzygium jambosa.* Khandekar *et al*. (2016) reported that inflorescence of all *Syzygium samarangense* cultivars *viz.,* Giant green, Masam manis pink and Jambu madu red had the cymose inflorescence.

In a study conducted by Jayasree (2019), on characterization and evaluation of *Syzygium sp.* concluded that number of flower buds per inflorescence varied from 5.30 to 14.40 with mean of 8.53. Maximum number of buds was observed in *S. samarangense* (white) (14.40) followed by *S. samarangense* (red) (11.20), *S. malaccense* (7.90) and minimum number of buds per inflorescence was observed in *S. jambos* (5.30).

Patil *et al*. (2009) observed that fruit weight of jamun ranged from 2.20 g in RNC-29 to 13.90 g in RNC-26 genotypes. Studies on variability in fruit characters of jamun at Banaras Hindu University, Varanasi reported that fruit weight ranged from 3.55 g to 14.55 g and higher fruit weight was recorded in Selection 1 while lowest in Selection 6 (Prakash *et al*., 2010). In jamun fruit weight was influenced by many factors; fruit length, fruit breadth, volume, size, pulp weight, pulp percent, pulp thickness, pulp to seed ratio, seed length, seed breadth, seed volume, seed size, seed weight and seed percent (Devi *et al*., 2016). Jayasree (2019) reported that *S. malaccense* had the highest fruit weight (54.40 g) followed by *S. samarangense* (red) (34.22 g), *S. samarangense* (white) (26.61 g) and the lowest fruit weight was observed in *S. jambos* (16.37 g), respectively.

Jayasree (2019) also reported that variability in *Syzygium sp*, was very high with respect to seed length and it varied from 1.50 cm in *S. jambos to* 3.80 cm in *S. malaccense*. In case of *S. samarangense* (white) seed length showed 1.67 cm whereas in S. *samarangense* (red) rudimentary seeds were observed. On the contrary seed width of fruit varied from 0 to 3.90

cm in *S. malaccense* and *S. jambos* showed a seed width of 1.80 cm. With respect to seed weight, variability was high and it varied from 1.50 to 5.20 g. *S. malaccense* showed the highest seed weight of 5.20 g while *S. samarangense* (red) recorded the lowest.

Patil *et al*. (2005) observed that maximum seed weight was noticed in RNC-11 among the accessions followed by V-3 and V-2 genotypes in jamun. Khandekar *et al*. (2011) observed seed weight in rose apple genotypes and concluded that Giant Green cultivar had the highest seed weight (4.41 g) followed by Jambu madu red and Masam manis pink with a weight of 0.04 and 0.02 g respectively. Among the jamun genotypes observed by the Devi *et al*. (2016), seed weight was maximum in AJG-20 (2.40 g) and minimum in TC-85 (0.56 g).

2.1.4 Multivariate analysis

Cluster analysis followed by principal component analysis (PCA) had been used to cluster *Cinnamomum sp*. into groups and to show relationship among the species on the basis of morphological characters (Ravindran *et al*., 1991).

The main purpose of PCA is to extract the important information from the table, to represent it as a set of new orthogonal variables called principal components, and to show the pattern of similarity of the observations and of the variables as points in maps (Abdi and Williams, 2010). According to Mahendran *et al*. (2015), higher the coefficients, regardless of the direction (positive or negative), the more effective they will be in discriminating between the accessions.

Characters with high variability are expected to provide high level of gene transfer during breeding programs (Aliyu *et al.*, 2000; Gana, 2006).

Morphological characterization as a way for the description and classification of germplasm and statistical methods like principal components analysis and/or cluster analysis can be used as useful tools for screening the genotypes of a collection (Hillig and Iezzoni, 1988). The selection of representative Minimum Data Set (Doran and Parkin, 1994) was done by the Principal Component Analysis (PCA) based on the assumption that principal components receiving higher values would best represent the system attributes. Among each

principal component, the one with the highest sum of correlation coefficients was chosen for the Minimum Data Set. The correlation coefficient between any two characters was estimated by the cosine of the angle between their vectors (Yan and Rajcan, 2002). PCA is commonly used to analyze large data sets and is used to evaluate germplasm (Sohrabi *et al*., 2012).

Principal component analysis, linear discriminant analysis and multidimensional scaling (MDS) are three most classical techniques for dimensionality reduction. PCA finds a low-dimensional embedding of the data points that best preserves their variance as measured in the high-dimensional input space. Classical MDS finds an embedding that preserves the interpoint distances, equivalent to PCA when those distances are Euclidean. MDS is widely used for the visualization of data and in molecular modeling (Xie *et al*., 2014).

Multi-Dimensional Scaling (MDS) is an ordering technique for dimensional reduction that allows one to map individuals as points in low-dimensional space (generally 2D or 3D). MDS is especially useful when the relation between individuals is unknown (not an unusual case in germplasm banks), but it is possible to estimate a detachment matrix between them (Manly, 2004; Borg and Groenen, 2005).

RAPD markers were used for the comparison of genetic divergence and variability of tall coconuts maintained in Coconut Germplasm Bank of Brazil. Cluster analysis and multidimensional scaling were used to group plants on the basis of a dissimilarity matrix calculated by Euclidean distance revealed that WAT (West African Tall), BRT (Brazil Tall) and RLT (Rennell Island Tall) represented homogenous and distinct populations of tall coconuts. WAT and BRT were closer to each other than they were to RLT and yet these populations was still distinct. The divergence observed within BRT was greater than detected within other ecotypes reflecting its greater level of polymorphism (Wadt *et al*., 1999)

In accessions of cocoa, Leal *et al*. (2008) used a hierarchical method together with an MDS solution to identify accession diversity. Studying genetic variability of *Tibouchina papyrus* populations, Telles *et al*. (2010) based their conclusions on the results of UPGMA and MDS analyses.

2.2 Standardization of pollinatiom techniques

2.2.1 Floral phenology and biology

2.2.1.1 Flower opening time

Wit (1969) has observed that clove flowers in Indonesia open in the early morning. According to Nair *et al*. (1974), anthesis in clove occurs in the afternoon at 1.30 pm with a peak between 3.30 pm and 4.30 pm. Sritharan and Bavappa (1981) have reported from Sri Lanka that the majority of clove flowers open in the afternoon.

In *Syzygium syzygiodes*, flowers are pale yellow in colour, open slowly with maximum flower opening occuring in the morning hours between 07.00-9.00 am (Lack and Kevan, 1984). Armstrong and Drummond (1986) revealed that anthesis in nutmeg occurs shortly after dark and 95 percent of all staminate flowers that blooms on a particular day opens within the first five hours after sunset. Pool and Bermawie (1986) concluded that majority of clove flowers open in the afternoon.

Chantaranothai and Parnell (1994) reported on the day preceeding full anthesis the ripe floral buds of both *Syzigium jambos* and *Syzigium megacarpum* start swelling during the early evening (after 6 pm) and continue swelling in the late evening reaching full expansion in the early morning of the next day. Nagarajan *et al*. (1998) observed anthesis in tamarind starts at 20:00 h and flowers completely open by 02:00 h. In *Syzygium saveri*, flowers start opening before sunrise and remained open for approximately 3 days at which point all anthers were lost or had become brown and withered (Boulter *et al.*, 2005).

In *Syzygium cuminii*, anthesis starts at around 8 am and lasts approximately 10 h peaking between 10 am - 12 noon (Singh, 2016). Thangaselvabai *et al*. (2009) reported that peak anthesis in cinnamon was from 11 am to 12 noon. Raju *et al*. (2014) reported that flowers in *Syzygium alternifloium* opened during 16-18 h with maximum flowering occuring at 17 h. Geethika and Sabu (2017) reported that flowers in *Syzygium caryophyllatum* started opening in the afternoon at 16.00 h and continued up to 04.00 h on next day.

In *Syzigium cuminii* maximum anthesis was observed between 8.00 to 9.00 am followed by 9.00 to 10.00 am and 7.00 to 8.00 am. There were some genotypic differences for anthesis period due to variation in flowering time accompanied with weather conditions (Singh, 2017).

In *Syzygium occidentale* flowers open at 16.00-19.00 h with maximum blooming at 18.00 h (Varghese and Sreekala, 2017). Aswathi *et al*. (2018) reported that both male and female flower in *Garcinia cambogia* opened at evening hours and lasted until 4:30-6:30 pm.

Kuriakose *et al*. (2018) reported that majority of *Syzygium occidentale* flowers opened at 20.00-23.00 h during the night.

2.2.1.2 Anther dehiscence

Wit (1969) has observed that the anthers of clove flowers in Indonesia have dehisced within a few hours of flower opening. Nair *et al*. (1974) reported that anthers in clove dehisce longitudinally and anther dehiscence commenced 24 h before anthesis. Sritharan and Bavappa (1981) reported from Sri Lanka that the anther dehiscence begins 24 h before flower opening and dehiscence peaks immediately. In *Syzygium syzygiodes*, anthers that formed a ring around the flower started dehiscence at around 08:30 h when the dew or rain evaporated (Lack and Kevan, 1984). Pool and Bermawie (1986) concluded that anther dehiscence in clove occured shortly after anthesis.

In *S. jambos* and *S. megacarpum* anthers of outer stamens begin to dehisce in the early evening of the first day while anthers of inner stamens dehisce and the stigma appears receptive early the following morning (Chantaranothai and Parnell, 1994). Boulter *et al.* (2005) reported that anthers in *Syzygium saveri* dehisce longitudinally and anther dehiscence begins few hours after flower opening. In *Syzygium cuminii* anther dehiscence begins immediately after opening the flowers (Singh *et al*., 2009). Raju *et al.* (2014) reported that anther dehiscence occurs immediately after anthesis in *Syzygium alternifolium*. Geethika and Sabu (2017) reported that flowers in *Syzygium caryophyllatum* start dehiscing anthers along with opening of the flowers.

In jamun (*Syzigium cuminii*) maximum anther dehiscence was observed between 8.00 to 9.00 am followed by 9.00 to 10.00 am and 7.00 to 8.00 am (Singh, 2017). Varghese and

Sreekala (2017) reported that anther dehiscence in *Syzygium occidentale* occurs one day before anthesis. Kuriakose *et al*. (2018) reported that anther dehiscence in *Syzygium occidentale* occurs 2 days before flower opening. Nevertheless, dehisced anthers do not touch the stigma in the bud stage due to the incurved nature of stamens and the position of stigma outer the stamen filaments.

2.2.1.3 Number of stamens per flower

Staminal structure in *Syzygium sp.* is briefly discussed by Chantaranthoi and Parwell (1994). Stamens of syzygium are usually numerous (40-50) per flower in *Syzygium balsameum* to 1200 in *Syzygium megacarpum* arranged in many whorls, the outermost of which is the longest (1.5 mm in *Syzygium bornese*) to 35 mm in (*Syzygium jambosa*). In *Syzygium saveri*, approximately 150-200 stamens/flower were tightly packed between the 2 calyx lobes but appeared to drop out easily when touched. Lower stamens started opening before sunrise and remained open for approximately 3 days at which point all anthers were lost or had become brown and withered (Boulter *et al.*, 2005). Varghese and Sreekala (2017) reported that average number of stamens in *Syzygium occidentale* was 502, which were white, free, filaments thin, 3.5-5 cm long.

In a study conducted by Jayasree (2019) on characterization and evaluation of *Syzygium sp.* the highest number of stamens were found in *S. samarangense* (white) (397.50), while *S. jambosa* had the lowest number of stamens (224). On the other hand *S. samarangense* (red) and *S. malaccense* had 352 and 249 stamens respectively.

2.2.1.4 Pollen characteristics

Bonnefille (1971) reported 3 ± 4-colporate pollen grains in *Syzygium guineense*. Huang (1972) described pollen grains of five *Syzygium* species in the flora of Taiwan as 3 (4) - colporate, scabrate and rugulate. Sowunmi (1973) reported 3 ± 4 - colporate pollen in *Syzygium guineense*. Gupta and Sharma (1986) and Chauhan and Bera (1990) described 3 – colporate and parasyncolpate pollen grains with an obscure exine pattern in *Syzygium cuminii*, *Syzygium sp.* pollen being usually isopolar, radially symmetrical, suboblate, 3-parasyncolporate. Nayar (1990) reported both parasyncolporate and tetrazonocolporate pollen grains with psilate

patterns in *Syzygium cuminii*. Tissot *et al*. (1994) described trisyncolporate and smooth grains in *Syzygium gardneri, Syzygium laetum, Syzygium mundagam, and Syzygium occidentale.*

Pickett and Newsome (1997) indicated that pollen of Myrtaceae is considered distinctive, being usually syncolpate or parasyncolpate. *Syzygium megacarpum* had the longest pollen grains (18.4 µm) and twice the size of *Syzygium foxworthianum* and *Syzygium jasminifolium* which had the smallest (7.7 µm) (Parnell, 2003). Pollen size of *Syzygium aromaticum* reported by (Parnell, 2003) was 13.28-15.88 µm (polar view) and 5.34 - 8.25 µm (apocolpigal edge) and apocolpium surface/area polar face (16.21 - 26.99 per cent).

In *Syzygium saveri* pollen grains were tricolpate with an average polar diameter of 14.1 ± 0.18 µm and an equatorial diameter of 14.1 ± 0.26 µm. The surface exine of *S. sayeri* was smooth (Shivanna and Rangaswamy, 1992). In *Syzygium saveri* pollen grains were triangular in polar view and oblate-elliptic in lateral view (Boulter *et al*., 2005). In *syzygium alternifolium* pollen grains were creamy white triangular, tricolporate triangular, 16.6 µm in size, powdery and fertile. Most of the pollen was dislodged as single grains and it entered the ambient environment by wind (Raju *et al.,* 2014).

Thornhill *et al*. (2012) reported that *Syzygium* pollen under SEM were tricolporate with a psilate/regulate or verrucate/scabrate exine. Pollen sides were convex, straight or concave and the colpal morphology was parasyncolpate with angular or arcuate colpi. Pollen length ranged from 11.3 to 18.4 µm.

Nayar (1990) and Tissot *et al*. (1994) conducted the exine ornamentation of various *Syzygium* species showed the exine ornamentation of pollen to be psilate. The study lead by Premathilake and Nilsson (2001) indicated the pollen grains to be faintly rugulate. Geethika and Sabu (2017) reported that pollen grains in *Syzygium caryophyllatum* were triangular, non-sticky, tricolporate and psilate type and were 17.6 ± 0.32 µm in diameter and suitable for anemophily. In *Syzygium occidentale* pollen grains were triangular, non-sticky, tricolporate, 17-18 µm in diameter and suitable for anemophily (Varghese and Sreekala, 2017).

2.2.1.5 Stigma receptivity

Sritharan and Bavappa (1981) had reported from Sri Lanka that maximum stigma receptivity in clove was attained on the day of anthesis, with the stigma remaining receptive for a further 48 h. Workers in the Andaman Islands also observed that the stigma does not become receptive until 3 days after anthesis, reaching peak receptivity 2 days later and remaining receptive for an additional 2 days (Nair *et al*., 1974).

Misra and Bajpai (1975) reported that in *Syzygium cuminii* flowers, stigma receptivity lasted a day after anthesis. In *Syzygium saveri* stigma remains receptive from the opening of flower in the morning and ends until 3 days (Boulter *et al*., 2005). In *Syzigium cuminii*, stigma remains receptive one day prior to anthesis and remains receptive upto 5 days after anthesis (Singh *et al*., 2009). Sanewski (2010) reported that stigma receptivity in *Syzygium leymannii* was maximum after two days of stigma emergence and coincided approximately with stamen emergence and pollen shedding. The stigma remained receptive for at least two days after stamens were shed.

Geethika and Sabu (2017) reported that stigma receptivity in *Syzygium caryophyllatum* occured after anther dehiscence. Cytochemical localization of esterases on stigmatic surfaces using α-napthylacetae indicated that stigma receptivity was maximum at 11 h. In *S. occidentale*, Varghese and Sreekala (2017) reported maximum stigma receptivity at the time of anthesis and it extended up to 24 h which was indicated by of *in vivo* pollen germination and peroxidase activity.

2.2.2 Pollination biology

2.2.2.1 Pollen viability and pollen fertility

The stainability of pollen in clove was 81 percent and stained pollen grain was larger in size and gave a higher percentage of germination (Sritharan and Bavappa, 1981). In *Syzigium cuminii*, during the beginning of the season pollen fertility was higher and goes on decreasing as the season advanced (Singh *et al*., 2009).

Sanewski (2010) reported that after four days of anthesis, peak pollen germination was seen in *Syzygium leymannii* and hence viability occurred on four days after anthesis. This

approximately coincided with two days after stamen emergence that was when anthers dehisced to release pollen. Geethika and Sabu (2017) reported that pollen grains in *Syzygium caryophyllatum* were 49.54 ± 2.98 percent viable at 13.00 h on the first day of anthesis. Varghese and Sreekala (2017) revealed that 80 per cent of pollen grains were fertile in acetocarmine staining technique. Pollen viability by (Flouro Chromatic Reaction (FCR) and Diaminobenzidine (DAB) test indicated that 85 per cent and 82 per cent of pollen grains were viable, respectively on the day of anthesis and its viability gradually decreased on successive days of anthesis.

2.2.2.2 Pollen storage

Pollen viability may be decreased under low humidity because of the injury caused from rapid loss of moisture from the pollen grains, sometimes due to rapid increase of physiological activities of the pollen grains under high humidity (Liskens and Schrauwen, 1966). In *Syzigium cuminii*, pollen could be stored successfully for 9 months at a temperature of 23ºC in completely dry atmosphere (Singh *et al*., 2009). The most common methods used to store the pollen involve reducing the water content of pollen and maintaining the pollen grains at low temperatures to avoid fluctuations. Ganeshan *et al*. (2008) reported that pollen viability of stored pollen was influenced by moisture and storage temperature in addition to physiological and genetic factors.

2.2.2.2 Hybridization and fruit set

The best period for pollination in clove was between fourth and sixth day after opening. Under artificial pollination, maximum fruit set obtained was 30 per cent, while under bagged conditions it was 28 percent. Self pollination appeared to be more probable in clove. A fertilized flower took about three months for maturity of fruits in clove (Krishnamoorthy and Rema, 1994; Nybe *et al*., 2006).

In *Syzygium leyhmannii*, emasculation of flowers generally improved fruit set. The fruit set for emasculated, open pollinated fruit was 250 per cent higher than that which naturally occurred. It was possible that part of this response might not be due to the emasculation but rather the fact that some flowers were removed to make emasculating easier. This ‘thinning’

of flower clusters might have led to better retention of the remaining flowers. Emasculated flowers were capable of setting seed if open pollinated naturally but not by hand pollination. Emasculation by itself did not reduce seed set but the hand pollination procedure used appeared less effective for fertilization compared to natural pollination (Sanewski, 2010).

Raju *et al.* (2014) reported that hand pollination experiments in *Syzygium alternifolium* indicated that autogamy and geitonogamy were non-functional while xenogamy was the only mode of pollination for fruit set. In this mode fruit set stood at 56 per cent while in open pollination mode, it was 11 per cent only in *Syzygium alternifolium*. In *Syzygium leyhmannii*, xenogamous pollination (cross pollination between different seedling trees) produced the highest fruit set, a 3.4 fold increase over self pollination. Without pollination of emasculated flowers produced the same percentage of fruit set as open or self pollination. Without pollination produced no seed whereas all forms of pollination produced the same percentage seedy fruit (Sanewski, 2010). In *Syzigium cuminii*, fruit set by hand pollination indicated that 45-50 percent occurs by self pollination and 30-40 percentage by cross pollination (Singh *et al*., 2009).

After self pollination in *S. jambosa* (rose apple), fruit set occurs with seeds but under cultivation 73 percent blossoms set fruits whereas apomixis and self fertilization occurs freely (Chantaranothai and Parnell, 1994). Parthenocarpic fruit set occurred with self, open, xenogamic and without pollination. Parthenocarpic fruit set varied from 6-14 percent (Sanewski, 2010). In *Syzygium jambosa* 25.21 per cent open pollinated flowers set fruits whereas no fruit set occured after netting and bagging the inflorescence (Bhattacharya and Mandal, 2000).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Survey conducted by the Department of Plantation Crops and Spices, College of Agriculture, Vellayani in different clove growing locations of Southern Kerala and Kanyakumari districts of Kerala from 2016 to 2018 has identified 20 clove accessions which showed high yield and special characters. The twenty accessions were evaluated for a period of two years and has identified twelve elite accessions. These twelve accessions (Plate 1 to Plate 3) were further evaluated for two more years for quantitative characters. Along with that observation of floral phenology and biology, method of emasculation, pollen collection, pollen storage and hybridization need to be standardized before attempting the hybridization of twelve elite accessions. Hence the project formulated titled "Evaluation of elite clove (*Syzigium aromaticum* (L) Merr. & Perry) accessions and standardization of pollination techniques" was carried out in twelve elite clove accessions at clove plantations at Thiruvananthapuram and Kollam districts of Kerala from February 2018 to May 2019. The location of the twelve accessions is furnished in Table 1.

3.1. EVALUATION OF CLOVE ACCESSIONS

3.1.1 Quantitative characters

3.1.1.1 Tree characters

3.1.1.1.1 Plant height

The height of the selected clove accession was measured from the base of the tree to the tip of tree canopy with the help of software application "Smart measure android application" (Smart measure 1.7.1 for android) and was confirmed by measuring with tape. Plant height was expressed in metres (Avinash, 2018).

3.1.1.1.2 Girth

The girth of the tree trunk was recorded at 30 centimetres height from the ground level with the help of measuring tape and values were expressed in centimetres.

Table 1 Selected clove accessions and their location details

Sl. no.	Accession ID	Location
1	BRC-1	Braemore estate, Braemore, Trivandrum
2	BRC-2	
3	BRC-3	
4	BRC-4	
5	MRC-5	Merchiston estate, Ponmudi, Trivandrum
6	MRC-6	
7	MRC-7	
8	MRC-8	
10	AMC-10	Ambanad estate, Aryankavu, Kollam
11	AMC-11	
12	AMC-12	
13	AMC-13	

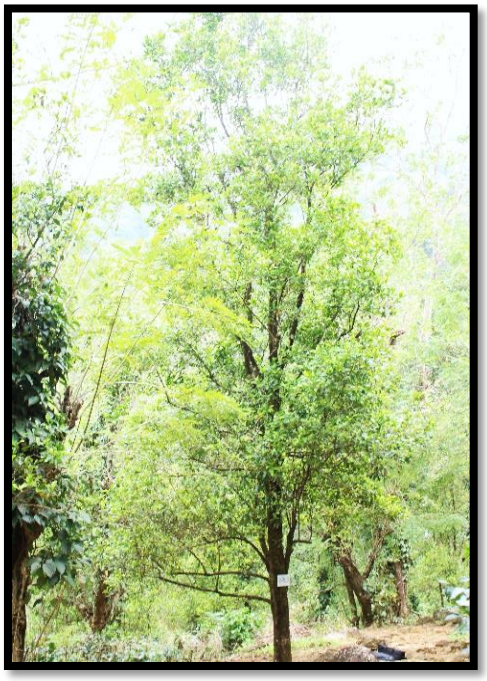
3.1.1.1.3 Canopy spread (N-S & E-W)

Canopy spread of the tree was measured in North – South and East – West direction using a measuring tape.

3.1.1.1.4 Number of branches

The number of main branches were counted in selected clove accessions and recorded.

Accession ID. BRC-1



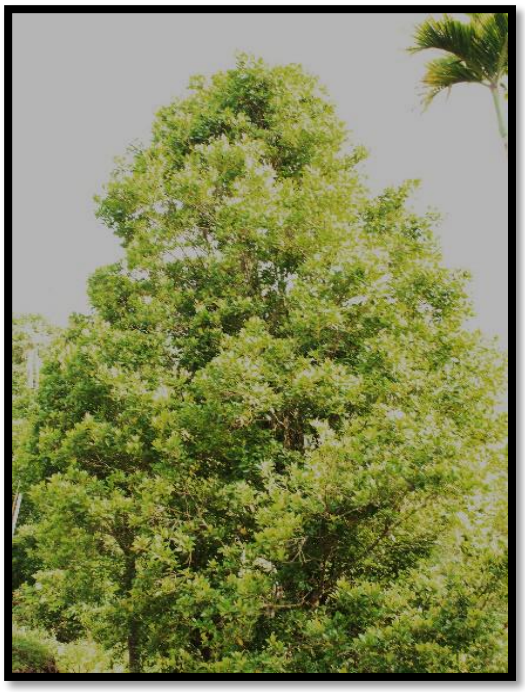
Accession ID. BRC-2

Accession ID. BRC-3

Accession ID. BRC-4

Plate 1. Accessions selected at Braemore estate

Accession ID. MRC-5



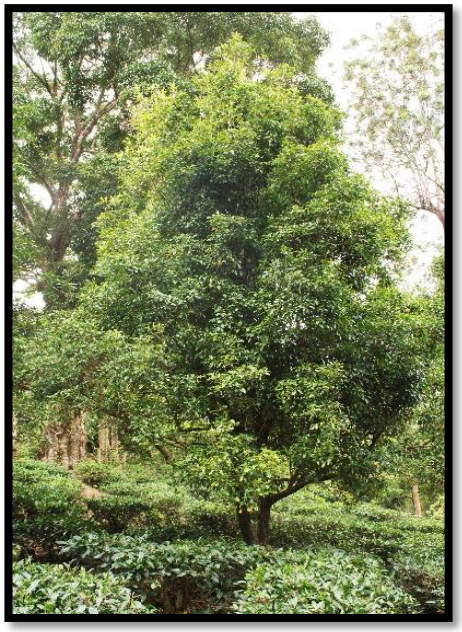
Accession ID. MRC-6

Accession ID. MRC-7

Accession ID. MRC-8

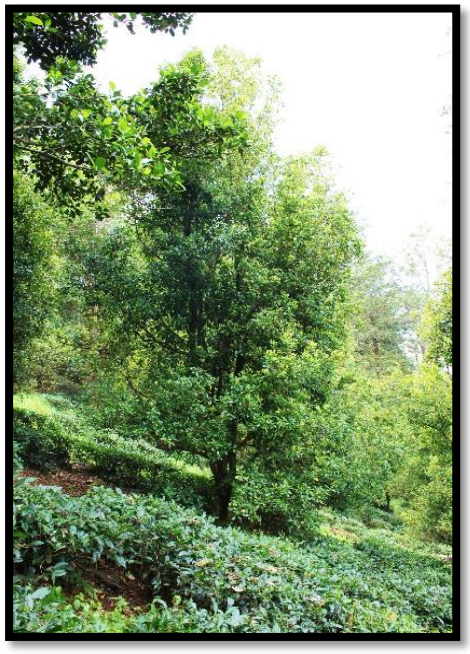
Plate 2. Accessions selected at Merchiston estate

Accession ID. AMC-10



Accession ID. AMC-11

Accession ID. AMC-12



Accession ID. AMC-13

Plate 3. Accessions selected at Ambanad estate

3.1.1.2 Leaf characters

3.1.1.2.1 Leaf length

Five fully opened and matured leaves, second from the tip of bearing shoot from four sides constituting twenty leaves were selected. Length of these twenty leaves of each clove accession was measured from the leaf base to the tip of lamina excluding petiole and the mean was found and expressed in centimetres (cm).

