VARIETAL EVALUATION AND DEVELOPMENT OF HYBRIDS IN *PHALAENOPSIS* ORCHIDS

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by

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THESIS

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2020

DECLARATION

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

Vellayani **Roshin Mariam George**

Date: (**2018 - 11- 029)**

CERTIFICATE

Certified that this thesis, entitled **"Varietal evaluation and development of hybrids in *Phalaenopsis* orchids"** is a record of research work done independently by **Ms. Roshin Mariam George (2018-11-029)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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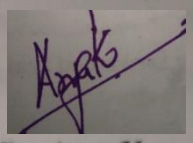

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Roshin Mariam George

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LIST OF ABBREVIATIONS AND SYMBOLS USED

°	Degree
°C	Degree Celsius
<	Less than
>	Greater than
%	Per cent
µL	Micro litre
½ MS	Half strength Murashige and Skoog medium
BA	Benzyl Adenine
BAP	Benzylaminopurine
CD	Critical Difference
cm	Centimetre
CRD	Completely Randomised Design
Cv	Cultivar
d.f	Degrees of freedom
et al.	And others
Fig.	Figure
g	Gram
g L^{-1}	Gram per litre
g^{-1}	Per gram

GCV	Genotypic coefficient of variation
i.e.	That is
IAA	Indole acetic acid
IBA	Indole butyric acid
KAU	Kerala Agricultural University
Kg	Kilo gram
L^{-1}	Per litre
mg	Milligram
mg g^{-1}	Milligram per gram
mg L^{-1}	Milligram per litre
ml L^{-1}	Millilitre per litre
NAA	Naphthalene acetic acid
No.	Number
PCV	Phenotypic coefficient of variation
ppm	Parts per million
S. E	Standard Error
Sl.	Serial
sp. or spp.	Species (Singular and Plural)
v/v	Volume / volume

Via	Through
viz.	Namely
w/v	Weight / volume

Introduction

1. INTRODUCTION

Orchids are the most beautiful plants and are without doubt an elegant creation with their unpredictable blossoms and stunning magnificence. The family Orchidaceae, is the biggest in the plant realm with around 600-800 genera, more than 25,000 species and more than a lakh and half man-made cross breeds. Taxonomically they are the most highly evolved monocotyledons. There occurs large variability in the floral characters in orchids exhibiting a wide range of flower colours and patterns, size and shape of the flowers. Despite the great diversity in orchids, only a very few genera *viz., Dendrobium, Cattleya, Phalaenopsis, Cymbidium, Aranda, Vanda, Mokara, Aranthera, Oncidium* and a couple of others have commercial significance. Orchid cultivation has become an international business covering around 10% of the world floriculture trade. Orchids are now dominating the cut flower and potted plant commerce due to its long lasting charm, high productivity, seasonal blooming, convenient packing and transportation (De *et al.*, 2014).

Orchids are mainly divided into two groups- monopodial or sympodial based on its growth habit. Monopodial orchids have a main stem which continues to grow year after year as in the case of *Phalaenopsis* and *Vanda* whereas sympodial orchids have a main stem which terminates growth at the end of each season like in *Cattleya* and *Cymbidium*.

Monopodials have gained popularity in the world market due to its wide range of variability in floral characters and the availability of large number of varieties and hybrids. *Phalaenopsis, Aranda, Mokara* and *Vanda* are the most popular among the monopodial orchids.

Phalaenopsis orchids or commonly called moth orchids are an important cut flower and potted plant orchid. The name *Phalaenopsis* was derived from two Greek words ‘Phalaena’ and ‘opsis’ meaning ‘resembling moth’. These plants are referred to as ‘Phals’ and have high value in floriculture because of their charming and long lasting flowers. The genus *Phalaenopsis* consists of about 60 species and contributes over 40,000 man-made hybrids, which is nearly 25 percent of the total orchid hybrids. *Phalaenopsis* orchids can be broadly classified as grandiflora (cut flower) and multiflora (pot plant) types based on their floral characters. Grandiflora types have long, arching inflorescence with large flowers, whereas, the multiflora types have short multiple inflorescence with numerous smaller sized flowers.