3.1.1.2.2 Leaf breadth

The leaves selected for taking the leaf length was utilized for measuring the leaf breadth. Leaf breadth was measured from the widest middle portion of leaf blade and the mean leaf breadth was expressed in centimetres (cm).

3.1.1.2.3 Leaf area

The leaf length and leaf breadth were measured and the leaf area was calculated based on length breadth method.

Leaf area = leaf length x leaf breadth x K (0.649) where K is a constant

Leaf area was expressed in cm^2 (Balakrishnan *et al*., 1998)

3.1.1.3 Bud, flower, fruit and seed characters

3.1.1.3.1 Number of inflorescence/m^2

Number of inflorescence/m^2 in clove accessions were measured by counting the number of inflorescence per m^2 area by fixing $1m^2$ quadrats on all four sides of the selected accessions and the mean was calculated.

3.1.1.3.2 Number of flower buds/inflorescence

Plate 4. Measuring mature bud length by Digital Vernier Caliper (HAWK HT0472)

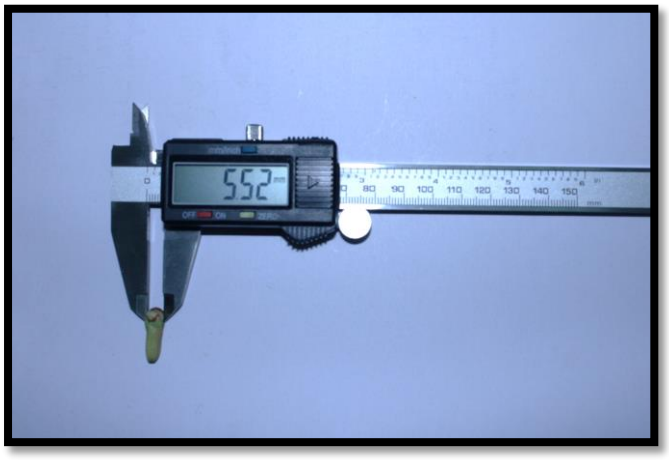


Plate 5. Measuring mature bud diameter by Digital Vernier Caliper (HAWK HT0472)

The number of flower buds/inflorescence was measured by counting the number of clove flower buds per inflorescence from all the four sides with a representative sample size of five inflorescence in each side of the selected accessions and the mean value was expressed.

3.1.1.3.3 Length of flower

The length of ten flowers on the day of opening was taken from the base of the hypanthium to the tip of stamen for each selected accession using the Digital Vernier Caliper (HAWK HT0472) and mean value was expressed in mm.

3.1.1.3.4 Breadth of flower

The breadth of ten flowers per accession was recorded on the same flower used for measuring the flower length. The widest part of the flower was measured using Digital Vernier Caliper (HAWK HT0472) and mean value was expressed in mm.

3.1.1.3.5 Length of sepal

Ten flowers which opened on the first day were selected and the length of the five sepals of each flower were taken using Digital Vernier Caliper (HAWK HT 0472). The length of ten sepals were measured from the tip of hypanthium where the sepal starts to the tip of sepal. The mean length of the sepals of each accession is worked out and expressed in mm.

3.1.1.3.6 Length of petal

Ten buds of each accession which were ready to open within one hour were collected. These buds were kept to open and when they burst opened the petals were collected and length of petals were recorded from the base to the tip of the petal with the help of Digital Vernier Caliper (HAWK HT 0472) and mean value of each accession was expressed in mm.

3.1.1.3.7 Single bud weight (fresh)

Single bud weight (fresh) was recorded by taking the fresh weight of twenty flower buds per accession using a weighing balance and the mean was calculated and expressed in mg.

3.1.1.3.8 Single bud weight (dry)

Single bud weight (dry) was recorded by taking the dry weight of 20 flower buds per accession after oven drying at 50^0C for 15 h till constant weight was obtained (Murni *et al*., 2017). Same flower buds which were used for measuring fresh weight of single buds were used for taking dry weight and the mean value was calculated and expressed in mg.

3.1.1.3.9 Mature bud length

The length of 20 mature buds were recorded from the base of the hypanthium to the tip of flower bud of each accession with the help of Digital Vernier Caliper (HAWKHT0472) and mean value was expressed in mm. Measurement of mature bud length by Digital Vernier Caliper (HAWK HT0472) is presented in plate 4.

3.1.1.3.10 Mature bud diameter

Diameter of the mature bud was measured by recording the head width of 20 flower buds of each accession using Digital Vernier Caliper (HAWKHT0472) and expressed in mm. Measurement of mature bud diameter by Digital Vernier Caliper (HAWK HT0472) is presented in plate 5.

3.1.1.3.11 Flowering period

To record the flowering period in individual accessions, proportions of flower buds and open flowers were monitored weekly, then daily from November 2019 to April 2020. Peak flowering time was defined as the time interval when between 25% and 75% of flowers in the canopy had opened (Adam *et al.*, 1999).

3.1.1.3.12 Bud yield per tree (fresh)

Fresh bud yield of selected accessions were recorded at the time of harvest during 2019 and 2020 and mean yield was expressed in kg/tree.

3.1.1.3.13 Bud yield per tree (dry)

Dry bud yield per tree of selected accessions were recorded after oven drying at 50ºC for 15 h till constant weight was obtained during 2019 and 2020 and mean yield expressed in kg/tree.

3.1.1.3.14 Fruit weight (fresh)

Fresh weight of twenty fruits per accession were recorded and mean weight was expressed as gram per fruit (g/fruit).

3.1.1.3.15 Ratio of fruit to seed

The fresh weight of twenty fruits and seeds per accession was weighed and the ratio of fruit to seed was found out and the mean of each accession was expressed.

3.1.1.3.16 Seed length

Seed length of ten fresh seeds per accession were recorded by extracting the seeds after soaking fresh fruits in water for 12 h. Seed length was measured from the base to the tip from the Digital Vernier Caliper (HAWKHT0472) (Plate 6) and mean expressed in cm.

3.1.1.3.17 Seed breadth

Seed breadth of ten fresh seeds per accession were recorded on the same seeds taken for the observation of seed length. Seed breadth was measured from the widest part of the seed using Digital Vernier Caliper (HAWKHT0472) and mean seed breadth expressed in cm.

3.1.1.3.18 Seed weight

Seed weight of twenty fresh seeds per accession were recorded and mean weight was expressed in gram per seed (g/seed).

Plate 6. Measuring Seed length by Digital Vernier Caliper (HAWK HT0472)

3.2 STANDARDIZATION OF POLLINATION TECHNIQUE

The standardization of pollination technique was carried out in 30-35 year old clove plants of Merchiston estate. 5 clove plants were selected for carrying out this experiment.

3.2.1 Floral phenology and biology

3.2.1.1 *Flower opening time*

A preliminary observation was undertaken in 20 flower buds of 5 selected clove trees to fix the time interval for recording the flower opening time. Based on the initial observation the time period for flower opening was fixed at 1 hour interval from 2:30 to 6:30 pm. To establish the time of flower opening in clove, four branches per tree having mature buds were labelled on 5 selected plants and the numbers of opened flowers were recorded daily on each branch at 2:30 pm-3:30 pm, 3:30 pm-4:30 pm, 4:30 pm-5:30 pm and 5:30 pm-6:30 pm for a period up to all flowers opened in all the branches. Based on the result maximum flower opening time in clove was determined.

3.2.1.2 *Anthesis duration*

To establish the anthesis duration in clove, four branches having mature buds were labelled on 5 selected plants and the numbers of opened flowers were recorded daily on each branch at 2:30 pm-3:30 pm, 3:30 pm-4:30 pm, 4:30 pm-5:30 pm and 5:30 pm-6:30 pm for a period till all flowers in all branches opened. Anthesis duration refers to the time period of opening of the flower. The time at which the first flower opened among the selected branches up to the last flower was recorded in time intervals and expressed as anthesis duration.

3.2.1.3 Number of stamens per flower

Ten flowers of clove were taken and the number of stamens in each flower was counted and mean value was expressed as number of stamens per flower.

3.2.1.4 Anther dehiscence starting time

A preliminary observation was undertaken to fix the time interval for studying the anther dehiscence starting time. Based on the preliminary observation 12 hour interval was fixed starting from 48 hour before the flower opening. Five flower buds were collected from five selected clove trees and anther dehiscence starting time was observed at 48 h, 36 h, 24 h and 12 h before flower opening. Anther dehiscence starting time was determined by observing the anthers collected at different duration under the stereomicroscope.

3.2.1.5 Anther dehiscence ending time

Five flower buds were collected from five selected clove trees and anther dehiscence ending time was observed on 0 h, 24 h and 48 h after flower opening. Anther dehiscence ending time was determined by observing the anthers collected at different duration under the stereomicroscope.

3.2.1.6 Pollen charactersitics

For pollen morphology studies, the anthers were collected from the buds which was expected to open after 12h was preserved immediately in 70% ethanol. Slide preparation for pollen morphology studies were made by acetolysis method proposed by Punt (1967).

The preserved material of anthers were transferred to a centrifuge tube and crushed with a glass rod. The dispersion was sieved through a brass mesh of 48 divisions per cm^2 and was collected in a glass centrifuge tube.

After centrifugation in centrifuge at 2000 rpm for 5 minutes, the supernatant was decanted and the pollengrains after washing in glacial acetic acid was treated with acetolysis mixture consisting of acetic anhydride and concentrated sulphuric acid (9:1) in the centrifuge tube. A glass rod was placed in each tube and was transferred to a water bath at 70-100°C for 3 to 5 minutes, till the medium became brown in colour. Centrifugation of this mixture was carried out in centrifuge at 2000 rpm for 5 minutes and the supernatant was decanted off and glacial acetic acid was added to the sediment and again centrifuged and supernatant was decanted. A drop of the sediment was placed on the glycerine medium in the centre of the slide

and covered by a coverslip. The permanent slide prepared was taken for observation of pollen shape, pollen size, viable and nonviable pollen using Scanning Electron Microscope of Sophisticated Instrumentation and Computation Centre, University of Kerala, Karyavattom, Thiruvananthapuram.

3.2.1.7 Stigma receptivity

Ten flowers each were collected from 10 selected clove trees for seven days from the day of anthesis. The stigma of those flowers were immersed in hydrogen peroxide (3%) for 3 min to observe the release of air bubbles (Zeisler, 1933). The number of bubbles of the stigma were counted on each day of anthesis and maximum number of bubbles indicated the maximum receptivity of the stigma.

3.2.1.8 Anther dehiscence duration

Anther dehiscence duration in clove plants was determined by checking both anther dehiscence starting time and anther dehiscence ending time.

3.2.2 Standardization of emasculation

3.2.2.1 Emasculation period

Emasculation period of clove was determined by standardization of anther dehiscence starting time, anther dehiscence ending time and stigma receptivity.

3.2.3 Pollen collection and storage

3.2.3.1 Pollen collection

The preliminary observation trial done to study the anther dehiscence starting time helped to identify the bud that will open after 12 h, 24 h and 36 h. Buds that will open on 12 h, 24 h and 36 h was taken to the laboratory, petals were removed and stamen exposed. Anthers

were crushed and pollen was collected and taken for analysis of pollen viability and pollen fertility.

Treatments

P_1-12 h before anthesis

P_2-24 h before anthesis

P_3-36 h before anthesis

Based on the maximum pollen viability and pollen fertility the time for pollen collection was standardized.

3.2.3.2 Short term pollen storage

Based on the experiment on pollen collection the time of pollen collection was standardized. The best treatment was taken for the short term pollen storage studies. Short term pollen storage was carried out with 7 treatments and 3 replications. The treatments are mentioned below.

Treatments

1. T_1- Pollen dried at 40°C for 2 hours and kept at room temperature in a dessicator for 2 months.
2. T_2- Pollen dried at 45°C for 2 hours and kept at room temperature in a dessicator for 2 months.
3. T_3- Pollen dried at 50°C for 2 hours and kept at room temperature in a dessicator for 2 months
4. T_4- Pollen dried at 40°C for 2 hours and stored under refrigeration (4^0C) for 2 months
5. T_5- Pollen dried at 45°C for 2 hours and stored under refrigeration (4^0C) for 2 months
6. T_6- Pollen dried at 50°C for 2 hours and stored under refrigeration (4^0C) for 2 months
7. T_7- Fresh pollen

Replication – 3

Design of experiment- Completely Randomized Design (CRD)

. Buds were collected randomly on selected 5 clove plants which opens after 12 hour. Anthers collected from 100 g of buds constituted 1 replication of the treatment. Altogether 2100g buds were used for collection of pollen for 7 treatments and 3 replications. The anther of each replication of each treatment were removed immediately and preserved in petriplates.

The petriplates were covered airtight with polythene film and labelled. The anthers kept in petriplates were dried at 40°C, 45°C and 50°C for a period of two hours. After drying the anthers, each petriplate with anthers dried at 40°C, 45°C and 50°C temperatures were kept in dessicator and refrigerator. Fresh pollen without drying stored in petriplate was taken as control. Pollen viability and pollen fertility tests was done at 0, 15, 30 and 60 days after storage by Iodine potassium iodide method and acetocarmine glycerine staining method (Sulisoglu and Cavusoglu, 2014; Marks, 1954)

3.2.3.3 Pollen viability

Pollen viability was tested by Iodine potassium iodide (IKI) staining technique test. Potassium iodide (1 g) and 0.5 g iodine were dissolved in 100 ml distilled water for preparing IKI solution. The preserved material of anthers were transferred to a centrifuge tube and crushed with a glass rod. Pollen grains were placed in IKI solution for 10 minutes and viability was accessed by counting the dark brown or red coloured pollen grains (Sulisoglu and Cavusoglu, 2014).

$$\text{Pollen viability percentage} = \frac{\text{Number of fully stained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

3.2.3.4 Pollen fertility

Pollen stainability is an index of fertility and was determined by staining pollen grains by acetocarmine glycerin staining technique. A modification of the technique followed by Marks (1954) was used for the study. Sample of anthers were kept in clean slide. To this one drop of acetocarmine was added and macerated to release the pollen grains. Debris were removed and one drop of glycerine was added over this and mixed once again using needle and covered by cover slip. After 10 minutes the slides were examined under stereo microscope. The number of deep red stained and unstained pollen grains were counted. The deep red stained pollen grains were considered as fertile whereas the unstained, undersized, partially stained and shriveled pollen grains were counted as sterile. The pollen fertility was calculated using the formula

Pollen fertility %= Number of fully stained pollen grains X 100

Total number of pollen grains

3.2.4 Hybridization

On selected 5 clove plants (P_1, P_2, P_3, P_4 and P_5) 20 buds were selected for hybridization. Based on the standardized emasculation technique, maximum stigma receptivity and pollen storage studies, hybridization was carried out and percentage fruit set worked out. The crossing was done in all combinations of 5 clove trees taking each as male and female parents. The cross combination taken for hybridization is detailed below.

Table 2. Cross combination of selected clove plants for hybridization

Sl No.	Female parent	Male parent	Crossing combination
1.	P_1	P_2	T_1 (P_1 X P_2)
2.	P_1	P_3	T_2 (P_1 X P_3)
3.	P_1	P_4	T_3 (P_1 X P_4)
4.	P_1	P_5	T_4 (P_1 X P_5)
5.	P_2	P_1	T_5 (P_2 X P_1)
6.	P_2	P_3	T_6 (P_2 X P_3)
7.	P_2	P_4	T_7 (P_2 X P_4)
8.	P_2	P_5	T_8 (P_2 X P_5)
9.	P_3	P_1	T_9 (P_3 X P_1)
10.	P_3	P_2	T_{10} (P_3 X P_2)
11.	P_3	P_4	T_{11} (P_3 X P_4)
12.	P_3	P_5	T_{12} (P_3 X P_5)
13.	P_4	P_1	T_{13} (P_4 x P_1)
14.	P_4	P_2	T_{14} (P_4 X P_2)
15.	P_4	P_3	T_{15} (P_4 X P_3)
16.	P_4	P_5	T_{16} (P_4 X P_5)
17.	P_5	P_1	T_{17} (P_5 X P_1)
18.	P_5	P_2	T_{18} (P_5 X P_2)
19.	P_5	P_3	T_{19} (P_5 X P_3)
20.	P_5	P_4	T_{20} (P_5 X P_4)

3.2.4.1 Procedure for artificial pollination

3.2.4.1.1 Selection of female and male parents

Five clove plants were selected and designated as P_1, P_2, P_3, P_4 and P_5. Each of them were taken both as female and male parent. Thus 20 cross combination were made.

3.2.4.1.2 Identification of buds for emasculation

Buds that open after 36h was selected in the inflorescence and tagged as female parent and remaining buds were removed. In an inflorescence 1 or 2 buds were selected and remaining buds were removed.

3.2.4.1.3 Emasculation and Bagging

On the selected buds petals were removed by using foreceps thus exposing the stamens. Stamens were removed using a needle by scooping the base of the stamens at their position of attachment to the calyx tube there by removing most of the stamens. The remaining stamens were removed by using foreceps. It is better to remove the stamen from the base to avoid falling of the anthers on the stigma. Emasculated flowers should be immediately enclosed in butter covers and tagged once again.

3.2.4.1.4 Pollination

The optimal time for pollination was six days after emasculation that is five days after anthesis. Buds that open after 12 hours were taken and petals were removed. The flowers were tapped on the petriplates to collect pollen. Then using a brush the pollen was brushed on the stigma of the emasculated clove flowers after removing the butter cover. This was once again covered with butter paper and labelled.

3.2.4.1.5 Percentage of fruit set

The percentage of fruit set was carried out by removing butter covers and checking whether the fertilized flowers exhibited a pronounced swelling of the calyx tube indicating the success of pollination. This was undertaken at 25 days after pollination.

$$\text{Percentage of fruitset} = \frac{\text{Fertilized flowers}}{\text{Number of flowers pollinated}} \times 100$$

3.2.5 Incidence of diseases

3.2.5.1 Scoring of diseases

The clove trees were observed to identify the incidence of any diseases. Clove trees were affected by *Colletotrichum gleosporoides* and the symptoms appeared as leafspot. Disease severity (Percentage Disease Index) was worked out in clove based on the score developed for leaf anthracnose of mango (Awa *et al*., 2012).

Scale 0 to 4 was used where

Scale 0 No disease symptoms on leaves
Scale 1 1-25% of leaf area covered with spots
Scale 2 26-50% of leaf area covered with spots
Scale 3 51-75% of leaf area covered with spots
Scale 4 76% or more

Disease severity (Percentage Disease Index) were obtained by the following formula:

$$\text{Disease severity (\%)} = \frac{\text{Sum of all diseased leaves ratings or scores}}{\text{Total number of leaves examined x maximum score}} \times 100$$

3.2.6 Incidence of pests

3.2.6.1 Scoring for pests

The selected trees were monitored for any incidence of pests during the experimental period of one year.

RESULTS

4. RESULTS

Survey conducted by the Department of Plantation Crops and Spices, College of Agriculture, Vellayani in different clove growing locations of Southern Kerala and Kanyakumari districts of Kerala from 2016 to 2018 has identified 20 accessions which showed high yield and special characters. The twenty accessions were evaluated for a period of two years and had identified twelve elite accessions. These twelve elite accessions were further evaluated for two more years for quantitative characters. Along with that observation of floral phenology and biology, method of emasculation, pollen collection, pollen storage and hybridization were standardized. The results of the study on "Evaluation of elite clove (*Syzigium aromaticum* (L) Merr. & Perry) accessions and standardization of pollination techniques" are presented in this chapter.

4.1 EVALUATION OF SELECTED CLOVE ACCESSIONS

4.1.1 Quantitative characters

4.1.1.1 Tree characters

Tree characters of selected clove accessions such as plant height, girth at 30 cm, canopy spread and number of branches are presented in Table 3.

4.1.1.1.1 Plant height

The plant height of twelve elite clove accessions extended from 6.15 m in AMC-13 to 15.30 m in MRC-7. The highest plant height among the accessions observed was in MRC-7 (15.30 m) followed by BRC-3 (14.10 m). Lowest plant height was recorded from the accession AMC-13 that showed 6.15 m.

4.1.1.1.2 Girth

The girth at 30 cm height varied from 70 cm in BRC-2 to 165 cm in the accessions BRC-3. BRC-3 had the highest girth at 30 cm height followed by MRC-7 (128 cm). The girth

at 30 cm for BRC-4 was 125 cm, MRC-6 was 124 cm, MRC-5 was 122 cm and BRC-1 was 120 cm.

4.1.1.1.3 Canopy spread

The canopy spread in North South extended from 4.80 m in AMC-12 which was the lowest to 7.70 m in MRC-7 which was the highest. The canopy spread in East West extended from 5.00 m in AMC-12 to 8.10 m in MRC-7. The canopy spread N-S and E-W was the highest for MRC-7 and the lowest for AMC-12.

4.1.1.1.4 Number of branches

The number of branches in the clove accessions ranged from 40 to 57. The highest number of branches was observed in AMC-11 (57) followed by BRC-3 (56) and the lowest number of branches is observed in AMC-12 (40). The number of branches for BRC-1 was 52, BRC-2 was 45, BRC-4 was 49, MRC-5 was 48, MRC-6 was 42, MRC-7 was 53, MRC-8 was 43, AMC-10 was 53 and AMC-13 was 42.

4.1.1.2 Leaf characters

The characters of leaf such as leaf length, leaf breadth and leaf area of elite clove accessions are presented in Table 4.

Table 3. Tree characters of selected clove accessions

Sl. no.	Accession	Plant height (m)	Girth at 30 cm	Canopy spread (m)		Number of branches
				N-S	E-W	
1	BRC-1	10.20	120	6.70	6.30	52
2	BRC-2	9.80	70	5.30	5.30	45
3	BRC-3	14.10	165	6.20	6.90	56
4	BRC-4	6.51	125	5.10	6.20	49
5	MRC-5	6.80	122	5.60	6.70	48
6	MRC-6	7.80	124	7.60	7.00	42
7	MRC-7	15.30	128	7.70	8.10	53
8	MRC-8	9.80	120	6.70	6.10	43
9	AMC-10	7.70	100	5.50	6.40	53
10	AMC-11	7.10	104	7.50	8.00	57
11	AMC-12	6.60	100	4.80	5.00	40
12	AMC-13	6.15	103	7.40	6.80	42

4.1.1.2.1 Leaf length

The leaf length of clove accessions varied from 9.59 cm in BRC-3 to 13.98 cm in MRC-5. The highest leaf length was observed in MRC-5 followed by AMC-11 (13.95 cm) and AMC-13 (13.56 cm).

4.1.1.2.2 Leaf breadth

The leaf breadth of clove accessions varied from 3.81 cm in AMC-12 to 4.75 cm in AMC-11. The highest leaf breadth was observed in AMC-11 followed by BRC-3 (4.65 cm) and BRC-4 (4.45 cm).

4.1.1.2.3 Leaf area

The leaf area extended from 25.35 cm^2 in MRC-7 to 43.00 cm^2 in AMC-11. The highest leaf area was observed in MRC-7 (43.00 cm^2) followed by MRC-5 (39.01 cm^2) and AMC-13 (36.78 cm^2).

4.1.1.3 Bud characters

Bud characters of selected clove accessions such as number of inflorescence/m^2, number of flower buds/inflorescence, single bud weight (fresh), single bud weight (dry), mature bud length and mature bud diameter are presented in Table 5.

4.1.1.3.1 Number of inflorescence/m^2

The number of inflorescence/m^2 was the highest in AMC-12 (156.25) followed by AMC-10 (117.10) and BRC-1 (115.25). The lowest number of inflorescence/m^2 was observed in MRC-8 (53.70).

Table 4. Leaf characters of selected clove accessions

Sl. No.	Accession	Leaf length (cm)	Leaf breadth (cm)	Leaf area (cm^2)
1	BRC-1	11.3	4.31	31.60
2	BRC-2	12.11	4.10	32.22
3	BRC-3	9.59	4.65	28.94
4	BRC-4	11.45	4.45	33.06
5	MRC-5	13.98	4.30	39.01
6	MRC-6	11.60	4.10	30.86
7	MRC-7	10.20	3.83	25.35
8	MRC-8	12.30	4.14	33.04
9	AMC-10	10.62	3.96	27.29
10	AMC-11	13.95	4.75	43.00
11	AMC-12	10.43	3.81	25.79
12	AMC-13	13.56	4.18	36.78

4.1.1.3.2 Number of flower buds/inflorescence

The number of flower buds/inflorescence is the highest in MRC-6 (17.25) and the lowest in BRC-3 (8.10). The number of flower buds/inflorescence in BRC-4 was 11.90, BRC-1 and MRC-5 was 10.50.

4.1.1.3.3 Single bud weight (fresh)

The single bud weight (fresh) among the clove accessions ranged from 219.30 mg to 398.72 mg. The highest single bud weight was observed in BRC-3 (398.72 mg) followed by MRC-5 (343.29 mg), MRC-6 (342.20 mg), BRC-1 (312.58 mg) and MRC-7 (310.57 mg). The least single bud weight (fresh) was observed in AMC-13 (219.30 mg).

4.1.1.3.4 Single bud weight (dry)

The single bud weight (dry) among the clove accessions ranged from 73.00 mg in AMC-13 to 127.26 mg in BRC-3 which recorded the highest single dry bud weight. The single bud weight (dry) of BRC-1 was 116.30 mg while that for MRC-8 was 108.67 mg and that for MRC-7 was 105.28 mg. The single dry bud weight of MRC-5 and MRC-6 were 94.74 mg and 90.24 mg, respectively.

4.1.1.3.5 Mature bud length

The mature bud length among the clove accessions ranged from 14.56 mm in BRC-2 which was the lowest to 19.34 mm in BRC-1 which was the highest. Mature bud length of BRC-4 was 18.82 mm and that for AMC-10 was 18.58 mm.

Table 5. Bud characters of selected clove accessions

Sl. No.	Accession	Number of inflorescence/m^2	Number of flower buds /inflorescence	Single bud weight (fresh) (mg)	Single bud weight (dry) (mg)	Mature bud length (mm)	Mature bud diameter (mm)
1	BRC-1	115.25	10.50	312.58	116.30	19.34	5.97
2	BRC-2	71.25	9.70	232.60	87.00	14.56	5.09
3	BRC-3	74.50	8.10	398.72	127.26	17.56	6.45
4	BRC-4	80.20	11.90	305.43	107.00	18.82	5.81
5	MRC-5	76.50	10.50	343.29	94.74	16.08	4.67
6	MRC-6	97.46	17.25	342.20	90.24	18.02	5.86
7	MRC-7	66.50	8.30	310.57	105.28	16.78	4.87
8	MRC-8	53.70	8.30	284.75	108.67	16.76	4.85
9	AMC-10	117.10	9.80	280.20	91.17	18.58	5.89
10	AMC-11	88.31	7.45	272.35	90.21	18.54	5.95
11	AMC-12	156.25	6.09	256.74	87.32	17.14	5.24
12	AMC-13	100.20	8.70	219.30	73.00	14.76	5.43

4.1.1.3.6 Mature bud diameter

The mature bud diameter among the clove accessions varied from 4.67 mm to 6.45 mm. Mature bud diameter was the highest in BRC-3 (6.45 mm) followed by BRC-1 (5.97 mm) and AMC-11 (5.95 mm). The mature bud diameter was the least in MRC-5 (4.67 mm).

4.1.1.4 Flower characters

The flower characters of selected clove accessions such as length of flower, breadth of flower, length of sepal, length of petal and flowering period are presented in Table 6.

4.1.1.4.1 Length of flower

Clove accessions selected showed varied length of flower ranging from 14.98 mm in AMC-12 to 21.86 mm in BRC-1. The length of flower in BRC-4 was 21.45 mm followed by MRC-6 with 21.43 mm.

4.1.1.4.2 Breadth of flower

The breadth of flower among the clove accessions in BRC-2 was 11.47 mm which was the least. Maximum breadth of flower is observed in BRC-3 (13.89 mm) followed by MRC-6 (13.76 mm).

4.1.1.4.3 Length of sepal

The length of sepal among the clove accessions ranged from 2.54 mm to 3.16 mm. Maximum length of sepal was observed in BRC-1 (3.16 mm) and minimum length of sepal was observed in MRC- 5 (2.54 mm).

Table 6. Flower characters of selected clove accessions

Sl. No.	Accession	Length of flower (mm)	Breadth of flower (mm)	Length of sepal (mm)	Length of petal (mm)	Flowering period
1	BRC-1	21.86	12.37	3.16	5.83	Dec-Feb
2	BRC-2	18.15	11.47	2.96	5.42	Jan-Mar
3	BRC-3	20.19	13.89	2.74	6.15	Jan-Mar
4	BRC-4	21.45	12.93	2.90	5.76	Jan-Mar
5	MRC-5	20.27	12.68	2.54	4.72	Dec-Feb
6	MRC-6	21.43	13.76	2.84	5.44	Jan-Mar
7	MRC-7	20.87	13.04	2.82	4.36	Jan-Mar
8	MRC-8	21.08	11.63	3.11	4.26	Jan-Mar
9	AMC-10	20.38	12.97	2.75	5.49	Jan-Mar
10	AMC-11	19.49	12.29	2.74	5.74	Jan-Mar
11	AMC-12	14.98	12.42	2.73	5.33	Jan-Mar
12	AMC-13	19.73	11.89	2.86	5.72	Dec-Feb

4.1.1.4.4 Length of petal

The length of petal among the clove accessions varied from 4.26 mm in MRC-8 to 6.15 mm in BRC-3.

4.1.1.4.5 Flowering period

. Flowering period of accessions BRC-1, MRC-5 and AMC-13 was from December to February. For the remaining accessions BRC-2, BRC-3, BRC-4, MRC-6, MRC-7, MRC-8, AMC-10, AMC-11 and AMC-12, flowering period started from January to March.

4.1.1.5 Yield characteristics of elite clove accessions

The bud yield per tree (fresh), bud yield per tree (dry) of elite clove accessions are presented in Table 7.

4.1.1.5.1 Bud yield/tree (fresh)

Table 7 represents fresh bud yield/tree and dry bud yield/tree. The fresh bud yield per tree during the year 2018-19 was 25.00 kg for BRC-1 followed by 24.50 kg for AMC-10, 24.00 kg for BRC-4 and 23.00 kg for MRC-7. The fresh bud yield/tree for MRC-5 and MRC-6 were 20.00 kg and 21.00 kg respectively for the year 2018-2019.

During the period of 2019-2020 the fresh bud yield per tree was the highest for MRC-7 (25.00 kg) followed by MRC-5 (24.00 kg). For all the other accessions such as BRC-1 (19.00 kg), BRC-2 (8.00 kg), BRC-3 (2.00 kg), BRC-4 (11.00 kg), MRC-6 (3.50 kg), MRC-8 (14.00 kg), AMC-10 (21.25 kg), AMC-11 (15.00 kg), AMC-12 (3.00 kg) and AMC-13 (15.00 kg) showed decreased yield compared to the previous year.

The pooled mean of fresh bud yield per tree of selected clove accessions ranged from 6.50 kg in AMC-12 to 24.00 kg in MRC-7. The higher yielders in terms of fresh bud yield per tree were MRC-7 (24.00 kg), AMC-10 (22.87 kg), BRC-1 (22.00 kg), MRC-5 (22.00 kg),

BRC-4 (17.50 kg) and AMC-11 (17.25 kg). Among the accessions BRC-1, MRC-5, MRC-7 and AMC-10 were giving consistent yield during 2018-2019 and 2019-2020.

4.1.1.5.3 Bud yield/tree (dry)

The dry bud yield/tree during the year 2018-2019 ranged from 3.40 kg to 8.50 kg. The dry bud yield per tree during the year 2018-19 was 8.50 kg for BRC-1 followed by 8.40 kg for BRC-4, 7.87 kg for AMC-10 and 7.79 kg for MRC-7. The dry bud yield/tree for MRC-5 and MRC-6 were 5.51 kg and 5.53 kg respectively for the year 2018-2019.

The dry bud yield/tree during the year 2019-2020 ranged from 0.63 kg to 8.47 kg. The dry bud yield per tree during the year was the highest for MRC-7 (8.47 kg) followed by AMC-10 (6.91 kg), MRC-5 (6.62 kg) and BRC-1 (6.46 kg).

The bud yield per tree dry was the highest for MRC-7 (8.13 kg/tree) followed by BRC-1 (7.48 kg/tree) and the least observed in AMC-12 (2.21 kg/tree).

Variability in yield among the selected clove accessions were analysed using ANOVA and it showed non-significance. The yield among the accessions was the highest in MRC-7 (8.13 kg) followed by BRC-1 (7.48 kg), AMC-10 (7.39 kg), BRC-4 (6.12 kg) and MRC-5 (6.06 kg).

Table 7. Yield characteristics of elite clove accessions

Sl. No.	Accession	Fresh bud yield per tree (Kg)			Dry bud yield per tree (Kg)		
		2018-2019	2019-2020	Pooled mean	2018-2019	2019-2020	Pooled mean
1	BRC-1	25.00	19.00	22.00	8.50	6.46	7.48
2	BRC-2	15.00	8.00	11.50	5.61	2.99	4.30
3	BRC-3	18.00	2.00	10.00	5.74	0.63	3.18
4	BRC-4	24.00	11.00	17.50	8.40	3.85	6.12
5	MRC-5	20.00	24.00	22.00	5.51	6.62	6.06
6	MRC-6	21.00	3.50	12.25	5.53	0.99	3.26
7	MRC-7	23.00	25.00	24.00	7.79	8.47	8.13
8	MRC-8	17.00	14.00	15.50	6.48	5.34	5.91
9	AMC-10	24.50	21.25	22.87	7.87	6.91	7.39
10	AMC-11	19.50	15.00	17.25	6.45	4.96	5.70
11	AMC-12	10.00	3.00	6.50	3.40	1.02	2.21
12	AMC-13	18.00	15.00	16.50	5.99	4.99	5.49
		NS			NS		

4.1.1.6 Fruit and Seed characters

The fruit characters including fruit weight (fresh), ratio of fruit to seed, and seed characters including seed length, seed breadth and seed weight are presented in Table 8.

4.1.1.6.1 Fruit weight (fresh)

The fruit weight (fresh) of clove accessions ranged from 1.21 g in AMC-12 to 3.54 g in BRC-3. The fruit weight (fresh) of AMC-11 was 3.38 g followed by BRC-4 with 2.87 g. The fruit weight of MRC-7 was 2.55 g while that for MRC-5, 6 and 8 were 2.48 g, 2.41 g and 2.43 g, respectively.

4.1.1.6.2 Ratio of fruit to seed

The ratio of fruit to seed was the highest in AMC-13 (3.07) and was the least in MRC-6 (2.38). The ratio of fruit to seed in various elite clove accessions such as BRC-4 (3.05), BRC-3 (2.95), AMC-10 (2.93), MRC-8 (2.92), BRC-2 (2.85), AMC-11 (2.81), MRC-5 (2.72), BRC-1 (2.64), MRC-7 (2.45), AMC-12 (2.42) and MRC-6 (2.38) were observed.

4.1.1.6.3 Seed length

The seed length of twelve elite clove accessions ranged from 13.47 mm in AMC-12 to 19.40 mm in BRC-3. The seed length of BRC-1 was 18.74 mm, BRC-2 was 16.75 mm, BRC-4 was 17.52 mm, MRC-5 was 17.61 mm, MRC-6 was 17.30 mm, MRC-7 was 16.40 mm, MRC-8 was 17.65 mm, AMC-10 was 16.68 mm, AMC-11 was 18.94 mm, AMC-12 was 13.47 mm and AMC-13 was 18.35 mm.

Table 8. Fruit and seed characters of selected clove accessions

Sl. No.	Accession	Fruit weight (fresh) (g)	Ratio of fruit to seed	Seed length (mm)	Seed breadth (mm)	Seed weight (g)
1	BRC-1	2.70	2.64	18.74	8.34	1.02
2	BRC-2	2.37	2.85	16.75	7.89	0.83
3	BRC-3	3.54	2.95	19.40	9.24	1.20
4	BRC-4	2.87	3.05	17.52	8.57	0.94
5	MRC-5	2.48	2.72	17.61	8.24	0.91
6	MRC-6	2.41	2.38	17.30	8.31	1.01
7	MRC-7	2.55	2.45	16.40	8.30	1.04
8	MRC-8	2.43	2.92	17.65	7.58	0.83
9	AMC-10	2.05	2.93	16.68	7.55	0.70
10	AMC-11	3.38	2.81	18.94	8.94	1.20
11	AMC-12	1.21	2.42	13.47	6.31	0.50
12	AMC-13	3.10	3.07	18.35	8.87	1.01

4.1.1.6.4 Seed breadth

The seed breadth of elite clove accessions ranged from 6.31 mm in AMC-12 to 9.24 mm in BRC-3. The seed breadth was the highest in BRC-3 followed by AMC-11 (8.94 mm), AMC-13 (8.87 mm) and BRC-4 (8.57 mm).

4.1.1.6.5 Seed weight

The seed weight recorded was the highest in BRC-3 and AMC-11 and was the least in AMC-12 which had only 0.50 g. The seed weight observed in BRC-1 was 1.02 g, BRC-2 was 0.83 g, BRC-3 was 1.20 g, BRC-4 was 0.94 g, MRC-5 was 0.91 g, MRC-6 was 1.01 g, MRC-7 was 1.04 g, MRC-8 was 0.83 g, AMC-10 was 0.70 g, AMC-11 was 1.20 g and AMC-13 was 1.01 g.

4.1.1.7 Incidence of diseases

During the evaluation of elite clove accessions, leaf spot due to *Colletotrichum gloeosporioides* was noticed in elite clove accessions. Percentage disease index or disease severity of twelve elite accessions is presented in Table 9. Disease severity was the highest in BRC-2 (28.33 per cent) and the lowest in MRC-6 (15.00 per cent).

4.1.1.8 Incidence of pests

There was no pest incidence in the selected clove accessions

Table 9. Disease severity of selected clove accessions

Sl.no	Accessions ID	Disease severity (%)
1.	BRC-1	18.75
2.	BRC-2	28.33
3.	BRC-3	17.50
4.	BRC-4	26.25
5.	MRC-5	23.75
6.	MRC-6	15.00
7.	MRC-7	22.50
8.	MRC-8	23.75
9.	AMC-10	16.60
10.	AMC-11	23.30
11.	AMC-12	16.60
12.	AMC-13	25.00

Table10. Descriptive statistics for quantitative characters of clove

Characters	Minimum	Maximum	Mean	Std. Deviation
Plant height	6.15	15.30	8.98	3.016
Girth	70.00	165.00	115.08	22.741
N-S canopy spread	4.80	7.70	6.34	1.062
E-W canopy spread	5.00	8.10	6.56	0.918
Number of Branches	40.00	57.00	48.33	5.882
Leaf length	9.59	13.98	11.75	1.474
Leaf breadth	3.81	4.75	4.21	0.295
Leaf area	25.35	43.00	32.24	5.311
Number of inflorescence/m^2	53.70	156.25	91.43	28.024
Number of buds/inflorescence	6.09	17.25	9.71	2.839
Length of Flower	14.98	21.86	19.99	1.876
Breadth of Flower	11.47	13.89	12.61	0.760
Length of Sepal	2.54	3.16	2.84	0.171
Length of Petal	4.26	6.15	5.35	0.597
Single Bud Weight fresh	219.30	398.72	296.56	50.338
Single Bud Weight dry	73.00	127.26	98.18	15.005
Mature bud length	14.56	19.34	17.24	1.544
Mature bud diameter	4.67	6.45	5.50	0.560
Bud yield fresh	6.50	24.00	16.48	16.489
Bud yield dry	2.21	8.13	5.43	5.437
Fruit weight	1.21	3.54	2.59	0.617
Ratio of Fruit to Seed	2.38	3.07	2.76	0.243
Seed length	13.47	19.40	17.40	1.554
Seed breadth	6.31	9.24	8.17	0.782
Seed weight	0.50	1.20	0.93	0.199

4.1.2 Descriptive statistics for quantitative characters of clove

4.1.2.1 Tree characters

The plant height of selected clove accessions ranged from 6.15 m in to 15.30 m in MRC-7 with average plant height (8.98 m) and standard deviation of 3.016. The girth at 30 cm height varied from 70 cm in BRC-2 to 165 cm in BRC-3 among the selected clove accessions with average girth (115.08 cm) and standard deviation of 22.741. The canopy spread in North-South extended from 4.80 m in AMC-12 to 7.70 m in MRC-7 with average canopy spread in North-South (6.34) and standard deviation of 1.062. The canopy spread in East-West extended from 5.00 m in AMC-12 to 8.10 m in MRC-7 with average canopy spread in East-West (6.56) and standard deviation of 0.918. The number of branches in the clove accessions ranged from 40 in AMC-12 to 57 in AMC-11 with average number of branches (48.33) and standard deviation of 5.882.

4.1.2.2 Leaf characters

The leaf length of clove accessions varied from 9.59 cm in BRC-3 to 13.98 cm in MRC-5 with average leaf length of 11.75 cm and standard deviation of 1.474. The leaf breadth of clove accessions varied from 3.81 cm in AMC-12 to 4.75 cm in AMC-11 with average leaf breadth of 4.21 cm and standard deviation of 0.295. The leaf area extended from 25.35 cm^2 in MRC-7 to 43.00 cm^2 in AMC-11 with average leaf area of 32.24 cm^2 and standard deviation of 5.311.

4.1.2.3 Bud characters

The number of inflorescence/m^2 was the highest in AMC-12 (156.25) and lowest in MRC-8 (53.70) with average number of inflorescence/m^2 (91.43) and standard deviation of 28.034. The number of flower buds/inflorescence is highest in MRC-6 (17.25) and lowest in BRC-3 (8.10) with average number of flower buds/inflorescence (9.71) and standard deviation of 2.839. The single bud weight (fresh) among the clove accessions ranged from 219.30 mg in AMC-13 to 398.72 mg in BRC-3 with average single bud weight fresh (296.56 mg) and standard deviation of 50.338. The single bud weight (dry) among the clove accessions ranged from 73.00 mg in AMC-13 to 127.26 mg in BRC-3 with average single dry bud weight (98.18

mg) and standard deviation of 15.003. The mature bud length among the clove accessions ranged from 14.56 mm in BRC-2 to 19.34 mm in BRC-1 with average mature bud length (17.24 mm) and standard deviation of 1.544. The mature bud diameter among the clove accessions varied from 4.67 mm inMRC-5 to 6.45 mm in BRC-3 with average mature bud diameter (5.50 mm) and standard deviation of 0.560.

4.1.2.4 Flower characters

Clove accessions selected showed varied length of flower ranging from 14.98 mm in AMC-12 to 21.86 mm in BRC-1 with average length of flower (19.99 mm) and standard deviation of 1.876. The breadth of flower among the clove accessions ranged from 11.47 mm in BRC-2 to 13.89 mm in BRC-3 with average breadth of flower (12.61 mm) and standard deviation of 0.760. The length of sepal among the clove accessions ranged from 2.54 mm in MRC-5 to 3.16 mm in BRC-1 with average length of sepal (2.84 mm) and standard deviation of 0.171. The length of petal among the clove accessions varied from 4.26 mm in MRC-8 to 6.15 mm in BRC-3 with average length of petal (5.35 mm) and standard deviation of 0.597).

4.1.2.5 Yield characters

The pooled mean of fresh bud yield per tree of selected clove accessions ranged from 6.50 kg in AMC-12 to 24.00 kg in MRC-7 with average fresh bud yield (16.48) and standard deviation of 16.489. The pooled mean of dry bud yield per tree of selected clove accessions ranged from 2.21 kg in AMC-12 to 8.13 kg in MRC-7 with average dry bud yield (5.43 kg) and standard deviation of 5.437.

4.1.2.6 Fruit and seed characters

The fruit weight (fresh) of clove accessions ranged from 1.21 g in AMC-12 to 3.54 g in BRC-3 with average fruit weight (2.59 g) and standard deviation of 0.617. The ratio of fruit to seed was the highest in AMC-13 (3.07) and was the least in MRC-6 (2.38) with average ratio of fruit to seed (2.76) and standard deviation of 0.243. The seed length of twelve elite clove accessions ranged from 13.47 mm in AMC-12 to 19.40 mm in BRC-3 with average seed length (17.40 mm) and standard deviation of 1.554. The seed breadth of elite clove accessions ranged from 6.31 mm in AMC-12 to 9.24 mm in BRC-3. The seed weight recorded was the highest

in BRC-3 (1.20 g) and AMC-11 (1.20 g) and was the least from AMC-12 which had only 0.5 g with average seed weight (0.93 g) and standard deviation of 0.199.

4.1.3 Quantitative characterization

A multivariate analysis of the quantitative characters *viz*., plant height, girth at 30cm height, canopy spread (N-S), Canopy spread (E-W), number of branches, leaf length, leaf breadth, leaf area, number of inflorescence/m^2, number of buds/inflorescence, length of flower, breadth of flower, length of sepal, length of petal, single bud weight (fresh), single bud weight (dry), mature bud length, mature bud diameter, bud yield/tree (fresh), bud yield/tree (dry), fruit weight (fresh), ratio of fruit to seed, seed length, seed breadth, seed weight were carried out by using STATA software. As observations were taken only from single tree, the clustering of the accessions based on quantitative characters were carried out using both Cluster analysis and Multidimensional scaling.

Multi Dimensional Scaling is a multivariate analysis technique that serves as a dimension reduction technique for large dimensional data. Multidimensional scaling was carried out to determine the selection criteria and for identification of elite genotype. Based on the scree plot of MDS, first 2 dimensions are selected for generating the biplot (Figure 1). The 25 variables with 12 observations in Multi Dimensional Scaling computed 10 dimensions.

The contribution of MDS Dimension-1 and Dimension-2 were 75.76 and 17.21 percent with cumulative variance of 92.97 per cent respectively. The details of eigen values of Multi Dimensional Scaling is presented in Table 11.

The eigen vectors designated as Dimension-1 and Dimension-2 with the respective component loadings of all 25 variable characters are given in the Table 12. Dimension-1 had negative loadings for number of inflorescence/m^2 and positive loadings like girth at 30cm height, single bud weight fresh, single bud weight dry. Dimension-2 had positive loadings for Number of inflorescence/m^2. Multi Dimensional Scaling Configuration is presented in Figure 2. Based on Multi Dimensional Scaling Configuration eight clusters of selected clove accessions were identified.

Table 11. Classical metric Multi Dimensional Scaling

Dimension	Eigen value	Absolute value (eigen value)		(eigen value)^2	
		Percent	Cumulative	Percent	Cumulative
1.	34929.373	75.76	75.76	94.88	94.88
2.	7934.4915	17.21	92.97	4.90	99.77
3.	1352.0084	2.93	95.90	0.14	99.91
4.	865.1567	1.88	97.78	0.06	99.97
5.	508.4887	1.10	98.88	0.02	99.99
6.	258.07833	0.56	99.44	0.01	100.00
7.	162.59568	0.35	99.79	0.00	100.00
8.	58.109002	0.13	99.92	0.00	100.00
9.	26.614756	0.05	99.97	0.00	100.00
10.	10.099913	0.02	99.99	0.00	100.00

4.1.4 Scree plot of Eigen values of MDS

A scree plot shows how much variation each dimension captures from the data. The y axis is eigenvalues, which essentially stand for the amount of variation. Use a scree plot to select the dimensions to keep. In Figure 1, just dimension1 and dimension 2 are enough to describe the data as it is showing variation and remaining dimensions are just flatten out.

4.1.5 Biplot coordinates for the first two dimensions of multidimensional scaling

Biplot coordinates for the first two dimensions of multidimensional scaling is presented in Table 12. Dimension-1 had negative loadings for number of inflorescence/m^2 (-2.443) and positive loadings like girth at 30 cm height (5.0396), single bud weight fresh (12.098), single bud weight dry (2.888). Dimension-2 had positive loadings for Number of inflorescence/m^2 (9.1827).

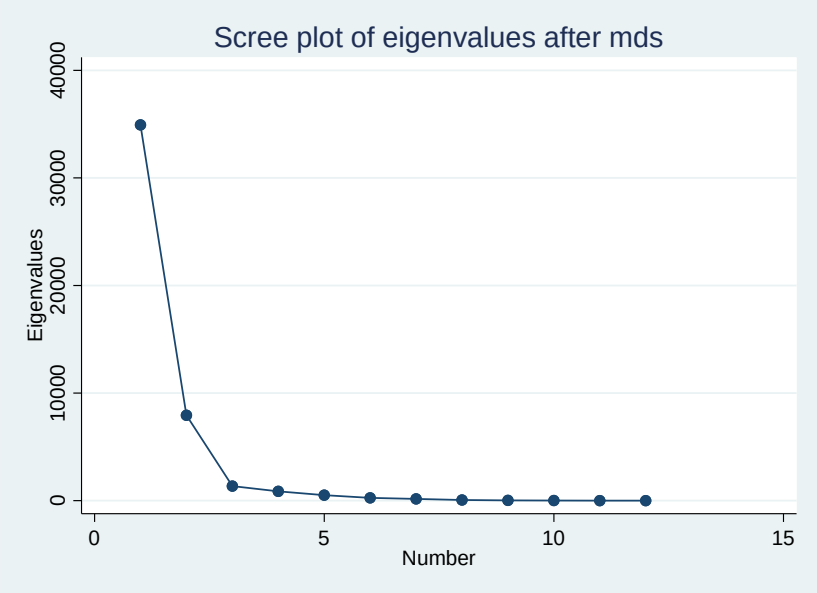


Figure 1. Scree plot of eigen values after mds

Table 12. Biplot coordinates for the first two dimensions of Multidimensional scaling

Sl. no	Characters	Dimension 1	Dimension 2
1.	Plant height	0.3806	-0.3048
2.	Girth	**5.0396**	0.4073
3.	N-S canopy spread	0.0337	-0.1036
4.	E-W canopy spread	0.0844	-0.090
5.	Number of Branches	0.6557	-0.2077
6.	Leaf length	-0.1314	-0.1936
7.	Leaf breadth	0.0285	-0.0217
8.	Leaf area	-0.1804	-0.6861
9.	Number of inflorescence/m^2	**-2.443**	**9.1827**
10.	Number of buds/inflorescence	0.1976	0.0189
11.	Single Bud Weight Fresh	**12.098**	1.8040
12.	Single Bud Weight dry	**2.888**	-0.4222
13.	Mean bud length	0.1536	0.2407
14.	Mean bud diameter	0.0447	0.0789
15.	Length of Flower	0.2358	-0.2571
16.	Breadth of Flower	0.1420	0.1010
17.	Length of Sepal	-0.0075	-0.0151
18.	Length of Petal	0.0049	0.0891
19.	Bud yield fresh	0.1249	-0.4687
20.	Bud yield dry	-0.0082	-0.2212
21.	Fruit weight	0.0286	-0.0996
22.	Ratio of Fruit to Seed	-0.0071	-0.0371
23.	Seed length	0.1598	-0.2223
24.	Seed breadth	0.0823	-0.1114
25.	Seed weight	0.0242	-0.027

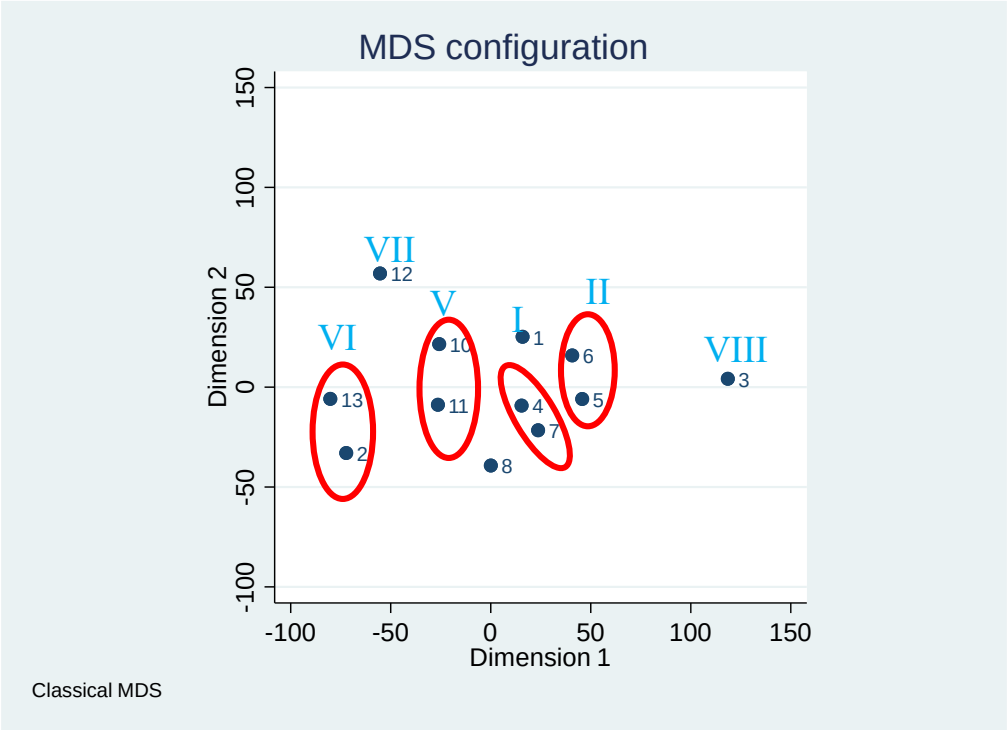


Figure 2. Identification of clusters based on MDS

(1- BRC-1, 2- BRC-2, 3- BRC-3, 4-BRC-4, 5- MRC-5, 6- MRC-6, 7- MRC-7, 8-MRC-8, 10-AMC-10, 11- AMC-11, 12-AMC-12 and 13-AMC-13)

Table 13. Identification of clusters of selected clove accessions based on Multidimensional Scaling.