The genus *Phalaenopsis* are monopodial epiphytic plants with long, coarse roots and short leafy stems which is hidden by overlapping leaf bases. In *Phalaenopsis*, the shoot apical meristem is deeply embedded in the terminal shoot, and the axillary bud develops in the axil of a leaf. The axillary bud becomes dormant as the leaf matures. Under the cool condition (about 20 °C), the dormant axillary bud will be activated and develop into a floral stem. In nature, the distribution of *Phalaenopsis* is widespread ranging from Assam and the Eastern Himalayas to Burma, the Andaman Islands, Malaya, Indonesia, Borneo, the Moluccas and Philippine Islands. In India, there are about 1300 species of orchids distributed across the north-eastern and north-western Himalayas, Maharashtra, Andaman and Nicobar Islands as well as in the Western Ghats (Rajeevan, 2007).

Some species of *Phalaenopsis* do grow at sea level and others at much higher elevations and the general climatic conditions under which they grow are relatively uniform. These plants prefer shaded areas and exhibit robust growth than in well-lit areas. *Phalaenopsis* species are generally fond of warm temperatures, flourishing in temperatures around 20 to 35 °C. *Phalaenopsis* requires high humidity of about 60–70% and low light of 12,000 to 20,000 lux. However, these orchids can adapt to lower humidity as well. They are also typically robust than other species of orchids, and this makes moth orchids particularly popular among first-time orchid growers. The major countries that are growing *Phalaenopsis* include Taiwan, Netherlands, United States of America, Japan, Germany and Thailand. In India, the cultivation of orchid is still in its emerging stage, which is limited to only a few farmers in Kerala and Karnataka.

The cultivation of *Phalaenopsis* is gaining popularity due to the ease in cultural practices, diverse flower colour, shape, size and delicacy (De *et al.*, 2019). These plants are among the most popular orchids sold as potted plants, owing to the ease of propagation and flowering under artificial conditions. The popularity of *Phalaenopsis* naturally led to the creation of many artificial hybrids. The breeding objectives include developing plants for better colour, size, and substance of the flower, to introduce perfect blending of colours in sepals, petals and lip, to create round and full form of sepals and petals with minimum fenestration and twists, to increase the length of inflorescence, to increase the number of flowers per inflorescence, to achieve compactness in flower facing on the spike, to develop

hybrids showing correct mode of display, to extend blooming period, to produce miniature forms, to produce fragrant varieties, to produce flowers with longer vase life, to develop types suitable as pot plants, to develop hybrids insensitive to strict climatic regime and to develop hybrids resistance to biotic stress like diseases particularly to viruses.

Phalaenopsis are unique in that in some species, the flowers turn into green leaves after pollination. As in other plants, the petals of these flowers serve to attract pollinating insects and protect essential organs. After pollination, petals usually will undergo senescence because it is metabolically expensive to maintain them. The flowers transform into capsules with millions of minute seeds.

Even though orchid seeds are produced in large number, as they lack endosperm and germinate very poorly, *in vitro* culture became the standard procedure for germinating orchid seeds. A large number of hybrids are produced in *Phalaenopsis* orchids, both interspecifically and intraspecifically through *in vitro* culture techniques. Under natural conditions, germination of the mature orchid seed is dependent upon the association of a mycorrhizal fungus. Since Knudson's discovery, orchid seeds can also germinate successfully in a culture medium in the absence of a mycorrhizal fungus. This method is known as asymbiotic germination, and is a useful propagation technique for most of the orchids (Yeung *et al.*, 1996). Asymbiotic germination is a useful and popular technique in obtaining seedlings for nursery culture, and to conserve our natural resources.

With the emergence of an urban class with higher disposable income, there is huge potential for commercial growth of *Phalaenopsis* in India. So, the objective of the present study is the improvement of characters through hybridization and selection, such as flower shape, colour, fragrance, longevity and biotic and abiotic resistance, and creation of novel variations in *Phalaenopsis* orchids and to develop healthy plantlets through *in vitro* techniques which are important to increase its commercial value.

Review of Literature

2. REVIEW OF LITERATURE

Orchids are the most beautiful of plants and their family Orchidaceae is the biggest in the plant kingdom with hundreds of genera, thousands of species and more than a lakh and half man-made cross breeds. They are highly valued in the international markets because of its wide range of flower colours and patterns, size and shape of the flowers. *Phalaenopsis* orchids or commonly called moth orchids are an important cut flower and potted plant orchid in the world market. The *c*ultivation of *Phalaenopsis* is gaining popularity due to the ease in cultural practices, diverse flower colour, shape, size and delicacy (De *et al*., 2019).