Cluster	Accessions
I	BRC-1
II	MRC-5, MRC-6
III	BRC-4, MRC-7
IV	MRC-8
V	AMC-10, AMC-11
VI	BRC-2, AMC-13
VII	AMC-12
VIII	BRC-3

4.1.6 Cluster analysis

Dendrogram representing the relationship among 12 elite accessions based on Euclidean distance are presented in Figure. 2. Dendrogram gives an idea about identifying the clusters of selected clove accessions.

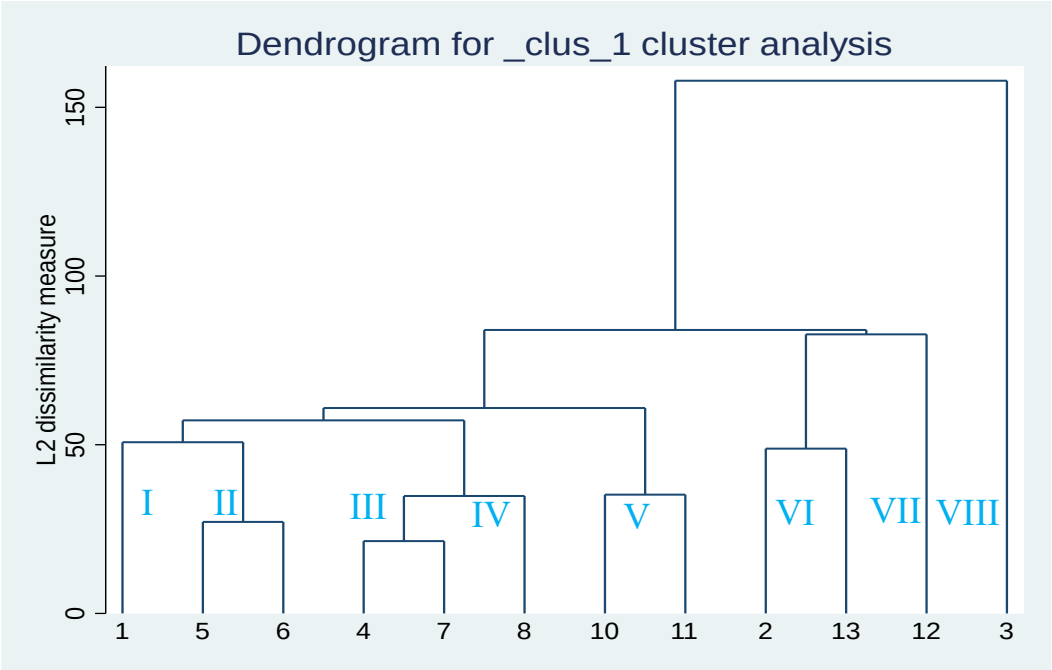


Figure 3. UPGMA dendrogram of quantitative characteristics of clove accessions

Table14. Cluster analysis of quantitative characters of clove based on UPGMA method

Quantitative cluster	Number of accessions	Cluster members
I	1	BRC-1
II	2	MRC-5, MRC-6
III	2	BRC-4, MRC-7
IV	1	MRC-8
V	2	AMC-10, AMC-11
VI	2	BRC-2, AMC-13
VII	1	AMC-12
VIII	1	BRC-3

4.1.7 Linkage between major quantitative characters of clove

The linkage was analysed based on the biplot of multidimensional scaling of first two dimensions of biplot coordinates. The biplot is justifiable by the fact that the first two dimensions explained 92.97 percent of variation. The biplot revealed that the characters number of inflorescence/m^2 (NOI), single bud weight fresh (SBWF), girth at 30 cm height (Girth), Single bud weight dry (SBWD) were the main characters. Among these the main independent character was number of inflorescence/m^2 (NOI). SBWF and NOI are independent characters. Thus the single bud weight (fresh) is independent of the number of inflorescence/m^2. Single bud weight fresh (SBWF), girth at 30 cm height (girth), single bud weight dry are associated to certain extent. The remaining variable characters are very closely related. The main causative feature for the increased bud weight (fresh and dry) was girth at 30 cm height. On the other hand, along with NOI, SBWF, Girth and SBWD, all other characters have great impact on the single bud weight (fresh).

The linkage analysed based on the biplot of two dimensions of multidimensional scaling of biplot coordinates revealed a strong positive association between most of the characters measured except, girth at 30 cm height, number of inflorescence per m^2, single bud weight fresh and single bud weight dry.

A near zero correlation was observed between number of inflorescence per $m^2$and single bud weight fresh, girth and single bud weight dry as indicated by perpendicular vectors.

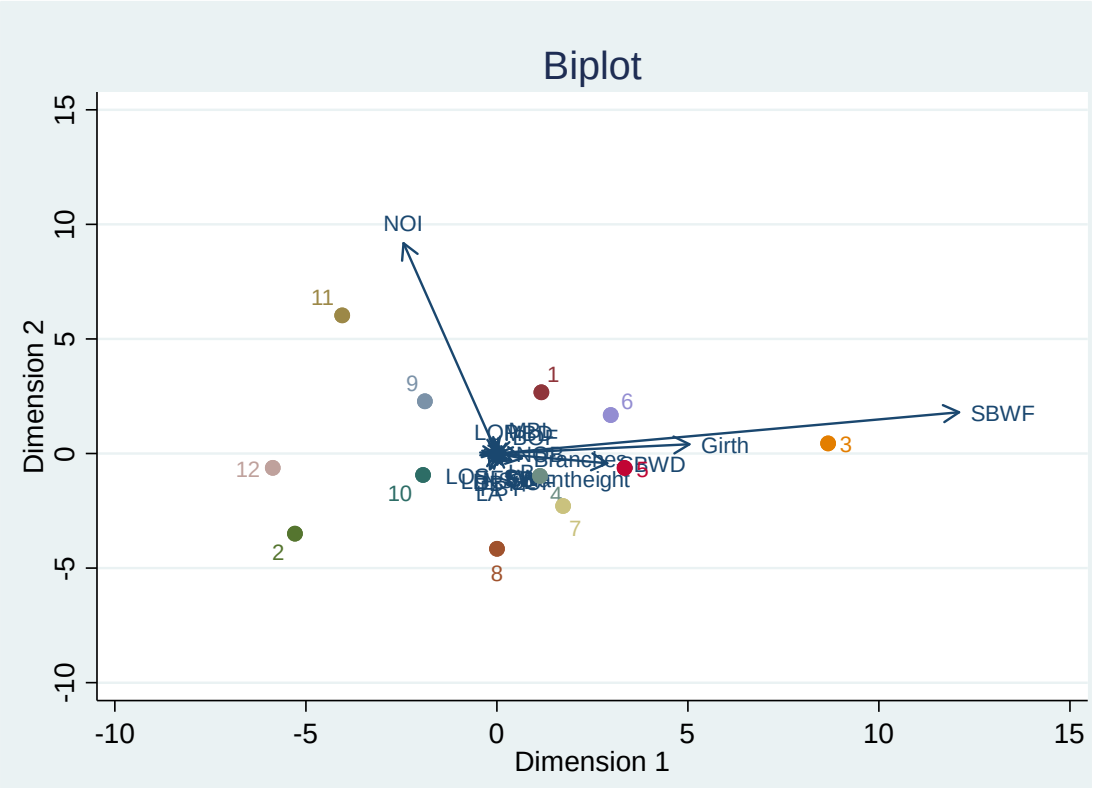


Figure 4. Linkage between major quantitative characters of clove based on Multidimensional scaling biplot

Minimum data set characters

Girth- Girth

SBWF- Single bud weight fresh

SBWD- Single bud weight dry

NOI- Number of inflorescence/m^2

(Plant height- Plant height, NS- North South canopy spread, EW- East West canopy spread, Branches- Number of branches, LL- Leaf length, LB- Leaf breadth, LA- Leaf area, NOI- Number of inflorescence/m^2 , NOB- Number of flower buds/inflorescence, SBWF-Single bud weight fresh, SBWD-Single bud weight dry, MBL- Mature bud length, MBD- Mature bud diameter, LOF- Length of flower, BOF- Breadth of flower, LOS-Length of sepal, LOP- Length of petal, LOS- Length of sepal, FBY- Fresh bud yield, DBY- Dry bud yield, FW- Fruit weight, RFS- Ratio of fruit to seed, SL-Seed length, SB- Seed breath, SW- Seed weight)
(1- BRC-1, 2- BRC-2, 3- BRC-3, 4-BRC-4, 5- MRC-5, 6- MRC-6, 7- MRC-7, 8-MRC-8, 10-AMC-10, 11- AMC-11, 12-AMC-12 and 13-AMC-13)

4.1.5 Minimum data set characters

Based on the values of the component loadings of the first two dimensions of multidimensional scaling that explained 92.97 per cent of the total variation, the minimum data set for recognizing a promising clove accession were generated. The data generated consisted of a vector and a singleton characteristic. The vector of characteristics were girth at 30 cm height, single bud weight fresh and single bud weight dry. The singleton characteristic which was different from other characters was number of inflorescence/m^2.

Data set-1

Tree girth at 30 cm height
Single bud weight (fresh)
Single bud weight (dry)

Data set-2

Number of inflorescence/m^2

Table15. Illustration of identification of an ideotype using existing data

Quantitative cluster	**Cluster members**	**Girth at 30 cm height**	**Single bud weight (fresh) (mg)**	**Single bud weight (dry) (mg)**	**Number of inflorescence/m^2**	**Effective yield (g/m^2)**	**Number of flower buds/ inflorescence**
I	BRC-1	120.00	312.58	116.30	115.25	140.73	10.50
II	MRC-5, MRC-6	123.00	342.74	92.49	86.98	111.60	13.87
III	BRC-4, MRC-7	126.50	308.00	106.14	73.35	78.63	10.10
IV	MRC-8	120.00	284.75	108.67	53.70	48.43	8.30
V	AMC-10, AMC-11	102.00	276.27	90.69	102.70	80.33	8.62
VI	BRC-2, AMC-13	86.50	225.95	80.00	85.72	63.08	9.20
VII	AMC-12	100.00	256.74	87.32	156.25	83.09	6.09
VIII	BRC-3	165.00	398.72	127.26	74.50	76.79	8.10

It revealed accessions BRC-1, MRC-5 and MRC-6 had better ideotype and can be suggested as elite or superior accessions. BRC-1 had 120 cm stem girth at 30 cm height, 312.58 mg the single bud weight fresh, 116.3 mg the single bud weight dry and the number of inflorescence/m^2 was 115.25. The effective yield worked out was 140.73 gm^{-2} which is a higher yield. On the other hand, MRC-5 and MRC-6 belonged to same cluster, cluster III had 123 cm stem girth at 30 cm height. The single bud weight fresh was 342.74 mg and single bud weight dry was 92.49 mg. The number of inflorescence m^{-2} was 86.98 and the effective yield was 111.60 gm^{-2}.

AMC-10, AMC-11, BRC-4, BRC-7 was considered as the best accessions which satisfied all the existing minimum data set characters such as girth, single bud weight (fresh), single bud weight (dry) and effective yield was greater than 75 gm^{-2}. AMC-12 and BRC-3 can also be considered as the best accessions as it satisfied all existing minimum data set characters except alternate bearing in nature.

4.2 STANDARDIZATION OF POLLINATION TECHNIQUE

4.2.1 Floral phenology and biology

4.2.1.1 Flower opening time

The flower opening time was taken from twenty branches of five selected clove plants constituting 403 flowers for a period till all flowers in all branches opened. The percentage of maximum flower opening time observed was at 4:30-5:30 pm (41.62 per cent) followed by 3:30-4:30 pm (37.68 per cent). Thus the maximum flower opened between 3.30- 5.30 pm. The details of mean flower opening time is shown in Table 16. The details of individual observation is shown in Appendix-1.

Table 16. Flower opening time in Clove

Time period	Flowers opened on each day (percentage)								Total
	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	
6:30pm-2:30pm	0	0	0	0	0	0	0	0	**0**
2:30pm-3:30pm	3.2	2.48	4.46	0.99	1.48	0.66	0.49	0.24	**14**
3:30pm-4:30pm	9.18	8.93	4.96	3.72	4.71	4.21	1.73	0.24	**37.68**
4:30pm-5:30pm	7.9	10.9	5.46	3.72	4.96	3.97	3.72	0.99	**41.62**
5:30pm-6:30pm	0.49	1.24	1.73	0.74	1.48	0.24	0.49	0	**6.41**
Total	**20.77**	**23.55**	**16.61**	**9.17**	**12.63**	**9.08**	**6.43**	**1.47**	**100**

4.2.1.2 Anthesis duration

Anthesis duration recorded is presented in Table 16. The opening of the flower was recorded from 0 to 24 hours. From 6.30 pm in the evening to 2.30 pm in the afternoon the flower buds did not open. All flower buds observed opened between 2.30 pm and 6.30 pm. Thus anthesis duration was from 2:30 pm to 6.30 pm.

4.2.1.3 Number of stamens per flower

Plate 7. Flower buds of clove at different stages

Plate 8. Branches selected for flower opening time

The details of mean number of stamens per flower is shown in Table 17. The number of stamens per flower as observed from 10 flowers of clove was counted and mean value was recorded. The number of stamens per flower varied from 167 to 343. The mean number of stamens per flower was 243.90.

Table 17. Mean number of stamens per flower in Clove

Sl.no	Number of stamens/flower
1	167
2	169
3	243
4	293
5	343
6	287
7	267
8	258
9	198
10	214
Mean	**243.9**

4.2.1.4 Anther dehiscence starting time

Anther dehiscence starting time was observed for a period of 48 h before the opening of bud is presented in Table 18. The rupturing of anthers started 36h before anthesis and continued till opening of the flowers. The percentage of maximum anthers dehisced between 36 to 24 h before flower opening and maximum dehiscence occured on 0 h of anthesis. Anther dehiscence at different stages is presented in plate 9. The details of percentage of anther dehiscence in clove is presented in Appendix 2.

4.2.1.5 Anther dehiscence ending time

Anther dehiscence ending time is presented in Table 18. Anther dehiscence noticed at 36, 24, 12 h before flower opening and 0 h of flower bud opening revealed all anthers were dehisced at the time of opening of flowers. The details of percentage of anther dehiscence in clove is presented in Appendix 2.

Table 18. Percentage of anthers dehisced in clove

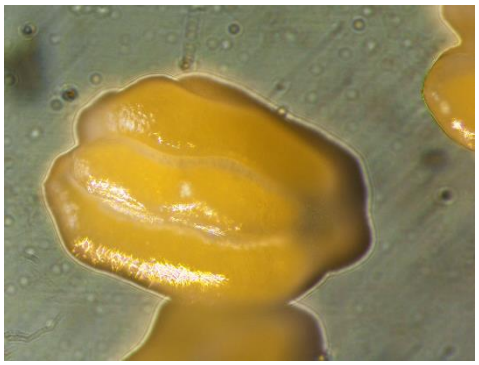
Time period	R1	R2	R3	R4	R5	Mean
48hour	0	0	0	0	0	**0**
36hour	17.96	18.93	16.46	13.31	14.28	**16.18**
24hour	89.8	75.65	81.3	85.85	92.52	**85.02**
12hour	95.5	94.59	97.34	94.33	95.76	**95.50**
0hour	98.29	98.15	98.36	98.26	98.59	**98.33**

4.2.1.6 Pollen characteristics

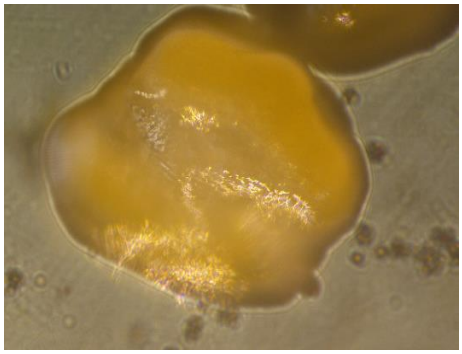
The pollen morphology studied using Scanning Electronic Microscope is presented in plate 10. The pollen grains were monad, radially symmetrical, triangular, trizonosyncolporate and were having exine ornamentation. Polar and equatorial axis of pollen grains of clove is depicted in Table 19. The polar diameter of pollen grain ranged from 17.06-18.22 µm and the equatorial diameter of pollen grain ranged from 19.40-20.70 µm.

Table 19. Polar and Equatorial axis diameter of five pollen grains of clove

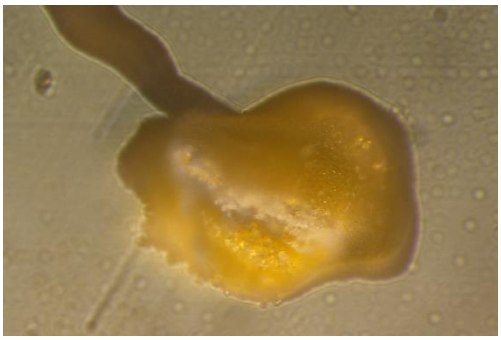
Sl. no	Polar axis diameter (µm)	Equatorial axis diameter (µm)
1	17.24	20.12
2	17.06	19.40
3	18.22	20.70
4	18.15	20.10
5	17.29	20.30
Mean	**17.59**	**20.12**



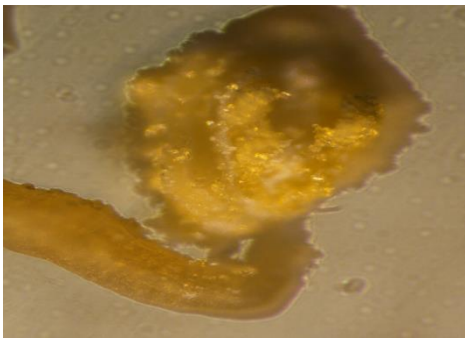
Anther at 48 h before anthesis



Anther at 36 h before anthesis



Anther at 24 h before anthesis



Anther at 12 h before anthesis

Plate 9. Anther dehiscence at different stages

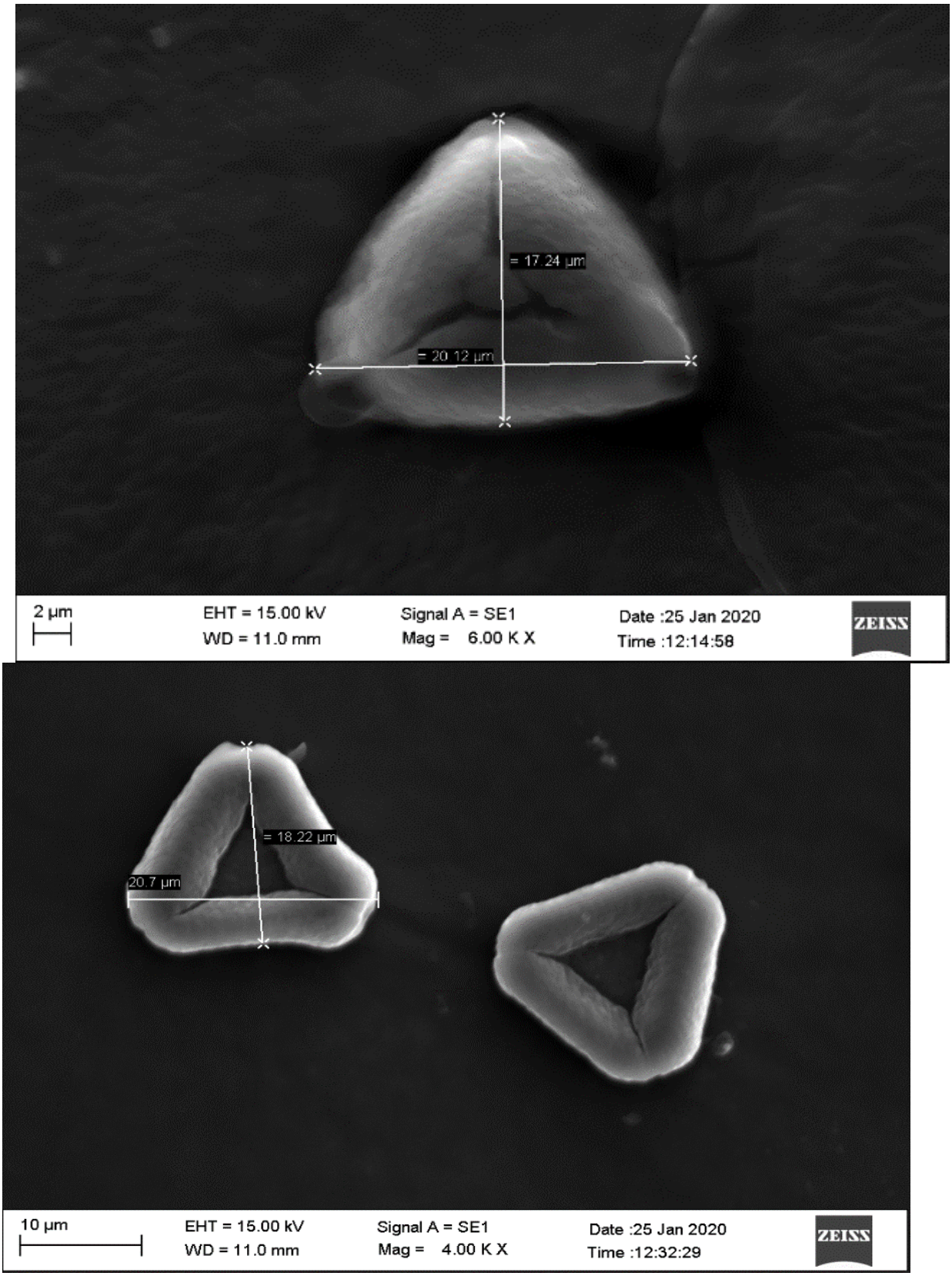


Plate 10. Equatorial and Polar diameter of pollen grains of clove

4.2.1.7 Stigma receptivity

Stigma receptivity was observed for 7 days from the day of flower opening and is presented in Table 20. The stigma receptivity by hydrogen peroxide test revealed that stigma was receptive upto six days from anthesis. Bubbles started appearing from second day of anthesis and maximum stigma receptivity was observed on fifth day of anthesis with highest number of bubbles (31.40 per cent). Stereomicroscopic image of stigma through hydrogen peroxide is shown in plate 11.

4.2.1.8 Anther dehiscence duration

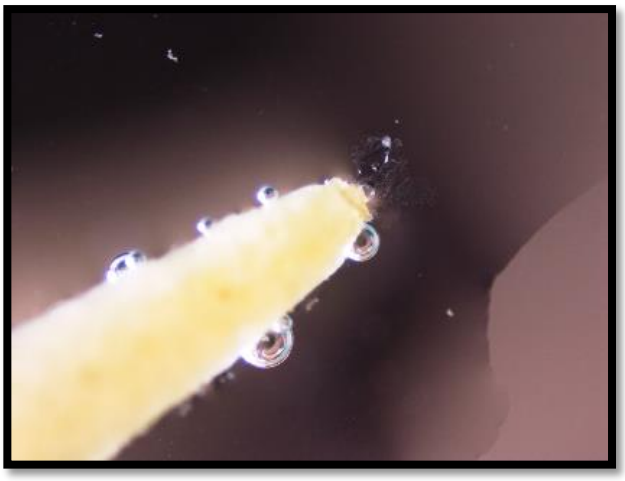
Anther dehiscence duration in clove plants as determined by checking both anther dehiscence starting time and anther dehiscence ending time is depicted in Table 18. Anther dehiscence begins 36 h before anthesis and ends at anthesis.

Table 20. Stigma receptivity through hydrogen peroxide test of Clove

No. of days from anthesis	Number of flowers										Mean number of bubbles	Percentage
	1	2	3	4	5	6	7	8	9	10		
First day	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Second day	16	15	14	15	16	13	14	16	17	14	15.00	15.20
Third day	17	15	18	16	14	17	19	15	20	17	16.80	17.12
Fourth day	23	24	23	26	21	28	24	25	22	25	24.10	24.56
Fifth day	28	35	29	30	32	28	35	31	29	37	**31.40**	**32.00**
Sixth day	14	8	6	9	13	10	12	12	11	13	10.80	11.00
Seventh day	0	0	0	0	0	0	0	0	0	0	0.00	0.00

4.2.2 Standardization of emasculation

4.2.2.1 Emasculation period



Bubbles seen on 5th day of anthesis

Bubbles seen on 5th day of anthesis

Bubbles seen on 5th day of anthesis

Plate 11. Stigma receptivity on different days of anthesis

Emasculation period of clove was standardized from the anther dehiscence starting time and stigma receptivity. Since anther dehiscence begins 36 h before anthesis and no more pollen was available in anthers after two days of anthesis. Stigma receptivity observed from second day of anthesis to sixth day of anthesis and maximum was observed on fifth day of anthesis. The emasculation should hence start from 48 h before flower bud opens which was revealed by the starting of separation of the petals.

4.2.3 Pollen collection and storage

4.2.3.1 Pollen collection

The pollen collected during different periods of anthesis (12, 24 and 36 h before anthesis) from the clove plants were tested for pollen viability and pollen fertility. Flower buds at different stages for pollen collection is presented in plate 12.

Table 21. Pollen viability in clove by iodine potassium iodide (IKI) method

Treatments	Mean
P_1-12 h before anthesis	94.02[a]
P_2 -24 h before anthesis	92.16[a]
P_3 -36 h before anthesis	72.47[b]
CD(0.05)	**3.522**

Pollen viability at different period of anthesis is shown in Table 21. Pollen viability by IKI method revealed that 12 hour before anthesis showed 94.02 per cent viability which was maximum among the treatments followed by 24 hour before anthesis which showed 92.16 per cent viability. The pollen viability increased with increase in anthesis time.

Table 22. Pollen fertility by acetocarmine method in clove

Treatments	Mean
P_1 -12 h before anthesis	82.83[a]
P_2 -24 h before anthesis	80.42[ab]
P_3 -36 h before anthesis	77.02[b]
CD(0.05)	**4.421**

Plate 12. Flower buds at different stages

Pollen fertility estimated by acetocarmine method is presented in Table 22. Pollen fertility was maximum on the pollen collected 12 hour before anthesis (82.83 per cent) followed by 24 hour before anthesis which showed 80.84 per cent fertility.

4.2.3.2 Pollen storage

The pollen viability and pollen fertility of dried pollen at different storage conditions like desiccator and refrigerator at different temperatures was compared with that of fresh pollen and the data is presented in Table 23, Table 24, Table 25 and Table 26.

Table 23. Percentage of pollen viability of clove by IKI method

Treatments	0 DAS	15 DAS	30 DAS	60 DAS
T_1 - Desiccator (40°C dried pollen)	90.24^{b}	83.11^{c}	80.83^{ab}	75.66^{ab}
T_2 - Desiccator (45°C dried pollen)	89.11^{b}	83.82^{c}	78.48^{bc}	76.64^{a}
T_3 – Desiccator (50°C dried pollen)	90.31^{b}	81.83^{c}	78.33^{bc}	77.12^{a}
T_4 – Refrigerator (40°C dried pollen)	90.24^{b}	85.62^{abc}	73.81^{c}	71.33^{c}
T_5 – Refrigerator (45°C dried pollen)	89.11^{b}	83.98^{bc}	75.37^{c}	72.84^{bc}
T_6 – Refrigerator (50°C dried pollen)	90.31^{b}	87.90^{a}	84.73^{a}	76.74^{a}
T_7 - Fresh pollen at room temperature (Control)	94.36^{a}	87.78^{ab}	74.97^{c}	69.90^{c}
CD(0.05)	**2.26**	**3.89**	**4.84**	**3.62**

(DAS- Days after Storage)

The pollen viability of fresh pollen on the day of collection (0 DAS) was 94.36 percent and was the maximum and superior to all other storage conditions. Treatment (T_6), which was pollen dried at 50 °C and stored in refrigerator after 15 days of storage showed 87.90 percent viability which was maximum and was on par with treatment (T_4), which was pollen dried at 40°C and stored in refrigerator and treatment (T_7), which fresh pollen was kept at room temperature. The pollen dried at 50 °C and stored for 30 days on refrigerator (T_6) showed 84.73 percent viability which was maximum and was on par with treatment (T_1) which was pollen dried at 40°C and stored in desiccator. The pollen dried at 50°C and stored in desiccator (T_3) for 60 days revealed 77.12 percent viability which was maximum and was on par with pollen dried at 50° C and stored in refrigerator (T_6) as well as with pollen dried at 45° C and

stored in desiccator (T_2). Fresh pollen stored at room temperature for 60 days showed minimum pollen viability of 69.90 per cent. Pollen viability by IKI method test is presented in plate 12.

Table 24. Two way mean table of percentage of pollen viability of clove by IKI method

Treatments (Factor A)	**Days (Factor B)**				**Treatment Mean A**
	0 DAS	**15 DAS**	**30 DAS**	**60 DAS**	
T_1-Desiccator (40°C dried pollen)	90.24	83.107	80.833	75.663	**82.46^{b}**
T_2 –Desiccator (45°C dried pollen)	89.107	83.817	78.483	76.64	**82.01^{b}**
T_3 –Desiccator (50°C dried pollen)	90.31	81.833	78.333	77.127	**81.90^{b}**
T_4- Refrigerator (40°C dried pollen)	90.24	85.623	73.813	71.333	**80.25^{b}**
T_5- Refrigerator (45°C dried pollen)	89.107	83.983	75.373	72.84	**80.32^{b}**
T_6 –Refrigerator (50°C dried pollen)	90.31	87.9	84.737	76.747	**84.92^{a}**
T_7 -Fresh pollen stored at room temperature (Control)	94.367	87.78	74.973	69.903	**81.75^{b}**
Days Mean B	**90.52^{a}**	**84.86^{b}**	**78.07^{c}**	**74.32^{d}**	
Treatment Mean A CD (0.05)	**1.743**				
Days Mean B CD (0.05)	**1.317**				
Factor (A x B) CD (0.05)	**3.485**				

From the above two way mean Table 24 of percentage of pollen viability of clove by IKI method, $T_{6,}$ pollen dried at 50°C and kept in refrigerator had 84.92 percent viability which was maximum and was significantly different among the treatments irrespective of days and all other treatments T_1-Desiccator (40 °C dried pollen), T_2 –Desiccator (45°C dried pollen), T_3 –Desiccator (50°C dried pollen), T_7 -Fresh pollen stored at room temperature (Control), T_5-Refrigerator (45°C dried pollen) and T_4- Refrigerator (40°C dried pollen) had 82.46, 82.01, 81.90, 81.75, 80.32 and 80.25 per cent which were significantly on par. Among the treatments the best one was pollen dried at 50°C and stored in refrigerator.

At 0 days after storage had 90.52 per cent viability which was maximum followed by 15 days after storage (84.86 per cent), 30 days after storage (78. 07 per cent) and 60 days after storage (74.32 per cent).

Table 25. Percentage of pollen fertility in clove by acetocarmine glycerine method

Treatments	0 DAS	15 DAS	30 DAS	60 DAS
T_1 - Desiccator (40°C dried pollen)	89.63	88.31^{a}	75.82^{bc}	68.60^{b}
T_2 - Desiccator (45°C dried pollen)	88.66	79.71^{d}	78.51^{ab}	77.10^{a}
T_3 – Desiccator (50°C dried pollen)	89.28	83.98^{bc}	82.12^{a}	78.19^{a}
T_4 – Refrigerator (40°C dried pollen)	89.63	86.84^{ab}	76.50^{bc}	75.75^{a}
T_5 – Refrigerator (45°C dried pollen)	88.67	85.96^{ab}	80.25^{ab}	78.29^{a}
T_6 – Refrigerator (50°C dried pollen)	89.28	81.69^{cd}	76.72^{bc}	74.86^{a}
T_7 - Fresh pollen at room temperature (Control)	90.18	78.69^{d}	73.17^{c}	68.82^{b}
CD(0.05)	**NS**	**3.55**	**4.49**	**3.96**

(DAS- Days after Storage)

Pollen fertility of pollen dried at different temperature and stored in desiccator and refrigerator for 60 days is presented in Table 25. Pollen fertility on the day of collection dried at different temperatures and stored in desiccator, refrigerator or only fresh pollen was at room temperature and not the one dried at different temperature did not differ significantly from each other. At 15 days after storage, pollen dried at 40° C in desiccator had 88.13 per cent fertility which was maximum among the treatments and was on par with pollen dried at 40° C as well

as 45 °C and stored at refrigerator (T_4 and T_5). At 30 days after storage pollen dried at 50°C and stored in desiccator had 82.12 per cent fertility which was maximum among the treatments and was on par with pollen dried at 45° C and stored in desiccator (T_2) as well as with pollen dried at 45° C and stored in refrigerator (T_5). At 60 days after storage, pollen dried at 45°C and stored in refrigerator produced maximum pollen fertility of 78.29 per cent and was on par with pollen dried at 50° C and stored in desiccator (T_3), pollen dried at 45°C and stored in desiccator (T_2) and pollen dried at 50°C and stored at refrigerator (T_6). The fresh pollen stored at room temperature for 60 days revealed 68.82 per cent pollen fertility. Pollen fertility by acetocarmine test is presented in plate 13.

Table 26. Two way mean table of percentage of pollen fertility in clove by acetocarmine glycerine method

Treatments (Factor A)	Days (Factor B)				Treatment Mean A
	0 DAS	15 DAS	30 DAS	60 DAS	
T_1-Desiccator (40°C dried pollen)	89.63	88.31	75.83	68.60	**80.59^{b}**
T_2 –Desiccator (45°C dried pollen)	88.67	79.72	78.51	77.10	**81.00^{b}**
T_3 –Desiccator (50°C dried pollen)	89.28	83.99	82.12	78.19	**83.39^{a}**
T_4- Refrigerator (40°C dried pollen)	89.63	86.85	76.50	75.75	**82.18^{ab}**
T_5- Refrigerator (45°C dried pollen)	88.67	85.96	80.26	78.30	**83.29^{a}**
T_6 –Refrigerator (50°C dried pollen)	89.28	81.69	76.73	74.86	**80.64^{b}**
T_7 -Fresh pollen stored at room temperature (Control)	90.18	78.69	73.17	68.82	**77.70^{c}**
Days Mean B	**89.34^{a}**	**85.24^{b}**	**77.59^{c}**	**74.52^{d}**	
Treatment Mean A CD (0.05)	**1.802**				
Days Mean B CD (0.05)	**1.362**				
Factor (A x B) CD (0.05)	**1.362**				

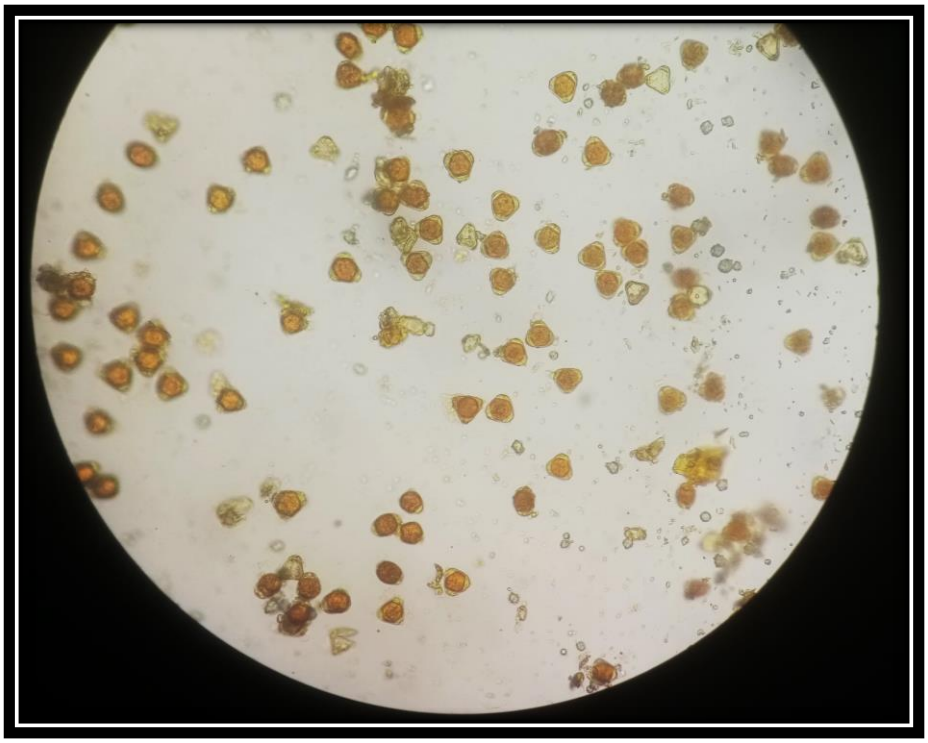


Plate 13. Pollen viability test by IKI method

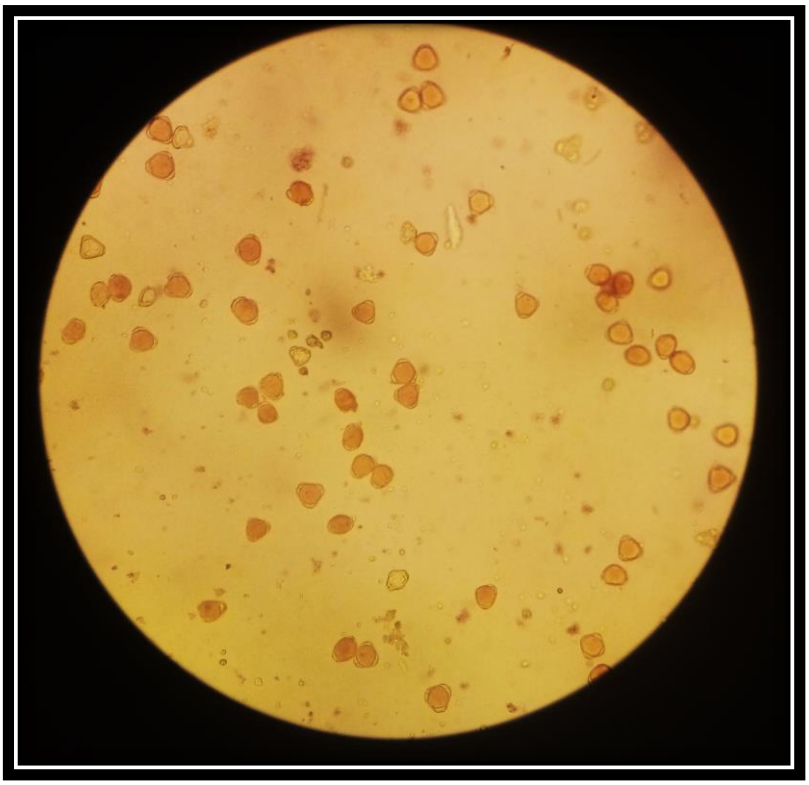


Plate 14. Pollen fertility test by Acetocarmine method

From the above mean Table 26, percentage of pollen fertility in clove by acetocarmine glycerine method, T_3, pollen dried at 50°C and stored in desiccator had 83.39 per cent fertility which was maximum and was on par with T_5- Refrigerator (45°C dried pollen) and T_4- Refrigerator (40°C dried pollen). Mean pollen fertility of T_1-Desiccator (40°C dried pollen) showed 80.59, while T_2 -81.00 and T_6 showed 80.64 per cent. T_7 Fresh pollen stored at room temperature (Control) had 77.70 per cent fertility which was lower among the treatments.

The pollen on the day of collection had 89.34 per cent fertility which was maximum followed by 15 days after storage (85.24 per cent), 30 days after storage (77.59 per cent) and 60 days after storage (74.52 per cent).

4.2.4 Hybridization

The percentage of fruit set in all the plant combinations selected is presented in Appendix-3. The mean percentage of fruit set of different plant combinations during crossing in clove is presented in Table 27. The percentage of fruit set ranged from 15 per cent to 42.5 per cent and average fruit set from all the plant combinations was 28.87 per cent.

Table 27. Fruit set percentage of different plant combinations during crossing in clove

Plant combinations	Mean percentage of fruit set
P_1 X P_2	27.50
P_1 X P_3	25.00
P_1 X P_4	35.00
P_1 X P_5	20.00
P_2 X P_1	40.00
P_2 X P_3	30.00
P_2 X P_4	22.50
P_2 X P_5	15.00
P_3 X P_1	25.00
P_3 X P_2	32.50
P_3 X P_4	42.50
P_3 X P_5	37.50
P_4 X P_1	42.50
P_4 X P_2	30.00
P_4 X P_3	27.50
P_4 X P_5	32.50
P_5 X P_1	20.00
P_5 X P_2	25.00
P_5 X P_3	27.50
P_5 X P_4	20.00
Mean	**28.87**

DISCUSSION

5. DISCUSSION

The study entitled "Evaluation of elite clove (*Syzigium aromaticum* (L) Merr. & Perry) accessions and standardization of pollination techniques" was undertaken in clove estates of Trivandrum and Kollam districts of Kerala from 2018-2020. Quantitative characters, floral biology, pollen biology, pollen storage and hybridization carried out are discussed here based on the results obtained.

5.1 EVALUATION OF ACCESSIONS

5.1.1 Quantitative characters

5.1.1.1 Tree characters

5.1.1.1.1 Plant height

The plant height of 12 selected clove accessions ranged from 6.15 m to 15.30 m. The highest plant height among the accessions observed was in MRC-7 (15.30 m) followed by BRC-3 (14.10 m). The lowest plant height was recorded from the accession AMC-13 (6.15 m). This was followed by BRC-4 with 6.51 m, AMC-12 with 6.60 m and MRC-5 with 6.80 m. However in a study conducted by Avinash (2018) on survey, characterization and evaluation of clove accessions concluded that plant height of the clove accessions selected for study ranged from 5.15 m to 15.25 m and the highest plant height among the accessions was BLC-18. Balakrishnamoorthy and Kennedy (1999) identified twelve high yielding types from the survey based on five years performance from a private estate at Nagercoil and reported that tree height varied from 25-30 m in clove. Four promising genotypes of clove were selected at Dapoli among the germplasm that were planted during 1996-97. In these accessions the plant height ranged from 5.89 to 7.15 m (AICRP, 2019).

In the present study, MRC-7 had good yield (8.14 kg) with the highest plant height (15.30 m). However the plant height of the clove tree varied with age of the tree as well as its genetic trait. The accessions in this study showed a variation in plant height (6.15-15.30 m). MRC-5 was of 30 years age with comparatively low plant height of 6.80 m while BRC-3 was having 14.10 m height at 35 years age.

5.1.1.1.2 Tree girth

Tree girth was the important morphological trait for assessing the productivity (Balakrishnan *et al.*, 1998) and it could be measured at 45 cm from the base of the tree (Kennedy and Nageswari, 2000). But some of the accessions selected for evaluation had produced branches at a plant height below 45 cm from the base of the tree. So the tree girth of selected clove accessions was measured at 30 cm height from the base of the tree in the present study. Girth is an important character that influences the yield. The data observed that the tree girth at 30 cm height from the base of the tree among the accessions ranged from 70 cm to 165 cm (Figure.5). The girth at 30 cm height from the base of the tree was the highest for BRC-3 (165 cm) followed by MRC-7 (128 cm). Both of them were good yielders. The accessions BRC-1, MRC-5 and MRC-6 that were identified as superior accessions showed the girth at 30 cm height as 120 cm, 122 cm and 124 cm respectively. In a study conducted by Avinash (2018) on survey, characterization and evaluation of clove accessions concluded that the girth at 45 cm varied from 44.10 cm to 138.10 cm and it was highest the for BRC-3. BRC-3 had showed the highest girth at 30 cm and 45 cm height from the base of the tree and the age of the plant was 35 years.

5.1.1.1.3 Canopy spread

Canopy spread of the tree is another important tree character. In this study, canopy spread (N-S) of the selected clove accessions extended from 4.80 m to 7.70 m (Table 3). The canopy spread (E-W) of the selected clove accessions varied from 5.00 m to 8.10 m (Table 3). MRC-7 had good canopy spread in N-S (7.70 m) and E-W (8.10 m). Variability was observed between the canopy spread (N-S and E-W) of selected accessions and E-W canopy spread is more correlated to yield compared to N-S canopy spread. The accessions BRC-1, MRC-5 and MRC-6 that were identified as superior accessions recorded the E-W canopy spread of 6.30 m, 6.70 m and 7.00 m respectively. Four promising genotypes of clove were selected at Dapoli among the germplasm that were planted during 1996-97 and the canopy spread in these accessions varied from 2.50 m to 3.05 m (AICRP, 2019). According to Avinash (2018) the canopy spread N-S ranged from 3.1 m to 7.42 m and it was the highest for MRC-7 and canopy spread E-W ranged from 2.95 m to 7.90 m and it was the highest for AMC-11.

5.1.1.1.4 Number of branches

The number of branches varied from 40 to 57 (Table 3) among the selected clove accessions. The highest number of branches was observed in AMC-11 (57) followed by BRC-3 (56). Among the 24 accessions of clove that were maintained at Pechiparai, SA-3 recorded the highest number of branches (16) (AICRP, 2019). The accessions BRC-1, MRC-5 and MRC-6 that were identified as superior accessions recorded 52, 48 and 42 branches respectively. In a study conducted by Avinash (2018) on survey, characterization and evaluation of clove accessions concluded that the number of branches ranged from 26 to 55 and AMC-11 showed maximum number of branches.

5.1.1.2 Leaf characters

5.1.1.2.1 Leaf length

The leaf length of clove accessions varied from 9.59 cm in BRC-3 to 13.98 cm in MRC-5 (Table 4). Among the 24 accessions of clove that were maintained at Pechiparai, the accession SA-3 recorded the highest leaf length (12.47 cm) and leaf breadth (7.46 cm) (AICRP, 2019). Avinash (2018) concluded that leaf length varied from 9.66 cm in AMC-20 to 13.93 cm in MRC-5. Among the characterization and evaluation of *Syzygium sp.*, *S. malaccense* had showed the highest leaf length (34.45 cm) followed by *S. samarangense* (white) (21.25 cm), *S. jambos* (19.81 cm) and *S. samarangense* (red) (17.25 cm) (Jayasree, 2019). The accessions of *S. aromaticum* selected for evaluation had average leaf length of 11.75 cm which was very less compared to above *Syzygium sp*.

5.1.1.2.2 Leaf breadth

Leaf breadth of selected clove accessions ranged from 3.81 cm to 4.75 cm (Table 4). The highest leaf breadth was observed in AMC-11 followed by BRC-3 (4.65 cm) and BRC-4 (4.45 cm). In a study conducted by Avinash (2018) on clove accessions concluded that leaf breadth extended from 3.55 cm in ANC-20 to 4.72 cm in AMC-11. Among the characterization and evaluation of *Syzygium sp., S. malaccense* had showed highest leaf breadth (9.38 cm) followed by *S. samarangense* (red) (6.39 cm), *S. samarangense* (white) (5.63 cm) and *S.*

jambos (3.09 cm) (Jayasree, 2019). The accessions of *S. aromaticum* selected for evaluation had average leaf breadth of 4.21 cm which was higher than *S. jambos* (3.09 cm) and lower than the above *Syzygium sp*.

5.1.1.2.3 Leaf area

Balakrishnan *et al*. (1998) reported that measurement of leaf area was one of the important method of assessing the variability and productivity in clove. The leaf area of the present study on selected clove accessions extended from 25.35 cm^2 in MRC-7 to 43.00 cm^2 in AMC-11. The highest leaf area was observed in MRC-7 (43.00 cm^2) followed by MRC-5 (39.01 cm^2) and AMC-13(36.78 cm^2) (Table 4). Avinash (2018) characterized and evaluated clove accessions and concluded that the leaf area varied from 23.07 cm^2 in ANC-20 to 42.93 cm^2 in AMC-11. The leaf area of *S. malaccense, S. samarangense* (white), *S. samarangense* (red) and *S. jambos* showed 320.35 cm^2, 173.45 cm^2, 111.30 cm^2 and 62.09 cm^2, respectively (Jayasree, 2019). The accessions of *S. aromaticum* selected for evaluation recorded an average leaf area of 32.24 cm^2 which was very less compared to the above *Syzygium sp*.

5.1.1.3 Bud characters

5.1.1.3.1 Number of inflorescence/m^2

The number of inflorescence/m^2 was the highest in AMC-12 (156.25) followed by AMC-10 (117.10) and BRC-1 (115.25) (Fig.6). The lowest number of inflorescence/m^2 was observed in MRC-8 (53.70). Number of inflorescence/m^2 had an influence on the yield. The number of inflorescence/m^2 showed high variability as inferred from the descriptive statistics where the standard deviation noted was 28.04. Krishnamoorthy and Rema (1992) reported that number of inflorescence per branch was one of the characters that determined the yield in clove. In a study conducted by Avinash (2018) on survey, characterization and evaluation of clove accessions, the number of inflorescence /m^2 was the highest in AMC-12 (155.5) and the lowest in ANC-20 (36.25).

5.1.1.3.2 Number of flower buds/inflorescence

The number of flower buds/inflorescence was the highest in MRC-6 (17.25) and the lowest in BRC-3 (8.10). The number of flower buds/inflorescence in BRC-4 was 11.90, BRC-1 and MRC-5 was 10.50 (Table 5). The accessions BRC-1, MRC-5 and MRC-6 that were identified as best accessions had higher number of buds per inflorescence. The average number of flower buds per inflorescence observed among the selected clove accessions was 9.71 (Table 10). Krishnamoorthy and Rema (1992) reported that number of buds per inflorescence was one of the characters that determined the yield in clove. The clove tree inflorescence is a terminal branching cyme of 3 to 20 hermaphrodite florets (Purseglove, 1968). According to Avinash (2018), the number of flower buds /inflorescence ranged from 6.08 to 18.21 in MRC-6.