The improvement of characters through selection and hybridization, such as flower shape, colour, fragrance, longevity and biotic and abiotic resistance, and creation of novel variations in *Phalaenopsis* orchids can increase its commercial value and help in the production of outstanding hybrids valued at international level.

A thorough understanding of floral biology, pollination, compatibility, *in vitro* seed germination and development, variability and hybrid development of *Phalaenopsis* orchids is essential for organizing its improvement through breeding programme. Therefore, a brief review of relevant literature has been presented here, based on related research works.

2.1. Floral Biology

Brown in 1833 and Darwin in 1862 have described the structure of orchid flowers. Orchid flowers are zygomorphic or bilaterally symmetrical. They are perfect flowers containing both androecium and gynoecium. They are mostly bisexual and rarely unisexual.

The flowers can be born as solitary, as a raceme or cyme inflorescence or even as a panicle. They are epigynous and have an inferior ovary. The colour range varies from pink, violet, orange, red, ivory, white to yellow and even their combinations. They can be large or small, pedicellate or sessile and may or may not have a characteristic scent.

2.1.1. Floral Morphology

A detailed description of orchid flower structure, the column, anther and stigma was described by Dressler (1993).

The orchid flowers are trimerous and have 3 sepals, 3 petals, 3 carpels and 6 stamens arranged in two whorls of three each (Abraham and Vatsala, 1981).

The flower is composed of perianth lobes and a column. The perianth lobes are further divided into petals and sepals that are arranged in 2 whorls. The 3 sepals are similar in size, shape and colour. They are referred to as 'petaloid' sepals as they are coloured like petals. Petals are 3 in number, two of them are similar in size and shape and the third petal is a highly evolved one known as the labellum or the lip. The labellum is trilobed with two lateral lobes and a mid-lobe which is triangular with two tendril like structures, a cirrus, and a bifid callus. The labellum or lip is usually larger and flashy than the lateral petals (Sheehan and Sheehan, 1979; Abraham and Vatsala, 1981). They act as a perfect landing place for the pollinators. The lip is oriented upward in the bud, but, as it later develops, the pedicel or ovary twists so that the lip is usually oriented downward, by a process called resupination, by the time the flower opens (Abraham and Vatsala, 1981).

In most orchids the anther is a cap like structure at the apex of the column. The anther of some of the more primitive orchids is superficially similar to that of a lily or amaryllis. The pollen grains are bound together by threads of a clear, sticky substance (viscin) in masses called pollinia and this is considered an important theme in the evolution of orchids. A single pollinium may contain more than a million pollen grains. This aggregation of pollen grains is an indication of the large number of ovules that could be fertilized in the orchid ovary. In many orchids there are four pollinia indicating four anther cells but in many other cases this is more or less united into two as in the case of *Phalaenopsis*. In most orchids, the anthers are seated in a clearly defined area at the end of the column (Dressler, 1993).

The column like structure situated in the center of the flower is 'gynostegium' and it is positioned above the labellum. The column is straight or slightly curved, subcylindric, and dilated toward the apex in *Phalaenopsis* orchids. The gynostegium

is a fusion of androecium and gynoecium. The gynostegium has the anther cap with a wall like structure called rostellum, which is the modified third stigma, covering the pollinia or the pollen mass. It separates the male and female organs restricting self-pollination. The rostellum secretes a sticky substance which holds the pollinia till they reach maturity. Below the rostellum, at the ventral side of the gynostegium is the functional stigma of the flower represented by a hollow cavity or depression which is composed of three stigmatic lobes however, the three lobes are fused together. It is actually the two fertile stigmas fused together. A viscous substance covers the surface of the hollow cavity and it helps to hold the pollinia when deposited on it at the time of pollination. Generally, the ovary in Orchidaceae family is tricarpellary, unilocular with parietal placentation and numerous ovules (Mukherjee, 1990). The ovary is not fully differentiated at flowering and develops only after a successful pollination especially in the case of epiphytes. Thus the plant spends only little energy in the ovaries of the unpollinated flowers (Dressler, 1993).

2.1.2. Resupination

The term resupinate can be used for any orchid flower that has the lip on the lower side (Dressler, 1993).

Resupination refers to the 180° developmental rotation in floral orientation (Arditti, 2002). The phenomenon of resupination changes the orientation of orchid flowers during anthesis so the lip, although initially in an adaxial or dorsal position, becomes the lowermost perianth organ (Mondragón-Palomino and Theißen, 2009).

Nyman *et al*. (1985) showed that experimental removal of column and pollinia indicate that resupination occurs as a response of the flower to gravity.

Resupination orientates the lip to provide the orchid's pollinator with a convenient landing pad. Resupination begins to occur as the buds open and is essentially complete by the time the floral segments are fully expanded. All of the commonly known genera including *Phalaenopsis*, *Dendrobium*, *and Cymbidium* have resupinate flowers.

2.1.3. Anthesis

The flower anthesis in orchids lasts from a few hours to several weeks (Endress, 1994). In the case of *Calypso bulbosa*, it lasts for 8–11 days (Proctor and Harder, 1995).

In principal, the length of flower anthesis depends on pollination and removal of the pollinium (Luit and Johnson, 2001).

During the cultivation of *Phalaenopsis violacea* and *P. bellina*, a daily difference in temperature of around 10°C might be the necessary condition to induce the inflorescence.

Anthesis time of the flowers ranged from 5:00 am to 2:00 pm among different varieties of *Phalaenopsis*. Elegant Yellow showed anthesis during 5:00 am -8:00 am whereas Violet was observed to open from 11:00 am - 2:00 pm (Rahi, 2017).

2.1.4. Stigma Receptivity

The stigma remained receptive for four consecutive days following anthesis in *Spathoglottis plicata*, for five days in *Aerides odoratum*, for six days in *Dendrobium amoenum* and for 12 days in *Phaius tankervilleae* (Devi and Deka, 1992).

In a study conducted in *Platanthera chlorantha* flowers, the stigma receptivity lasts 15 days on average. The flowers showed receptivity between the bud stage and the end of anthesis (Stpiczynska, 2003).

Pollination was more successful in morning hours, because stigma is more receptive in morning hours in many orchid genera including *Dendrobium* (Dongarwar and Thakur, 2014).

In a study conducted, it was observed that the maximum number of days for stigma receptivity in *Phalaenopsis* Winter Spot was 3-12 days and *Phalaenopsis* Violet had a minimum of 1-5 days (Rahi, 2017).

2.2. Compatibility Analysis

It was observed that out of 520 crosses conducted with the aim of developing white Taisuco *Phalaenopsis*, only 46.2 per cent cross combinations produced viable seeds as reported by Chen *et al.* (2000).

Compatibility studies in 116 combinations in monopodial orchids were conducted and it was observed that a total of 58 combinations were successful and gave harvestable green capsules. Progeny from 24 combinations were successfully deflasked (Ninitha, 2003).

The compatibility relationships among 18 monopodial orchid genotypes were studied. The study concluded that the capsules at seed inoculation maturity were successfully harvested from 67 combinations out of the 324 possible combinations. These included 61 cross and 6 self-combinations. The pollinated flowers or immature capsules from 257 unsuccessful combinations fell off at different stages of development from immediately after pollination to just before capsule harvest depending on the relative intensity of incompatibility (Lekha Rani and Udaya, 2008).

In a study conducted, 160 cross combinations were made among *Sedirea japonica*, *Phalaenopsis*, *Doritaenopsis* and *Neofinetia falcata*. High incompatibility was observed from most of crosses as evidenced by traits such as early dropping of seed pods after pod formation or no seed formation after pod maturation (Kim *et al*., 2015).

Inter varietal crosses made in all possible cross combinations between the selected 6 varieties of *Phalaenopsis* namely Elegant Yellow, Elegant Purple, Elegant Fairy, Pink, Violet and Winter Spot resulted in pod set of six crosses. Successful combinations were Winter Spot X Elegant Purple, Winter Spot X Pink, Elegant Purple X Pink, Pink X Winter Spot, Pink X Elegant Purple and Violet X Elegant Purple (Rahi, 2017).

In a study, twelve cultivars of *Phalaenopsis* were selected for their highly variable morphology and used in six direct and reciprocal hybrid combinations. And eight out of 12 hybrid combinations produced capsules, while the other four did not produce any capsules (Cordea *et al*., 2019).

2.3. Pollination Biology

Orchids have exclusive relationships with their pollinators. These include bees, wasps, and flies, but many orchids also utilize moths, butterflies, or birds to cross-pollinate their flowers. While the ways that various species attract pollinators to visit their flowers and carry their pollinia off to a flower on another plant vary, they often employ complex and deceptive strategies to achieve success in pollination.