5.1.1.3.3 Bud characteristics

The characters of bud is important parameter as bud is an economic part of clove. The single bud weight (fresh) among the clove accessions ranged from 219.30 mg in AMC-13 to 398.72 mg in BRC-3 (Figure 7). The highest single bud weight was observed in BRC-3 (398.72 mg) followed by MRC-5 (343.29 mg), MRC-6 (342.20 mg), BRC-1 (312.58 mg) and MRC-7 (310.57 mg). The least single bud weight (fresh) was observed in AMC-13 (219.30 mg). The single bud weight (dry) among the clove accessions ranged from 73.00 mg in AMC-13 to 127.26 mg in BRC-3 which recorded the highest single dry bud weight. The single bud weight (dry) of BRC-1 was 116.30 mg while that for MRC-8 was 108.67 mg and that for MRC-7 was 105.28 mg. The single dry bud weight of MRC-5 and MRC-6 were 94.74 mg and 90.24 mg respectively. Single bud weight (fresh) and single bud weight (dry) influenced by the yield. The accessions that were identified as best had high single bud weight (fresh) and single bud weight (dry). Single bud weight (fresh) and single bud weight (dry) was highly correlated with the girth of the tree. Thus greater the girth of the tree higher the bud weight. According to Avinash (2018), single bud weight (fresh), single bud weight (dry) was the highest for BRC-3 which comply to the same status observed in the present experiment. According to him, single bud weight dry ranged from 66.50 g to 128.50 g.

The mature bud length among the clove accessions ranged from 14.56 mm in BRC-2 which was the lowest to 19.34 mm in BRC-1 which was the highest. Mature bud length of BRC-4 was 18.82 mm and that for AMC-10 was 18.58 mm. The mature bud diameter among the clove accessions varied from 4.67 mm to 6.45 mm. Mature bud diameter was the highest in BRC-3 (6.45 mm) followed by BRC-1 (5.97 mm) and AMC-11 (5.95 mm). The mature bud diameter was the least in MRC-5 (4.67 mm). Mature bud diameter, single bud weight (fresh) and single bud weight (dry) was the highest for BRC-3 showing the large size of the bud. Balakrishnamoorthy and Kennedy (1999) reported three different types of clove flower buds *viz.*, small, medium and big. The accession BRC-1 had 1.93 cm which was greater than king clove and accessions except BRC-2 and AMC-13 recorded bud length less than 1.6 cm bud length of king clove. However, the breadth did not fall into the category of king clove. The bud breadth recorded varied from 0.46 cm to 0.64 cm. In a study conducted by Avinash (2018), characterization and evaluation of clove accessions were made and the mature bud length of the accessions varied from 14.94 mm to 19.06 mm while the mature bud diameter ranged from 4.9 mm in AMC-9 to 6.41 mm in BRC-3. The higher size of the bud may be a genetic character and the buds with high bud length and diameter, BRC-1 and BRC-3 seemed to be a better genotype.

5.1.1.3.4 Flower characteristics

Clove accessions selected showed varied length of flower ranging from 14.98 mm in AMC-12 to 21.86 mm in BRC-1. The breadth of flower among the clove accessions in BRC-2 was 11.47 mm which was the least. Maximum breadth of flower was observed in BRC-3 (13.89 mm) followed by MRC-6 (13.76 mm). The length of sepal among the clove accessions ranged from 2.54 mm to 3.16 mm. The length of petal among the clove accessions varied from 4.26 mm in MRC-8 to 6.15 mm in BRC-3. Avinash (2018) reported that the length of flower ranged from 16.36 mm to 21.84 mm and the breadth of flower ranged from 9.85 mm to 14.82 mm. The length of sepal ranged from 2.49 mm to 3.15 mm.

5.1.1.3.5 Flowering period

Flowering period of accessions BRC-1, MRC-5 and AMC-13 was from December to February. For the remaining accessions BRC-2, BRC-3, BRC-4, MRC-6, MRC-7, MRC-8, AMC-10, AMC-11 and AMC-12, flowering period was from January to March. Flowering

season varies from September-October in plains to December-January at high altitude (Thangaselvabai *et al*., 2010).

5.1.1.3.6 Yield characteristics

5.1.1.3.6.1 Bud yield/tree (fresh)

The bud yield in clove stabilizes only after 15 years. The selected clove accessions for evaluation belonged to the age group of 25-40 years. Bud yield/tree (fresh) of selected clove accessions was recorded for two years 2019 and 2020 sequentially (Fig.8). The pooled mean of fresh bud yield per tree of selected clove accessions ranged from 6.50 kg in AMC-12 to 24.00 kg in MRC-7. The higher yielders in terms of fresh bud yield per tree were MRC-7 (24.00 kg), AMC-10 (22.87), BRC-1 (22.00), MRC-5 (22.00 kg), BRC-4 (17.50 kg) and AMC-11 (17.25 kg). Among the accessions, BRC-1, MRC-5, MRC-7 and AMC-10 gave consistent yield during 2018-2019 and 2019-2020. In a study conducted by Avinash (2018) on accessions observed that the pooled mean of fresh bud yield per tree varied from 2.70 kg to 40.25 kg. However BRC-1, MRC-5, MRC-6, MRC-8, AMC-10 and MMC-15 were good yielders yielding more than 30 kg/tree as revealed from pooled mean.

5.1.1.3.6.2 Bud yield/tree (dry)

The dry bud yield/tree during the year 2019-2020 ranged from 0.63 kg to 8.47 kg. The dry bud yield per tree during the year 2019-2020 was the highest for MRC-7 (8.47 kg) followed by AMC-10 (6.91 kg), MRC-5 (6.62 kg) and BRC-1 (6.46 kg). The pooled mean of dry bud yield per tree was the highest for MRC-7 (8.13 kg/tree) followed by BRC-1 (7.48 kg/tree) and the least observed in AMC-12 (2.21 kg/tree). MRC-7 had higher bud yield (fresh) (24.00 kg) and bud yield (dry) (8.13 kg/tree) among the selected clove accessions. The accessions selected showed biennial nature in terms of yield and it is influenced by weather parameters like rainfall and the last year crop harvested. According to Pursglove *et al*. (1981), it was occasional to get good yield sequentially due to physical shock of picking. However the selected accessions like

BRC-1, BRC-4, MRC-5, MRC-7 and AMC-10 showed good yield in two successive years. In a study conducted by Avinash (2018), on accessions observed that the pooled mean of fresh bud yield per tree varied from 2.70 kg to 40.25 kg. However BRC-1, MRC-5, MRC-6, MRC-8, AMC-10 and MMC-15 were good yielders yielding more than 30 kg/tree as revealed from pooled mean. The bud weight per tree dry was the highest for MRC-6 (12.25kg/tree) followed by MRC-5 (12.20 kg/tree). Most of the accessions showed biennial nature, while the yield gap was less in accessions like AMC-12 and BLC-16.

5.1.1.4 Fruit characteristics

Clove fruit is single seeded drupe known as mother of clove. The colour of the fruit is reddish purple, 2.5-3.5 cm long and 1.2-1.5 cm in diameter (Purseglove *et al*., 1981). Ravindran *et al*. (2006) reported that clove fruit contains one oblong shaped fruit of about 1.5 cm length. The fruit weight (fresh) of clove accessions recorded ranged from 1.21 g in AMC-12 to 3.54 g in BRC-3. The ratio of fruit to seed was the highest in AMC-13 (3.07) and was the least in MRC-6 (2.38). Studies on variability in fruit characters of jamun at Banaras Hindu University, Varanasi reported that fruit weight ranged from 3.55 g to 14.55 g and higher fruit weight was recorded in Selection 1 while the lowest in Selection 6 (Prakash *et al*., 2010).

Avinash (2018) observed that fruit weight of clove accessions ranged from 1.2 g in ANC-20 to 3.53 g in BRC-3 and the ratio of fruit to seed ranged from 2.41 to 3.64. In a study conducted by Jayasree (2019) on characterization and evaluation of *Syzygium sp.* concluded that *S. malaccense* had the highest fruit weight (54.40 g) followed by *S. samarangense* (red) (34.22 g), *S. samarangense* (white) (26.61 g) and the lowest fruit weight was observed in *S. jambos* (16.37 g). However in the selected accessions fruit weight of *S. aromaticum* varied from 1.20 g to 3.54 g. The average fruit weight of selected accessions of *S. aromaticum* (2.59 g) was comparatively less than *S. cuminii* (3.55 g-14.55 g), *S. malaccense* (54.40 g), *S. samarangense* (red) (34.22 g), *S. samarangense* (white) (26.61 g) and *S. jambos* (16.37 g), respectively.

5.1.1.5 Seed characters

5.1.1.5.1 Seed length

The seed length of twelve elite clove accessions ranged from 13.47mm in AMC-12 to 19.40 cm in BRC-3. According to Avinash (2018), the highest seed length corresponded to the highest fruit length and was noted in BRC-3, the seed length varied from 1.32 cm to 1.93 cm in the selected accessions. Jayasree (2019) on characterization and evaluation of *Syzygium sp.* observed that variability was very high with respect to seed length and it varied from 1.50 cm in *S. jambos to* 3.80 cm in *S. malaccense*. In case of S. *samarangense* (white), seed length showed 1.67 cm whereas *S. samarangense* (red) rudimentary seeds were observed. The average seed length of selected clove accessions showed 1.74 cm which was comparatively higher than *S. jambos* (1.50 cm), *S. samarangense* (white) (1.67 cm), *S. samarangense* (red) (rudimentary seeds) and lower than *S. malaccense* (3.80 cm).

5.1.1.5.2 Seed breadth

The seed breadth of elite clove accessions ranged from 6.31mm in AMC-12 to 9.24 mm in BRC-3. According to Avinash (2018), seed breadth of clove seeds varied from 0.61 cm to 0.919 cm in the selected clove accessions observed. On the contrary seed breadth of fruit varied from 0 to 3.90 cm in *S. malaccense*, *S. jambos* showed 1.80 cm whereas rudimentary seeds were observed in *S. samarangense* (red) (Jayasree, 2019).

5.1.1.5.3 Seed weight

The seed weight recorded was the highest in BRC-3 and AMC-11(1.20g) and was the least from AMC-12 which had only 0.50 g. Seed characters of progenies of 14 elite clove trees revealed no appreciable variation for 100 fruit weight, 100 seed weight, fruit breadth, fruit length, seed breadth and seed length (Krishnamoorthy and Rema, 1994). According to Avinash (2018) the seed weight was the highest for BRC-3 (1.18 g) followed by 1.10g in AMC-11. Jayasree (2019) on characterization and evaluation of *Syzygium sp.* concluded that with respect to seed weight, variability was high and it varied from 1.50 g to 5.20 g. *S. malaccense* showed 5.20 g weight which was the highest, and lowest seed weight was observed in *S. samarangense* (red) which had rudimentary seeds.

5.1.1.6 Incidence of diseases

Common diseases like leaf spot, leaf rot and sudden death disease were found in clove. During the evaluation of elite clove accessions, leaf spot due to *Colletotrichum gloeosporioides* was noticed in elite clove accessions. Disease severity was the highest in BRC-2 (28.33 per cent) and the lowest in MRC-6 (15.00 per cent). Surveys conducted at Keeriparai and Pechiparai areas in Kanyakumari district by Horticulture Research Centre, Pechiparai noticed that leaf spot of clove was high in these areas (AICRP, 2005).

5.1.1.7 Incidence of pests

There were no pest incidence in the selected clove accessions.

5.1.2 Quantitative characterization

Multi-Dimensional Scaling (MDS) is an ordering technique for dimensional reduction that allows one to map individuals as points in low-dimensional space (generally 2D or 3D). MDS is especially useful when the relation between individuals is unknown (not an unusual case in germplasm banks), but it is possible to estimate a detachment matrix between them (Manly, 2004; Borg and Groenen, 2005).

In this study, the multidimensional scaling of 25 quantitative characters resulted in the first two dimensions explaining 92.97 per cent variation (Table 11). The eigen vectors designated as Dimension-1 and Dimension-2 with the respective component loadings of all the 25 characters were taken. MDS detects the most important traits for the grouping. Dimension-1 and Dimension -2 explained the whole variability in accessions i.e. 92.97% of the total variance. The most important traits for the separation are those with the biggest loading on Dimension-1 and Dimension-2. Characters like tree girth at 30 cm height, single bud weight (fresh), single bud weight (dry) and number of inflorescence/m^2 showed high loadings. The characters which contributed to high variation in Dimension-1 had negative loadings for number of inflorescence/m^2 and positive loadings like girth at 30 cm height, single bud weight (fresh) and single bud weight (dry). Dimension-2 had positive loadings for number of inflorescence/m^2.

According to Mahendran *et al.* (2015), higher the coefficients, regardless of the direction (positive or negative), the more effective they will be in discriminating between the accessions. Thus, the prominent characters in different dimensions contributing towards explaining the variability have the tendency to remain together. Hence this may be kept into consideration during utilization of these characters in breeding programme. Characters with high variability are expected to provide high level of gene transfer during breeding programs (Aliyu *et al*., 2000; Gana, 2006). Hence selection of clove accessions with these characters (girth, number of inflorescence/m^2, single bud weight (fresh and dry) may be carried out and these characters provide a base for further improvement in crops.

5.1.3 Scree plot of Eigen values of MDS

A scree plot shows how much variation each dimension captures from the data. The y axis is eigenvalues, which essentially stand for the amount of variation. A scree plot is used to select the dimensions to keep. In Figure 5, just dimension-1 and dimension-2 were enough to describe the data as it showed variation and remaining dimensions were just flattened out. Scree plot decides the number of dimensions to consider for describing the data and in this study 2 dimensions were selected for describing the data.

5.1.4 Multi dimensional scaling configuration

Multi dimensional scaling configuration could be plotted as graph which showed dissimilarities of the accessions by using components of Dimension-1 and Dimension-2. Based on the graph of multi dimensional configuration eight clusters of selected clove accessions were identified. Clustering based on quantitative characters resulted in Cluster I containing BRC-1, Cluster II containing MRC-5 and MRC-6, Cluster III containing BRC-4 and MRC-7, Cluster IV containing MRC-8, Cluster V containing AMC-10 and AMC-11, Cluster VI containing BRC-2 and AMC-13, Cluster VII containing AMC-12 and Cluster VIII contained BRC-3. Multi Dimensional Scaling Configuration is presented in Figure 5.

5.1.5 Cluster analysis

Cluster analysis based on 25 quantitative characters revealed that all the twelve accessions could be grouped into 8 clusters at 80 percent similarity by plotting a Dendrogram. Dendrogram gives an idea about identifying the clusters of selected clove accessions. Dendrogram representing the relationship among 12 elite accessions based on Euclidean distance are presented in Figure. 2. The clusters that identified based on multi dimensional scaling configuration was similar to that of clusters identified based on the dendrogram. Clustering based on quantitative characters resulted in Cluster I containing BRC-1, Cluster II containing MRC-5 and MRC-6, Cluster III containing BRC-4 and MRC-7, Cluster IV containing MRC-8, Cluster V containing AMC-10 and AMC-11, Cluster VI containing BRC-2 and AMC-13, Cluster VII containing AMC-12 and Cluster VIII contained BRC-3. Accessions in Cluster I containing BRC-4 and MRC-7 were identical in tree characters, bud characters, flower characters, fruit and seed characters except leaf and yield characters. Accessions in Cluster III containing MRC-5 and MRC-6 were identical in all characters except some tree characters, leaf characters and yield characters. Accessions in Cluster IV containing AMC-10 and AMC-11 were identical in all characters except leaf, fruit and seed characters. The remaining clusters were different with respect to most of the characters.

5.1.6 Linkage between major quantitative characters of clove

The linkage was analysed based on the biplot coordinates of Dimension-1 and Dimension-2. The biplot is justifiable by the fact that the first two dimensions explained 92.97 per cent of variation.

The correlation coefficient between any two characters was estimated by the cosine of the angle between their vectors (Yan and Rajcan, 2002). The correlation coefficients among the quantitative traits with relatively large loadings on Dimension-1 and Dimension-2 axis were high. The linkage analysed based on the biplot coordinates of Dimension-1 and Dimension-2 revealed a strong positive association between all quantitative characters except girth at 30 cm height, number of inflorescence per m^2, single bud weight fresh and single bud weight dry.

A near zero correlation was observed between number of inflorescence per m^2 and single bud weight fresh and single bud weight dry as indicated by perpendicular vectors. Biplot analysis between Dimension-1 and Dimension-2 axis revealed that vector SBWF (single bud weight fresh) was the longest with positive loading for Dimension 1. Second in magnitude was

NOI (number of inflorescence per m^2) with negative loadings for Dimension-1 and positive loadings for If a character had a biplot coordinate score of near zero it has small interaction effects and is considered as stable. Thus, characters except SBWF, NOI, girth, Dimension-2. SBWD, plant height, number of buds/inflorescence all other characters have less co-efficients and they do not possess any strong interaction effect. Those characters with large co-efficients exerts a strong interaction.

5.1.7 Minimum Data Set characters

The selection of representative Minimum Data Set (Doran and Parkin, 1994) was done by the Principal Component Analysis (PCA) based on the assumption that principal components receiving higher values best represent the system attributes. Among each principal component, the one with the highest sum of correlation coefficients was chosen for the Minimum Data Set. In the same way, the dimensions of Multidimensional scaling receiving higher values best represent the system attributes. Among each dimension, the one with the highest sum of correlation coefficients was chosen for the Minimum Data Set.

The minimum data set for identifying a promising clove accession were generated based on the values of biplot coordinates of the first two dimensions of Multidimensional scaling, that explained 92.97 per cent of the total variation. The data generated consisted of a vector and a singleton characteristic. The vector of characteristics were girth at 30 cm height, single bud weight fresh and single bud weight dry. The singleton characteristic constituted of number of inflorescence/m^2.

5.1.8 Illustration of identification of an ideotype using existing data

Identification of ideotype based on existing data was worked out. This suggested that accessions BRC-1, MRC-5 and MRC-6 had better ideotype and could be suggested as elite or superior accessions. BRC-1 had 120 cm stem girth at 30 cm height, 312.58 mg single bud weight fresh, 116.30 mg single bud weight dry, the number of inflorescence/m^2 was 115.25 and 10.50 number of flower buds / inflorescence. The effective yield worked out was 140.73 g/m^2 which was a higher yield. On the other hand, MRC-5 and MRC-6 belonged to same cluster, Cluster II, which had 123 cm stem girth at 30 cm height. The single bud weight fresh

was 342.74 mg and single bud weight dry was 92.49 mg. The number of inflorescence/m^2 was 86.98, effective yield was 111.60 g/m^2 and number of flower buds/inflorescence was 13.87.

AMC-10, AMC-11, BRC-4, MRC-7 were considered as the best accessions which satisfied all the existing minimum data set characters such as girth, single bud weight (fresh), single bud weight (dry) and effective yield was greater than 75 g/m^2. AMC-12 and BRC-3 can also be considered as best accessions as it satisfied all existing minimum data set characters except their alternate bearing nature.

5.1.9 Identification of a better accession

An investigator should first identify a clove tree which possess a desirable set of quantitative characters and the quantitative characteristics should be mapped accordingly. In the bearing season the minimum data set characters should be observed. This could optimally sort out ideotype for elite accessions.

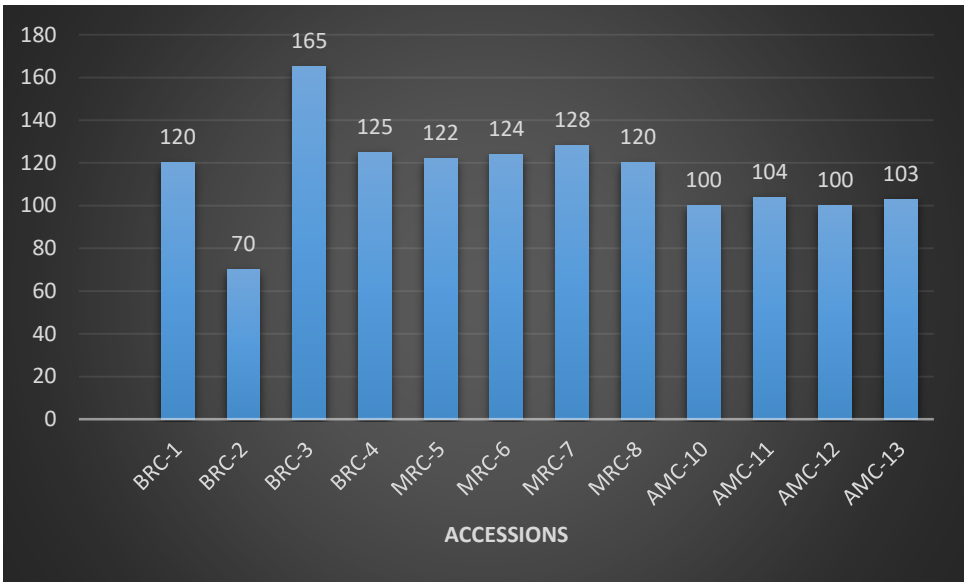


Fig 5. Girth at 30 cm height of selected clove accessions

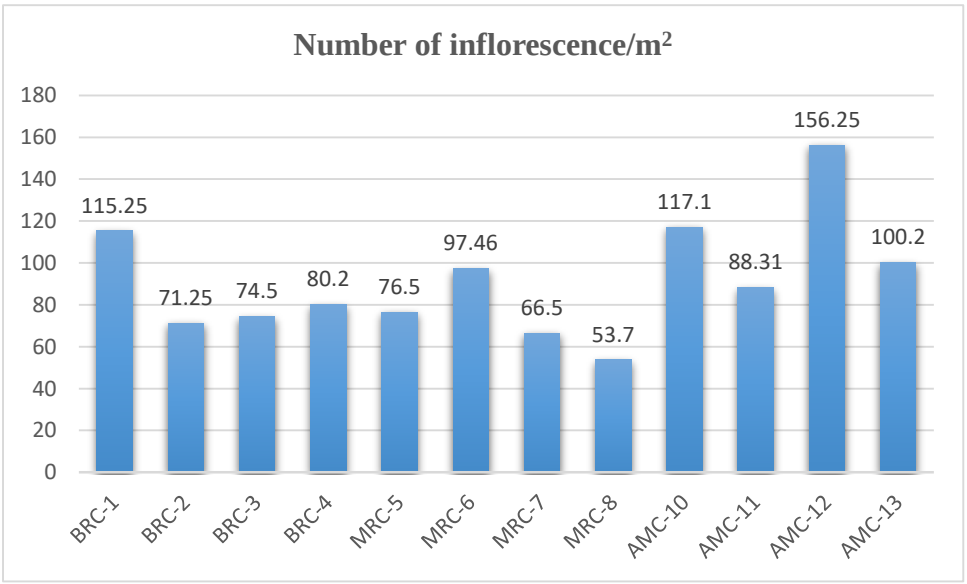


Fig 6. Number of inflorescence/m^{2}of selected clove accessions

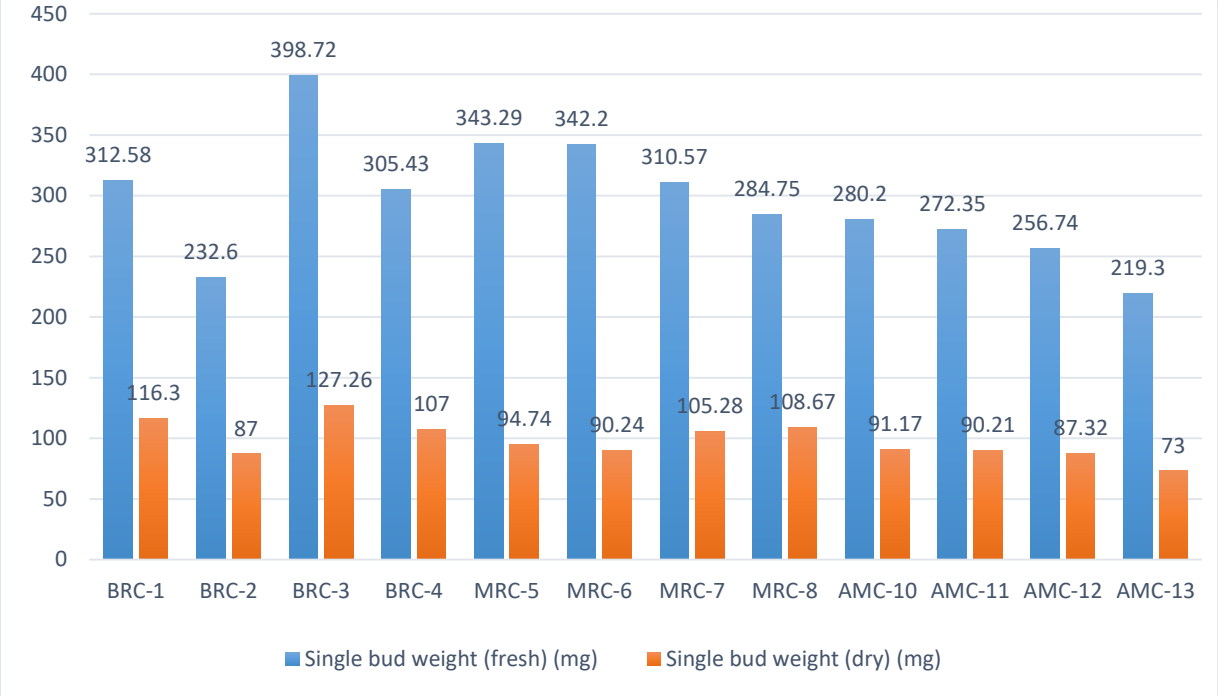


Fig 7. Single bud weight fresh and dry of selected clove accessions

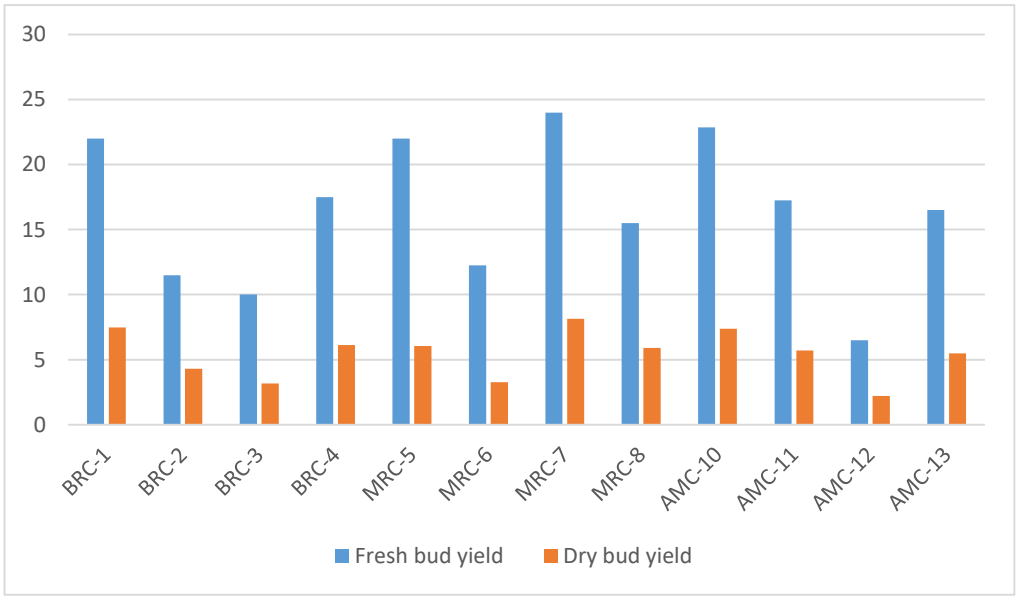


Fig 8. Pooled mean of fresh bud yield/tree and dry bud yield/tree (2018-2019 to 2019-2020) of selected clove accessions

5.2 STANDARDIZATION OF POLLINATION TECHNIQUE

5.2.1 Floral phenology and biology

5.2.1.1 Flower opening time

The flower opening time taken from twenty branches of five selected clove plants constituting 403 flowers for a period till all flowers in all branches opened. The percentage of maximum flower opening time observed was at 4:30-5:30 pm (41.62 per cent) followed by 3:30-4:30 pm (37.68 per cent). Thus the maximum flower opened between 3.30- 5.30 pm. The details of mean flower opening time is shown in Table 16. The details of individual observation is shown in Appendix-1. Wit (1969) has observed that clove flowers in Indonesia opened early in the morning. According to Nair *et al*. (1974), anthesis in clove occurs in the afternoon at 1.30 pm, with a peak between 3.30 pm. and 4.30 pm. Sritharan and Bavappa (1981) have reported from Sri Lanka that the majority of clove flowers open in the afternoon. However, flower opening time varied with respect to different locations in the world.

Water movement to the floral parts was important for opening of the flower since a blockage in the basal stem resulted in the impairment of flowering. In cotton and chrysanthemum, the continuum of water in the flower was separated from that in the stem, and the flower could open even when leaves were wilting due to desiccation (Trolinder *et al*., 1993; van Doorn and van Meeteren, 2003).

Floral buds open due to petal expansion and, to a lesser extent, stamen elongation. Flower opening is typically rapid and is completed within 5–30 min. In most species it occurs due to uncovering of the petals or petal movements thereby removal of physical constraints through the abscission of bracts and sepals. Petal movements can be controlled by cell expansion or shrinkage *via* osmoregulation. In most species petal movements occur due to differential growth rate of the epidermis on the two sides (i.e. abaxial or adaxial sides). A flower opens when the adaxial side of petals grows more than the abaxial side. This differential growth is mostly mediated by differential osmoregulation; carbohydrates accumulate, from either mobilization of storage carbohydrates or sucrose import (van Doorn and van Meeteren, 2003).

The petals of morning glory (*Ipomoea tricolor*) open early morning hours and senesence in the afternoon of the same day. The senescence starts by rolling of the corolla, curling the rib starting from the distal tip margins. This process is induced by ethylene, which enhances ion (Rb+) and sucrose efflux from the rib; the rolling could be due to asymmetric turgor changes, since the petals could be unrolled when turgor pressure was eliminated in a strong hyperosmotic treatment that plasmolysed cells (Hanson and Kende, 1975). Young flowers contain high levels of starch and solute levels increase prior to flower opening through the uptake of sugars from the apoplast and the conversion of polysaccharides to monosaccharides. This was supported by the fact that inhibition of starch degradation prevented petal growth in lily (Bieleski *et al*., 2000). In the case of tulip, in which the flower opens diurnally according to the daily temperature fluctuations, petal expansion is regulated at the level of aquaporin protein activity. The petals open when the temperature rises in the morning, and they close in the evening when the temperature drops. It has been shown that warm temperature activates water transport into the petal cells *via* the phosphorylation of PIP2:2 aquaporin in a Ca^{2+}-dependent manner (Azad *et al*., 2004, 2008). Aquaporins play central roles in petal cell expansion, and they mediate ethylene-dependent inhibition of flower opening. Ethylene seems to regulate aquaporin expression and ultimately petal cell expansion, *via* miRNA164-mediated post-transcriptional regulation and the NAC-domain transcription factor RhNAC100 (Pei *et al*., 2013).

According to Arnold (1959), if the flower opens in the late afternoon or at night high RH may have the stimulating effect on its opening. Apparently, a positive effect on opening of a decrease in RH has not been reported, indicating that opening in the morning is generally independent of RH. Floral opening in several species is independent of external regulation as it occurs at any time of the day while in other species, belonging to a large number of families, show a relationship with the time of day. This dependence on the time of the day may be regulated by external cues such as temperature, humidity, and light and/or by an internal rhythm (van Doorn and van Meeteren, 2003).

Oxalis plants were exposed to temperature and light (about 12 W/m^2) was also switched on, opening was hastened. In plants continuously held in darkness at 20 °C or 25 °C, light alone was sufficient for opening. However light had no effect in plants held at 15 °C or lower.

These data suggest that the ambient temperature determined whether flower opening in this species depends on an increase in temperature, light or both (Tanaka *et al*., 1989).

The flower opening due to increased elongation or local ion accumulation, differential enlargement of petal and stamen, accumulation of monosaccharides leading to difference in turgour pressure and differential changes in hormones might have resulted in the opening of the flower. The environmental factors, like relative humidity, temperature and light might also have influenced its flower opening. The sufficient temperature, light and relative humidity might have attained by 3.30 to 5.30 pm and this might have resulted in maximum opening of flower at that time.

5.2.1.2 Anthesis duration

Anthesis duration recorded is presented in Table 16. The opening of the flower was recorded from 0 to 24 hours. From 6.30 pm in the evening to 2.30 pm in the morning the flower buds did not open. All flower buds observed opened between 2.30 pm and 6.30 pm. Thus anthesis duration was from 2:30 pm to 6.30 pm. According to Nair *et al*. (1974), anthesis in clove occurs in the afternoon at 1.30 pm with a peak between 3.30 pm and 4.30 pm.

5.2.1.3 Number of stamens per flower

The number of stamens per flower varied from 167 to 343. The mean number of stamens per flower was 243.9. In a study conducted by Jayasree (2019) on characterization and evaluation of *Syzygium sp.* the highest number of stamens were found in *Syzygium samarangense* (white) (397.50), while *Syzygium jambosa* had the lowest number of stamens (224). On the other hand, *Syzygium samarangense* (red) and *Syzygium malaccense* had 352 and 249 stamens, respectively. Stamens of *Syzygium* are usually numerous (40-50 per flower in *Syzygium balsameum* to 1200 in *Syzygium megacarpum* (Chantaranthoi and Parwell, 1994). In *Syzygium saveri* approximately 150-200 stamens/flower were tightly packed between the 2 calyx lobes but appeared to drop out easily when touched (Boulter *et al.*, 2005). The literature of number of stamens in all the above *Syzygium sp.* including *Syzygium aromaticum* showed lot of variability.

5.2.1.4 Anther dehiscence

Anther dehiscence starting time and Anther dehiscence ending time observed for a period of 48 h before flower opening to anthesis is presented in Table 18. The rupturing of anthers started 36 h before anthesis and continued till opening of the flowers. Maximum anthers dehisced between 36 to 24 h before flower opening and maximum dehiscence occurs on 12 h before anthesis. Anther dehiscence noticed at 36, 24, 12 and 0 h of flower bud opening revealed all anthers were dehisced at the time of opening of flowers. Wit (1969) observed that the anthers of clove flowers in Indonesia dehisced within a few hours of flower opening. Nair *et al*. (1974) reported that anthers in clove dehisced longitudinally and anther dehiscence commenced 24 h before anthesis. Sritharan and Bavappa (1981) reported from Sri Lanka that the anther dehiscence begins 24 h before flower opening and dehiscence peaks immediately. Pool and Bermawie (1986) concluded that anther dehiscence in clove occurred shortly after anthesis. However, in this study anther dehiscence commenced from 36 h before flower opening and ends immediately after anthesis.

According to Cardarelli and Costantino (2018) jasmonic acid and auxin plays an important role in anther dehiscence. Xu *et al*. (2019) observed that AUXIN RESPONSE FACTOR17 (ARF17) plays a crucial role in pollen wall pattern formation, tapetum development, and auxin signal transduction in anthers as revealed from experiments on *Arabidopsis*. ARF17 is also involved in anther dehiscence. The *Arabidopsis* (*Arabidopsis thaliana*) *arf17* mutant exhibits defective endothecium lignification, which leads to defects in anther dehiscence. The expression of *MYB108*, which encodes a transcription factor important for anther dehiscence, was dramatically down-regulated in the flower buds of *arf17*. The expression pattern of both *ARF17* and *MYB108* is consistent with the function of these genes in anther dehiscence. The ARF17-MYB108 pathway is involved in regulating anther dehiscence.

5.2.1.5 Pollen characteristics

The pollen morphology studied using Scanning Electronic Microscope is presented in plate 10. The pollen grains were monad, radially symmetrical, triangular, trizonosyncolporate and were having exine ornamentation. Polar and equatorial axis of pollen grains of clove is

depicted in Table 19. The polar diameter of pollen grain ranged from 17.06-18.22 µm and the equatorial diameter of pollen grain ranged from 19.40-20.70 µm. Pickett and Newsome (1997) indicated that pollen of Myrtaceae is considered distinctive, being usually syncolpate or parasyncolpate. *Syzygium megacarpum* had the longest pollen grains (18.4 µm) and twice the size of *Syzygium foxworthianum* and *Syzygium jasminifolium* which had the smallest (7.7 µm) (Parnell, 2003). However in the study pollen grain size ranged from 17.08-18.02 µm which is almost similar to *Syzygium megacarpum* and larger than *Syzygium foxworthianum* and *Syzygium jasminifolium.*

5.2.1.6 Stigma receptivity

Stigma receptivity was observed for 7 days from the day of flower opening and is presented in Table 20. The stigma receptivity by hydrogen peroxide test revealed stigma was receptive was six days from anthesis. Bubbles started appearing from second day of anthesis and maximum stigma receptivity was observed on fifth day of anthesis with the highest number of bubbles (31.40 per cent). Sritharan and Bavappa (1981) had reported from Sri Lanka that maximum stigma receptivity in clove was attained on the day of anthesis, with the stigma remaining receptive for further 48 h. Workers in the Andaman Islands also observed that the stigma does not become receptive until 3 days after anthesis, reaching peak receptivity 2 days later and remaining receptive for an additional 2 days (Nair *et al*., 1974). However, in this study stigma receptivity started 2 days after anthesis and maximum stigma receptivity attained on fifth day of anthesis and ends on sixth day of anthesis. Jamshed *et al*. (2020) reported that in *Arabidopsis*, an extremely functionally redundant mitogen-activated protein kinase (MAPK) cascade is required for maintaining stigma receptivity to accept compatible pollen. Genetic analyses demonstrated that in stigmas, five MAPK kinases (MKKs), MKK1/2/3/7/9 are required to transmit upstream signals to two MPKs, MPK3/4, to mediate compatible pollination.

5.2.1.7 Standardization of emasculation

Emasculation period of clove was standardized from the anther dehiscence starting time and stigma receptivity. Since anther dehiscence begins 36 h before anthesis and no more pollen was available in anthers after two days of anthesis. Stigma receptivity observed from second day of anthesis to sixth day of anthesis and maximum was observed on 5^{th} day of anthesis. The emasculation should hence start from 48 h before flower bud opening which was revealed by

the starting of separation of the petals. However, anther dehiscence ends immediately after anthesis, stamens fall after 2 days of anthesis and stigma receptivity started second day of anthesis. Hence emasculation of buds may be avoided. But research in this field is meagre. In *Syzygium leyhmannii*, emasculation of flowers generally improved fruit set. Fruit set for emasculated, open pollinated fruit was 250 per cent higher than that which naturally occurred. It was possible that part of this response might not be due to the emasculation but rather the fact that some flowers were removed to make emasculating easier. This 'thinning' of flower clusters might have led to better retention of the remaining flowers. Emasculated flowers were capable of setting seed if open pollinated naturally but not by hand pollination. Emasculation by itself did not reduce seed set but the hand pollination procedure used appeared less effective for fertilisation compared to natural pollination (Sanewski, 2010).

5.2.2 Pollen collection

The pollen collected during different periods of anthesis (12, 24 and 36 h before anthesis) from the clove plants were tested for pollen viability and pollen fertility. Pollen viability at different period of anthesis is shown in Table 21. Pollen viability by IKI method revealed that 12 h before anthesis showed 94.02 per cent viability which was maximum among the treatments followed by 24 h before anthesis which showed 92.16 per cent viability. The pollen viability increased with increase in anthesis time. Pollen fertility by acetocarmine method is presented in Table 22. Pollen fertility was maximum on the pollen collected 12 h before anthesis (82.83 per cent) followed by 24 h before anthesis which showed 80.84 per cent fertility. As pollen viability and pollen fertility was maximum on pollen collected 12 h before anthesis, pollen collection is standardized on 12 h before anthesis. The stainability of pollen in clove was 81 per cent and stained pollen grain was larger in size and gave a higher percentage of germination (Sritharan and Bavappa, 1981; Bagade *et.al*., 1996). In *Syzigium cuminii*, during the beginning of the season pollen fertility was higher and goes on decreasing as the season advanced (Singh *et al*., 2009). Sanewski (2010) reported that after four days of anthesis, peak pollen germination was seen in *Syzygium leymannii* and hence viability occurred on four days after anthesis. This approximately coincided with two days after stamen emergence that was when anthers dehisced to release pollen.

5.2.3 Pollen storage

Pollen storage of clove by finding the pollen viability and pollen fertility of dried pollen at different storage conditions like desiccator and refrigerator at different temperatures was compared with fresh pollen and the data is presented in Table 23, Table 24, Table 25 and Table 26.

5.2.3.1 Percentage of pollen viability of clove by IKI method

The pollen viability of fresh pollen on the day of collection (0 DAS) was 94.36 percent and was the maximum and superior to all other storage conditions. Treatment (T_6), which was pollen dried at 50 °C and stored in refrigerator after 15 days of storage showed 87.90 per cent viability which was maximum and was on par with treatment (T_4), which was pollen dried at 40 °C and stored in refrigerator and treatment (T_7), which fresh pollen was kept at room temperature. The pollen dried at 50 °C and stored for 30 days on refrigerator (T_6) showed 84.73 per cent viability which was maximum and was on par with treatment (T_1) which was pollen dried at 40°C and stored in desiccator. The pollen dried at 50°C and stored in desiccator (T_3) for 60 days revealed 77.12 per cent viability which was maximum and was on par with pollen dried at 50° C and stored in refrigerator (T_6) as well as with pollen dried at 45° C and stored in desiccator (T_2). Fresh pollen stored at room temperature for 60 days showed minimum pollen viability of 69.90 per cent.

As indicators of pollen viability, staining tests are often preferred because they are faster and easier compared to pollen germination, but they tend to overestimate the viability and real germination of pollen grains (Gaaliche *et al*., 2013).

Pollen of peony cultivars stored for more than one year under four storage conditions (4 °C, −4 °C, −20 °C, and −76 °C) revealed that pollen stored at −76 °C showed a significantly slower rate of viability reduction compared to all the other storage conditions. The differential rates of reduction in pollen viability stored at different temperature regimes might be the result of temperature-dependent rates of metabolic activities in the pollen (Du *et al*., 2019).

Regarding shelf-life during storage and the pollen tube growth, the most important factors are temperature and humidity (Qiao *et al*., 2010). Another important factor affecting pollen shelf-life is the moisture content (Shi *et al*., 1987; Wang *et al*., 2003). Drying is the most frequently used method for pollen preservation as it can prevent the growth and reproduction of microorganisms and minimize many of the moisture-mediated degradation reactions to enhance shelf-life (Wang and Wang, 2017). Moreover, longevity and storage stability of pollen may be connected to the presence of substances such as proteins and starches (Li and Chen, 1998).

5.2.3.2 Two way mean table of percentage of pollen viability of clove by IKI method

From the above two way mean Table 24 of percentage of pollen viability of clove by IKI method, $T_{6,}$ pollen dried at 50°C and kept in refrigerator had 84.92 per cent viability which was maximum and was significantly different among the treatments irrespective of days and all other treatments T_1-Dessicator (40°C dried pollen), T_2 –Desiccator (45°C dried pollen), T_3 –Desiccator (50°C dried pollen), T_7 -Fresh pollen stored at room temperature (Control), T_5-Refrigerator (45°C dried pollen) and T_4- Refrigerator (40°C dried pollen) had 82.46, 82.01, 81.90, 81.75, 80.32 and 80.25 per cent which were significantly on par. Among the treatments the best one was pollen dried at 50°C and stored in refrigerator.

At 0 days after storage had 90.52 per cent viability which was maximum followed by 15 days after storage (84.86 per cent), 30 days after storage (78. 07 per cent) and 60 days after storage (74.32 per cent).

5.2.3.3 Percentage of pollen fertility in clove by acetocarmine glycerine method

Pollen fertility of pollen dried at different temperature and stored in desiccator and refrigerator for 60 days is presented in Table 25. Pollen fertility on the day of collection dried at different temperatures and stored in desiccator, refrigerator or under room temperature did not differ significantly from each other. At 15 days after storage, pollen dried at 40° C in

desiccator had 88.13 per cent fertility which was maximum among the treatments and was on par with pollen dried at 40 °C and as well as 45 °C and stored at refrigerator (T_4 and T_5). At 30 days after storage pollen dried at 50 °C and stored in desiccator had 82.12 per cent fertility which was maximum among the treatments and was on par with pollen dried at 45° C and stored in desiccator as well as with pollen dried at 45° C (T_2) and stored in refrigerator (T_5). At 60 days after storage, pollen dried at 45°C and stored in refrigerator produced maximum pollen fertility of 78.29 per cent and was on par with pollen dried at 50° C and stored in desiccator (T_3), pollen dried at 45 °C and stored in desiccator (T_2) and pollen dried at 50°C and stored at refrigerator (T_6). The fresh pollen stored at room temperature for 60 days revealed 68.82 per cent pollen fertility.

The activities of cytochrome oxidase (CO) and malate dehydrogenase (MDH) of anthers of maize lines with male-sterile T, S and C cytoplasms as well as normal (N) cytoplasm measured indicated CO activity could be used as an index of pollen fertility (Ohmasa, 1984). Non-functional pollen production by plant reduces effectiveness of pollination. A study carried out at Cocoa Research Institute of Nigeria, Ibadan to determine the influence of rainfall and temperature on flowering intensity of selected clones of Upper Amazon cocoa , as well as its pollen fertility revealed that pollen stainability was the highest for clones T86/ 45, C64 and C77, implying that their pollen grains were fertile while clone T12/5 showed the lowest pollen fertility (81.06 per cent) which suggested that clones with low pollen fertility would not be good candidates for hybrid seed production in cacao (Omolaja *et al*., 2009). Genetic studies in *Arabidopsis* and rice identified a large number of evolutionally conserved genes that are required for pollen development and male fertility (Shi *et al*., 2015a).

5.2.3.4 Two way mean table of percentage of pollen fertility in clove by acetocarmine glycerine method

From the above mean Table 26, percentage of pollen fertility in clove by acetocarmine glycerine method, T_3, pollen dried at 50°C and stored in desiccator had 83.39 per cent fertility which was maximum and was on par with T_5- Refrigerator (45°C dried pollen) and T_4- Refrigerator (40°C dried pollen), T_2 –Desiccator (45°C dried pollen), T_6 –Refrigerator (50°C dried pollen). Mean pollen fertility of T_1-Dessicator (40°C dried pollen) showed 80.59, while T_2 -81.00, T_4- 82.18, T_5-83.29 and T_6 showed 80.64 per cent. T_7 Fresh pollen stored at room

temperature (Control) had 77.70 percent fertility which was lower among the treatments. Among the treatments the best one was pollen dried at 50°C and stored in desiccator.

The pollen on the day of collection had 89.34 percent fertility which was maximum followed by 15 days after storage (85.24 per cent), 30 days after storage (77.59 per cent) and 60 days after storage (74.52 per cent).

Pollen viability may be decreased under low humidity because of the injury caused from rapid loss of moisture from the pollen grains, sometimes due to rapid increase of physiological activities of the pollen grains under high humidity (Liskens and Schrauwen, 1966). In *Syzigium cuminii*, pollen could be stored successfully for 9 months at a temperature of 23°C in completely dry atmosphere (Singh *et al*., 2009). The most common methods used to store the pollen involve reducing the water content of pollen and maintaining the pollen grains at low temperatures to avoid fluctuations. On the contrary pollen viability of stored pollen was influenced by moisture and storage temperature in addition to physiological and genetic factors (Ganeshan *et al*., 2008).

5.2.4 Hybridization

5.2.4.1 Percentage of fruit set

The percentage of fruit set in all the plant combinations selected is presented in Appendix-3. The mean percentage of fruit set of different plant combinations during crossing in clove is presented in Table 27. The percentage of fruit set ranged from 15 per cent to 42.5 per cent and average fruit set from all the plant combinations was 28.87 per cent. In *Syzygium jambosa* 25.21 per cent open pollinated flowers set fruits whereas no fruit set occurred after netting and bagging the inflorescence (Bhattacharya and Mandal, 2000). Raju *et al.* (2005) reported that hand pollination experiments in *Syzygium alterifolium* indicated that autogamy and geitonogamy were non-functional while xenogamy was the only mode of pollination for fruit set. In this mode, fruit set stood at 56 per cent while in open pollination mode, it was 11 per cent only in *Syzygium alternifolium*. In *Syzigium cuminii*, fruitset by hand pollination indicated that 45-50 per cent occurs by self pollination and 30-40 percentage by cross pollination (Singh *et al*. 2009). In *Syzygium leyhmannii*, xenogamous pollination (cross

pollination between different seedling trees) produced the highest fruit set, a 3.4 fold increase over self pollination. Without pollination of emasculated flowers produced the same percentage of fruit set as open or self pollination. Without pollination produced no seed whereas all forms of pollination produced the same percentage seedy fruit (Sanewski, 2010).

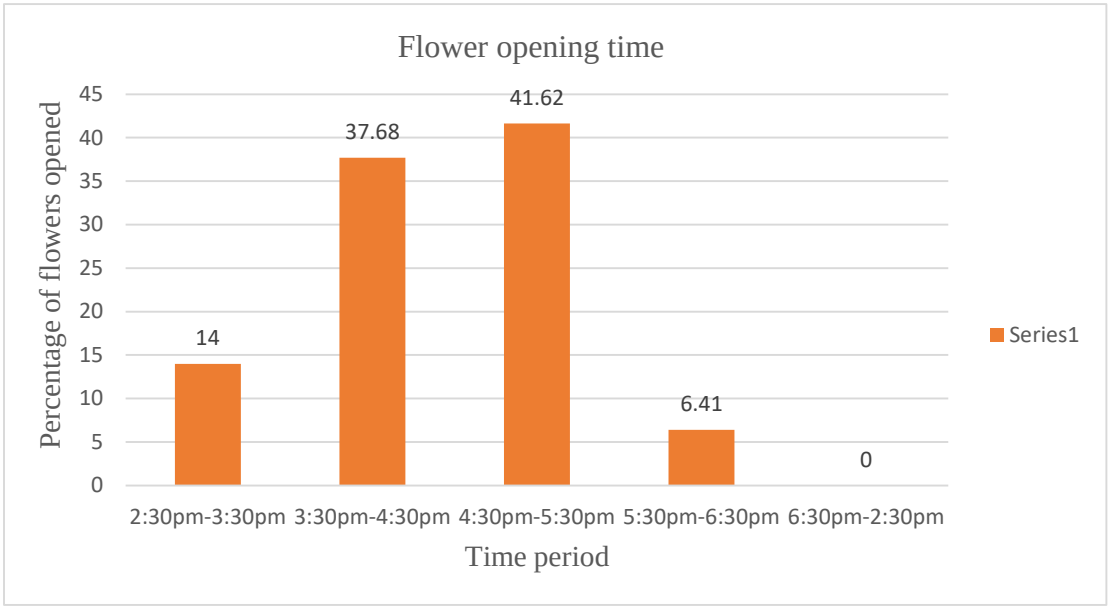


Fig 9. Percentage of flowers opened at different time periods

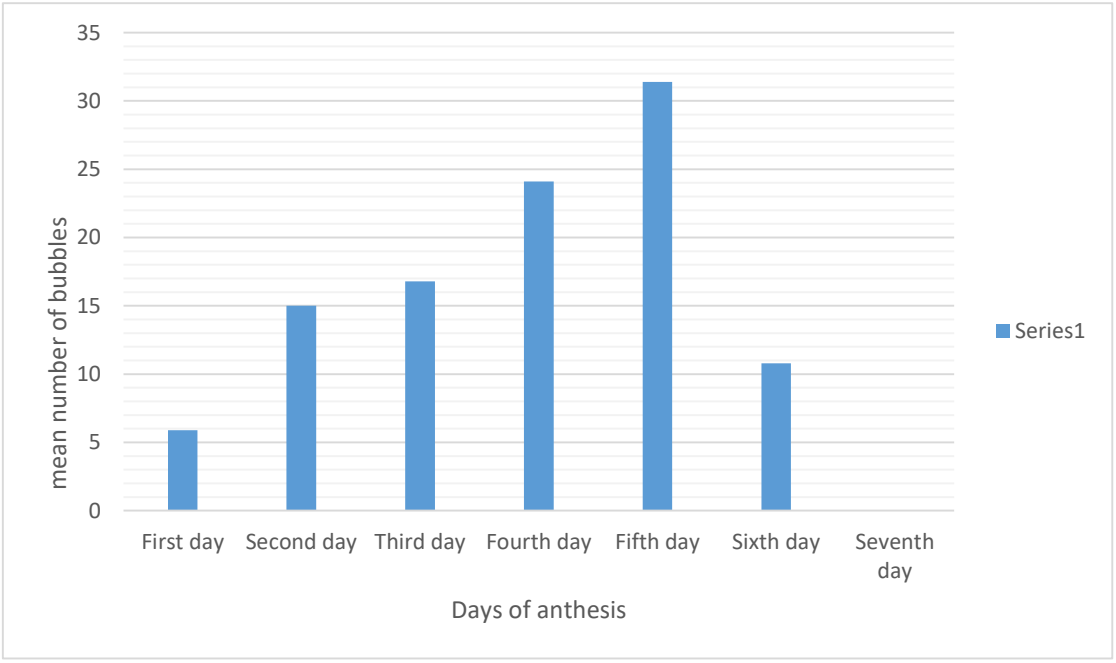


Fig 10. Mean number of bubbles at different days of anthesis

SUMMARY

6. SUMMARY

The present study entitled "Evaluation of elite clove (*Syzigium aromaticum* (L) Merr. & Perry) accessions and standardization of pollination techniques'' was carried out in the Department of Plantation Crops and Spices, College of Agriculture, Vellayani from 2018-2020. The objective of the study was to evaluate elite clove accessions and to standardize pollination techniques for hybridization.

Twelve elite clove accessions were evaluated for two years for quantitative characters from the twenty clove accessions identified from variability studies of clove conducted by Avinash during 2016-2018 in the Department. The floral phenology and biology were studied and the method of emasculation, pollen collection, pollen storage and hybridization was standardized as part of the study.

The twelve elite accessions identified from estates of Trivandrum and Kollam districts of Kerala were BRC-1, BRC-2, BRC-3, BRC-4, MRC-5, MRC-6, MRC-7, MRC-8, AMC-10, AMC-11, AMC-12 and AMC-13. Quantitative characters of these accessions were recorded for two consecutive years, 2018-2019 and 2019-2020. Quantitative characters of tree, leaf, bud, flower, fruit and seed characters were recorded. The tree characters included plant height, girth at 30 cm, canopy spread and number of branches. The plant height of twelve elite clove accessions extended from 6.15 m in AMC-13 to 15.30 m in MRC-7 and the girth at 30 cm height varied from 70 cm in BRC-2 to 165 cm in the accessions BRC-3. The canopy spread in North South extended from 4.80 m in AMC-12 which was the lowest to 7.70 m in MRC-7 which was the highest and the canopy spread in East West extended from 5.00 m in AMC-12 to 8.10 m in MRC-7. The number of branches in the clove accessions ranged from 40 in AMC-12 to 57 in AMC-11. The leaf characters observed were leaf length, leaf breadth and leaf area. The leaf length of clove accessions varied from 9.59 cm in BRC-3 to 13.98 cm in MRC-5. The leaf breadth of clove accessions varied from 3.81 cm in AMC-12 to 4.75 cm in AMC-11 and the leaf area extended from 25.35 cm^2 in MRC-7 to 43.00 cm^2 in AMC-11.

The bud characters observed were number of inflorescence/m^2, number of flower buds/inflorescence, single bud weight (fresh), single bud weight (dry), mature bud length, and mature bud diameter. The number of inflorescence/ m^2 was the highest in AMC-12(156.25) followed by AMC-10 (117.10) and BRC-1 (115.25) and the lowest number of inflorescence/m^2

was observed in MRC-8 (53.70). The number of flower buds/inflorescence was the highest in MRC-6 (17.25) and the lowest in BRC-3 (8.10). The single bud weight (fresh) among the clove accessions ranged from 219.30 mg in AMC-13 to 398.72 mg in BRC-3. The single bud weight (dry) among the clove accessions ranged from 73.00 mg in AMC-13 to 127.26 mg in BRC-3 which recorded the highest single dry bud weight. The mature bud length among the clove accessions ranged from 14.56 mm in BRC-2 which was the lowest to 19.34 mm in BRC-1 which was the highest, whereas the mature bud diameter among the clove accessions varied from 4.67 mm to 6.45 mm.

Flower characters recorded were length of flower, breadth of flower, length of sepal, length of petal and flowering period. Clove accessions selected showed varied length of flower ranging from 14.98 mm in AMC-12 to 21.86 mm in BRC-1 while the breadth of flower among the clove accessions extended from 11.47 mm in BRC-2 to 13.89 mm in BRC-3. Maximum length of sepal was observed in BRC-1 (3.16 mm) and minimum length of sepal was observed in MRC- 5 (2.54 mm). The length of petal among the clove accessions varied from 4.26 mm in MRC-8 to 6.15 mm in BRC-3. Flowering period of accessions BRC-1, MRC-5 and AMC-13 was from December to February whereas the remaining accessions BRC-2, BRC-3, BRC-4, MRC-6, MRC-7, MRC-8, AMC-10, AMC-11 and AMC-12, flowering period was from January to March.

The pooled mean of two years data revealed that fresh bud yield per tree of selected clove accessions ranged from 6.50 kg in AMC-12 to 24.00 kg in MRC-7. The higher yielders in terms of fresh bud yield per tree were MRC-7 (24.00 kg), AMC-10 (22.87 kg), BRC-1 (22.00 kg), MRC-5 (22.00 kg), BRC-4 (17.50 kg) and AMC-11 (17.25 kg). The bud yield per tree dry was the highest for MRC-7 (8.13 kg/tree) followed by BRC-1 (7.48 kg/tree) and the least observed in AMC-12 (2.21 kg/tree).

The fruit characters recorded included fruit weight (fresh) and ratio of fruit to seed. The fruit weight (fresh) of clove accessions ranged from 1.21 g in AMC-12 to 3.54 g in BRC-3 and the ratio of fruit to seed was the highest in AMC-13 (3.07) and was the least in MRC-6 (2.38). Seed characters such as seed length, seed breadth and seed weight were observed. The seed length of twelve elite clove accessions ranged from 13.47 mm in AMC-12 to 19.40 mm in BRC-3, seed breadth of elite clove accessions ranged from 6.31 mm in AMC-12 to 9.24 mm

in BRC-3 and the seed weight recorded was the highest in BRC-3 and AMC-11 and was the least in AMC-12 which had only 0.50 g. During the evaluation of elite clove accessions, leaf spot due to *Colletotrichum gloeosporioides* was noticed in elite clove accessions. Disease severity was the highest in BRC-2 (28.33 per cent) and lowest in MRC-6 (15 per cent). There was no pest incidence in the selected clove accessions.

Multi Dimensional Scaling is a multivariate analysis technique that serves as a dimension reduction technique for large dimensional data. Multidimensional scaling was carried out to determine the selection criteria and for identification of elite genotype. Based on the scree plot of MDS, first 2 dimensions were selected for generating the biplot (Figure 1). The 25 variables with 12 observations in Multi Dimensional Scaling computed 10 dimensions and the contribution of MDS Dimension-1 and Dimension-2 were 75.76 and 17.21 per cent with cumulative variance of 92.97 per cent respectively. Based on Multi Dimensional Scaling Configuration eight clusters of selected clove accessions were identified. Dendrogram representing the relationship among 12 elite accessions based on Euclidean distance identified same eight clusters of clove accessions that were identified in MDS configuration. The linkage analysed based on the biplot of two dimensions of multidimensional scaling of biplot coordinates revealed a strong positive association between most of the characters measured except, girth at 30 cm height, number of inflorescence per m^2, single bud weight fresh and single bud weight dry. Minimum Data Set for identifying a promising clove accession was generated. It revealed accessions BRC-1, MRC-5 and MRC-6 had better ideotype and can be suggested as elite or superior accessions.

The experiment on floral phenology and biology was carried out in clove plants at Braemore estate, Trivandrum district. Flower opening time in clove varied from 2.30 pm to 6.30 pm and maximum percentage of flower opening was observed between 4:30-5:30 pm (41.62 per cent) followed by 3:30-4:30 pm (37.68 per cent). Thus the maximum flower opened between 3.30-5.30 pm. The anthesis duration was from 2:30 pm to 6.30 pm. The number of stamens per flower varied from 167 to 343. The rupturing of anthers started 36 h before anthesis and maximum anther dehiscence occurred between 36 h before anthesis till anthesis. The pollen grains were monad, radially symmetrical, triangular, trizonosyncolporate and were having exine ornamentation. The polar diameter of pollen grain ranged from 17.06-18.22 µm and the equatorial diameter of pollen grain ranged from 19.40-20.70 µm. The stigma

receptivity by hydrogen peroxide test revealed stigma was receptive upto six days from anthesis and maximum stigma receptivity was observed on fifth day of anthesis with the highest number of bubbles (31.40 per cent). The emasculation should hence start from 48 h before flower bud opens which was revealed by the starting of separation of the petals.

Pollen viability by IKI method revealed that 12 h before anthesis showed 94.02 per cent viability which was maximum followed by 24 h before anthesis which showed 92.16 per cent viability. Pollen fertility was maximum for the pollen collected 12 h before anthesis (82.83 per cent) followed by 24 h before anthesis which showed 80.84 per cent fertility. Thus the pollen collection should be done 12 h before anthesis.

Pollen storage studies revealed pollen stored in refrigerator had maximum pollen viability whereas pollen fertility was maximum in desiccator as revealed from two way Table 24 and 26. Pollen dried at 50°C had maximum pollen viability and fertility compared to 45°C and 40°C. Pollen viability and pollen fertility was maximum on the day of collection and decreased with increase in number of days.

The hybridization carried out with selected five clove plants as male and female parents. The percentage of fruit set among the combinations ranged from 15.00 per cent to 42.50 per cent and average fruit set from all the plant combinations was 28.87 per cent.

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ABSTRACT

EVALUATION OF ELITE CLOVE (*Syzigium aromaticum* (L) Merr. & Perry) ACCESSIONS AND STANDARDIZATION OF POLLINATION TECHNIQUES

by

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ABSTRACT

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ABSTRACT

The present study entitled "Evaluation of elite clove (*Syzigium aromaticum* (L) Merr. & Perry) accessions and standardization of pollination techniques" was taken up with the specific objective to evaluate elite clove accessions and to standardize pollination techniques for hybridization.

The twelve elite accessions identified from estates of Trivandrum and Kollam districts of Kerala designated as BRC-1, BRC-2, BRC-3, BRC-4, MRC-5, MRC-6, MRC-7, MRC-8, AMC-10, AMC-11, AMC-12 and AMC-13 were evaluated during the period of 2018-2020. Quantitative characterization was done with 25 quantitative characters including tree, leaf, bud, flower, yield, fruit and seed characters. The girth of the tree at 30 cm height was maximum in BRC-3 (165 cm). Highest number of inflorescence/m^2 was recorded in AMC-12 (156.25). The number of flower buds/inflorescence was maximum in MRC-6 (17.25) while single bud fresh and dry weight was highest in BRC-3 with 398.72 mg and 127.26 mg respectively. The mature bud length among the clove accessions was the maximum in BRC-1 (19.34 mm) whereas the mature bud diameter was maximum in BRC-3 (6.45 mm). The dry bud yield per tree was the highest in MRC-7 (8.13 kg) followed by BRC-1 (7.48 kg). Quantitative characters summarized based on the descriptive statistics revealed wider range of variability in single bud weight fresh, number of inflorescence/m^2, single bud weight dry and girth of the tree.

Multi Dimensional Scaling (MDS), a multivariate analysis done on 25 variables with 12 observations revealed 10 dimensions and the contribution of MDS Dimension-1 and Dimension-2 were 75.76 and 17.21 percent with cumulative variance of 92.97 percent respectively. Based on the scree plot of MDS, first 2 dimensions were selected for generating the biplot. Based on MDS configuration eight clusters of selected clove accessions were identified. Dendrogram representing the relationship among 12 elite accessions based on Euclidean distance also identified same eight clusters of clove accessions that identified in MDS configuration. The linkage analysed based on the biplot of two dimensions of multidimensional scaling of biplot coordinates revealed a strong positive association between most of the characters measured except, girth at 30 cm height, number of inflorescence per m^2, single bud weight fresh and single bud weight dry. Minimum Data Set for identifying a

promising clove accession generated revealed accessions BRC-1, MRC-5 and MRC-6 had better ideotype and can be suggested as elite or superior accessions.

The experiment on floral phenology and biology was carried out in clove plants at Braemore estate, Trivandrum district. The flower opening time of the clove flowers observed were between 2:30-6:30 pm. However the percentage of flower opened was maximum between 3.30- 5.30 pm. The number of stamens per flower varied from 167 to 343. The rupturing of anthers started 36 hours before anthesis and maximum anther dehiscence occurred between 36 h before anthesis till anthesis. The pollen grains were monad, radially symmetrical, triangular, trizonosyncolporate and were having exine ornamentation. The polar diameter of pollen grain ranged from 17.06-18.22 µm and the equatorial diameter of pollen grain ranged from 19.40-20.70 µm. The stigma receptivity by hydrogen peroxide test revealed stigma receptivity was there upto six days from anthesis and maximum stigma receptivity was observed on fifth day of anthesis with highest number of bubbles (31.40 per cent). The emasculation should start from 48 hour before flower bud opens which was revealed by the starting of separation of the petals since anthers started rupturing from 36 hour before anthesis.

Pollen viability by iodine potassium iodide method revealed that pollen viability was maximum at 12 hour before anthesis (94.02 per cent). Pollen fertility was also maximum on the pollen collected 12 hour before anthesis (82.83 per cent). Thus the pollen collection should be undertaken 12 hour before anthesis. Pollen collected and dried at 50°C and stored upto two months of storage in refrigerator had maximum pollen viability whereas pollen fertility was maximum in pollen dried at 50°C and stored in desiccator.

The hybridization was carried out on selected five clove plants as male and female parents. The percentage of fruit set ranged from 15 per cent to 42.5 per cent and average fruit set from all the plant combinations was 28.87 per cent.

The study on "Evaluation of elite clove (*Syzigium aromaticum* (L) Merr. & Perry) accessions and standardization of pollination techniques" revealed that clove accessions such as BRC-1, MRC-5 and MRC-6 had superior ideotype and superior in effective yield. The floral phenology of clove was studied and pollen collection, storage and artificial hand pollination in clove was standardized.

APPENDIX

Appendix 1

Flower opening time of different days

Day 1 flower opening time

Selected branches	Total number of flowers in a branch	Time					
		6:30pm-2:30pm	2:30pm-3:30pm	3:30pm-4:30pm	4:30pm-5:30pm	5:30pm-6:30pm	Number of flowers opening per day
P_1B_1	24	0	0	2	1	0	3
P_1B_2	10	0	0	3	2	0	5
P_1B_3	16	0	0	0	3	1	4
P_1B_4	38	0	2	1	1	0	4
P_2B_1	35	0	0	3	2	0	5
P_2B_2	22	0	0	2	2	0	4
P_2B_3	15	0	0	2	3	0	5
P_2B_4	14	0	2	3	0	0	5
P_3B_1	42	0	1	4	2	0	7
P_3B_2	17	0	0	2	3	0	5
P_3B_3	13	0	2	2	1	0	5
P_3B_4	18	0	0	0	0	0	0
P_4B_1	15	0	0	0	2	0	2
P_4B_2	17	0	0	1	3	0	4
P_4B_3	13	0	2	3	1	0	6
P_4B_4	26	0	1	2	1	0	4
P_5B_1	16	0	0	3	1	0	4
P_5B_2	22	0	0	2	3	0	5
P_5B_3	19	0	1	1	1	0	3
P_5B_4	11	0	2	1	0	1	4
Mean		0	0.65	1.85	1.6	0.1	

Day 2 flower opening time

Selected branches	Total number of flowers in a branch	Time					
		6:30pm-2:30pm	2:30pm-3:30pm	3:30pm-4:30pm	4:30pm-5:30pm	5:30pm-6:30pm	Number of flowers opening per day
P_1B_1	21	0	0	2	3	0	5
P_1B_2	5	0	0	1	2	0	3
P_1B_3	12	0	2	3	1	0	6
P_1B_4	34	0	1	2	4	0	7
P_2B_1	30	0	0	4	2	0	6
P_2B_2	18	0	0	2	4	0	6
P_2B_3	10	0	0	1	4	0	5
P_2B_4	9	0	2	1	0	0	3
P_3B_1	35	0	0	4	2	0	6
P_3B_2	12	0	0	2	2	1	5
P_3B_3	8	0	2	1	0	0	3
P_3B_4	18	0	0	2	3	1	6
P_4B_1	13	0	0	1	2	0	3
P_4B_2	13	0	0	3	2	0	5
P_4B_3	7	0	1	1	0	0	2
P_4B_4	22	0	0	4	6	0	10
P_5B_1	12	0	0	1	2	0	3
P_5B_2	17	0	1	0	2	1	4
P_5B_3	16	0	0	1	1	1	3
P_5B_4	7	0	1	0	2	1	4
Mean		0	0.5	1.8	2.2	0.25	

Day 3 flower opening time

Selected branches	Total number of flowers in a branch	Time					
		6:30pm-2:30pm	2:30pm-3:30pm	3:30pm-4:30pm	4:30pm-5:30pm	5:30pm-6:30pm	Number of flowers opening per day
P_1B_1	16	0	4	0	1	0	5
P_1B_2	2	0	0	0	0	0	0
P_1B_3	6	0	0	0	2	0	2
P_1B_4	27	0	2	3	2	0	7
P_2B_1	24	0	0	2	3	0	5
P_2B_2	12	0	1	1	2	0	4
P_2B_3	5	0	0	1	2	0	3
P_2B_4	6	0	1	1	0	1	3
P_3B_1	29	0	1	3	2	1	7
P_3B_2	7	0	1	0	0	1	2
P_3B_3	5	0	1	0	1	0	2
P_3B_4	12	0	0	2	2	0	4
P_4B_1	10	0	0	2	1	0	3
P_4B_2	8	0	1	0	1	1	3
P_4B_3	5	0	0	0	1	1	2
P_4B_4	12	0	1	1	0	1	3
P_5B_1	9	0	2	3	1	0	6
P_5B_2	13	0	1	0	1	0	2
P_5B_3	13	0	2	1	0	1	4
P_5B_4	3	0	0	0	0	0	0
Mean		0	0.9	1	1.1	0.35	

Day 4 flower opening time

Selected branches	Total number of flowers in a branch	Time					
		6:30pm-2:30pm	2:30pm-3:30pm	3:30pm-4:30pm	4:30pm-5:30pm	5:30pm-6:30pm	Number of flowers opening per day
P_1B_1	11	0	0	2	1	0	3
P_1B_2	2	0	0	0	0	0	0
P_1B_3	4	0	0	1	0	0	1
P_1B_4	20	0	1	2	2	0	5
P_2B_1	19	0	0	3	1	1	5
P_2B_2	8	0	0	0	2	0	2
P_2B_3	2	0	0	1	0	0	1
P_2B_4	1	0	0	0	1	0	1
P_3B_1	22	0	0	3	2	0	5
P_3B_2	5	0	0	0	1	0	1
P_3B_3	3	0	0	1	0	0	1
P_3B_4	8	0	1	0	1	0	2
P_4B_1	7	0	0	1	1	0	2
P_4B_2	5	0	0	0	0	0	0
P_4B_3	3	0	0	0	0	0	0
P_4B_4	9	0	1	1	0	1	3
P_5B_1	3	0	0	0	0	0	0
P_5B_2	11	0	1	0	1	1	3
P_5B_3	9	0	0	0	2	0	2
P_5B_4	3	0	0	0	0	0	0
Mean		0	0.2	0.75	0.75	0.15	

Day 5 flower opening time

Selected branches	Total number of flowers in a branch	Time					
		6:30pm-2:30pm	2:30pm-3:30pm	3:30pm-4:30pm	4:30pm-5:30pm	5:30pm-6:30pm	Number of flowers opening per day
P_1B_1	8	0	1	2	1	0	4
P_1B_2	2	0	0	1	1	0	2
P_1B_3	4	0	1	2	0	1	4
P_1B_4	15	0	0	3	2	0	5
P_2B_1	15	0	0	1	4	1	6
P_2B_2	6	0	1	0	1	1	3
P_2B_3	2	0	0	1	1	0	2
P_2B_4	1	0	0	0	0	0	0
P_3B_1	17	0	1	2	4	1	8
P_3B_2	4	0	0	0	1	0	1
P_3B_3	1	0	0	0	1	0	1
P_3B_4	6	0	0	2	1	0	3
P_4B_1	5	0	1	0	0	1	2
P_4B_2	5	0	0	1	1	0	2
P_4B_3	3	0	0	0	0	0	0
P_4B_4	6	0	1	1	0	0	2
P_5B_1	3	0	0	0	0	0	0
P_5B_2	8	0	0	2	0	1	3
P_5B_3	7	0	0	1	1	0	2
P_5B_4	3	0	0	0	1	0	1
Mean		0	0.3	0.95	1	0.3	

Day 6 flower opening time

Selected branches	Total number of flowers in a branch	Time					
		6:30pm-2:30pm	2:30pm-3:30pm	3:30pm-4:30pm	4:30pm-5:30pm	5:30pm-6:30pm	Number of flowers opening per day
P_1B_1	4	0	0	1	1	0	2
P_1B_2	0	0	0	0	0	0	0
P_1B_3	0	0	0	0	0	0	0
P_1B_4	10	0	1	2	0	0	3
P_2B_1	9	0	1	3	2	0	6
P_2B_2	3	0	0	1	2	0	3
P_2B_3	0	0	0	0	0	0	0
P_2B_4	1	0	0	0	1	0	1
P_3B_1	9	0	1	1	3	0	5
P_3B_2	3	0	0	0	2	0	2
P_3B_3	0	0	0	0	0	0	0
P_3B_4	3	0	0	0	1	0	1
P_4B_1	3	0	1	0	1	0	2
P_4B_2	3	0	0	2	0	0	2
P_4B_3	3	0	0	0	0	0	0
P_4B_4	4	0	0	2	0	1	3
P_5B_1	3	0	0	1	1	0	2
P_5B_2	5	0	0	0	2	0	2
P_5B_3	5	0	0	3	0	0	3
P_5B_4	2	0	0	1	0	0	1
Mean		0	0.2	0.85	0.8	0.05	

Day 7 flower opening time

Selected branches	Total number of flowers in a branch	Time					
		6:30pm-2:30pm	2:30pm-3:30pm	3:30pm-4:30pm	4:30pm-5:30pm	5:30pm-6:30pm	Number of flowers opening per day
P_1B_1	2	0	0	0	2	0	2
P_1B_2	0	0	0	0	0	0	0
P_1B_3	0	0	0	0	0	0	0
P_1B_4	7	0	0	1	3	0	4
P_2B_1	3	0	1	0	0	1	2
P_2B_2	0	0	0	0	0	0	0
P_2B_3	0	0	0	0	0	0	0
P_2B_4	0	0	0	0	0	0	0
P_3B_1	5	0	0	1	2	0	3
P_3B_2	1	0	0	0	1	0	1
P_3B_3	0	0	0	0	0	0	0
P_3B_4	2	0	0	1	1	0	2
P_4B_1	1	0	0	1	0	0	1
P_4B_2	1	0	0	0	1	0	1
P_4B_3	3	0	0	1	0	1	2
P_4B_4	1	0	0	0	1	0	1
P_5B_1	1	0	0	0	1	0	1
P_5B_2	3	0	0	1	2	0	3
P_5B_3	2	0	1	1	0	0	2
P_5B_4	1	0	0	0	1	0	1
Mean		0	0.1	0.35	0.75	0.1	

Day 8 flower opening time

Selected branches	Total number of flowers in a branch	Time					
		6:30pm-2:30pm	2:30pm-3:30pm	3:30pm-4:30pm	4:30pm-5:30pm	5:30pm-6:30pm	Number of flowers opening per day
P_1B_1	0	0	0	0	0	0	0
P_1B_2	0	0	0	0	0	0	0
P_1B_3	0	0	0	0	0	0	0
P_1B_4	3	0	1	0	2	0	3
P_2B_1	1	0	0	0	1	0	1
P_2B_2	0	0	0	0	0	0	0
P_2B_3	0	0	0	0	0	0	0
P_2B_4	0	0	0	0	0	0	0
P_3B_1	2	0	0	1	1	0	2
P_3B_2	0	0	0	0	0	0	0
P_3B_3	0	0	0	0	0	0	0
P_3B_4	0	0	0	0	0	0	0
P_4B_1	0	0	0	0	0	0	0
P_4B_2	0	0	0	0	0	0	0
P_4B_3	0	0	0	0	0	0	0
P_4B_4	0	0	0	0	0	0	0
P_5B_1	0	0	0	0	0	0	0
P_5B_2	0	0	0	0	0	0	0
P_5B_3	0	0	0	0	0	0	0
P_5B_4	0	0	0	0	0	0	0
Mean		0	0.05	0.05	0.2	0	

Appendix 2

Anther dehiscence of individual anthers

Time period	R1	R2	R3	R4	R5	Mean percentage of anthers dehisced
48hour (Total number of anthers)	210	193	168	243	203	
(Number of anthers dehisced)	0	0	0	0	0	
(Percentage of anthers dehisced)	0	0	0	0	0	**0**
36hour (Total number of anthers)	167	169	243	293	343	
(Number of anthers dehisced)	30	32	40	39	49	
(Percentage of anthers dehisced)	17.96	18.93	16.46	13.31	14.28	**16.188**
24hour (Total number of anthers)	287	267	258	198	214	
(Number of anthers dehisced)	258	202	210	170	198	
(Percentage of anthers dehisced)	89.8	75.65	81.3	85.85	92.52	**85.024**
12hour (Total number of anthers)	246	296	188	212	189	
(Number of anthers dehisced)	235	280	183	200	181	
(Percentage of anthers dehisced)	95.5	94.59	97.34	94.33	95.76	**95.504**
0 hour (Total number of anthers)	234	217	184	289	214	
(Number of anthers dehisced)	230	213	181	284	211	
(Percentage of anthers dehisced)	98.29	98.15	98.36	98.26	98.59	**98.33**

Appendix 3

Percentage of fruit set in all plant combinations

Plant combinations	Total number of flowers pollinated	Total number of flowers set fruit	Mean percentage of fruitset
P1 X P2	40	11	27.5
P1 X P3	40	10	25
P1 X P4	40	14	35
P1 X P5	40	8	20
P2 X P1	40	16	40
P2 X P3	40	12	30
P2 X P4	40	9	22.5
P2 X P5	40	6	15
P3 X P1	40	10	25
P3 X P2	40	13	32.5
P3 X P4	40	17	42.5
P3 X P5	40	15	37.5
P4 X P1	40	17	42.5
P4 X P2	40	12	30
P4 X P3	40	11	27.5
P4 X P5	40	13	32.5
P5 X P1	40	8	20
P5 X P2	40	10	25
P5 X P3	40	11	27.5
P5 X P4	40	8	20
Mean			**28.875**