Most orchids provide a pollinator reward (e.g. nectar) like other angiosperms, but 8000-10 000 species act by deceit, *i.e.* are frauds that provide no reward (Anders Nilsson, 1992). The mechanisms of deception in orchids include generalized food deception, food-deceptive floral mimicry, brood-site imitation, shelter imitation, pseudo antagonism, rendezvous attraction and sexual deception. Generalized food deception is the most common mechanism which is reported in 38 genera, followed by sexual deception in 18 genera (Jersáková, 2006).

The mechanism for pollination works only when the insect of right shape and size enters the flower. The pollinia are positioned such that they get stuck to the pollinator as it exits the flower. When it visits the next flower and moves down the nectary, the pollen sticks on to the stigma (Northen, 1970).

Vanda tricolor entice the pollinators by the odour of its flowers and visual image (Arditti, 1967).

Many orchids are normally pollinated by a single class of pollinators and many others are promiscuous, that is, they are pollinated by pollinators of several classes (Dressler, 1993). It was observed that noctuid moths have high potential to facilitate hybridization among the two orchids *Platanthera bifolia* and *P. chlorantha* (Esposito *et al*., 2017).

Phalaenopsis bellina and its genetically as well as morphologically related species, *P. violacea*, are usually used in the breeding of scented cultivars. The floral scent profiles of *P. bellina* are dictated by monoterpenes, including linalool, geraniol

and their derivatives which help in emitting particular scent to attract pollinators in a circadian rhythm (Chuang *et al*., 2017).

2.3.1. Artificial Pollination and Hybridization

Orchids were probably hand-pollinated for the first time in 1797-1799 by the German botanist, J. K. Wachter in *Platanthera bifolia.* Attempts to pollinate *Vanilla planifolia* in Europe, Indonesia and the Indian Ocean island of Reunion played an important role in the development of hand pollination methods for orchids. The first horticultural orchid hybrid, Calanthe x Dominyi was produced by John Lindley in 1858 (Arditti, 1984).

The best time of pollination should be decided before initiating seed production in orchids. Usually pollination is done after the flowers have been open for a few days, but in species where the flowers are short lived or live for only one or two days, they must be pollinated immediately. In general, the best time for pollination is halfway through the life of a flower (Mercy and Dale, 1997). When there are many flowers in an inflorescence, mature flowers present towards the base are pollinated. If inflorescence is large and several flowers can be pollinated simultaneously it is to be noted that it increases the maturation time of the developing capsules.

Another aspect in *Phalaenopsis* breeding is production of blue flowers. The two species that contributed to the development of blue *Phalaenopsis* are *Doritis pulcherrima* var. *coerulea* and *Phalaenopsis violacea* var. *coerulea* (Mountford, 2001).

Multi-generic hybrids have been evolved by crossing *Phalaenopsis* with other genera like *Aerides, Arachnis, Doritis, Neofinetia, Rhyncostylis, Renanthera* and *Vanda* (De *et al*., 2014).

Improvement of *Phalaenopsis* orchids mainly evolved around the development of pure whites, pure pinks and white with pink lips. *P. amabilis, P. formosana* and *P. aphrodite* were used for evolving outstanding white hybrids (Thomas, 2001). For the development of pink coloured hybrids, *P. lueddemanniana*,

P. sanderiana and *P. schileriana* contributed prominently. White coloured flowers with pink lip were obtained in *P. intermedia* due to natural hybridization between *P. aphrodite* and *P. equestris*. *Phalaenopsis 'Doris'* developed in 1940 is one of the outstanding white hybrids. Yellow and red *Phalaenopsis* breeding initiated with a cross between 'Doris', and *P. manni* to produce *P. 'Golden Louis'* (De *et al*., 2019).

A new *Phalaenopsis* cultivar, 'Yellow Green' which has small, deep-yellow flowers with a red lip, and was introduced by artificial crossing of the *Phalaenopsis* cultivar 'Sogo Pride F-1016' and the *Phalaenopsis* cultivar 'Tzu Ching Glint' (Vo *et al*., 2019).

Artificial pollination was performed in the first days after flower opening in 12 varieties of *Phalaenopsis*, by detaching the anther cap and pollinia using a toothpick and placing the pollinia over the stigma of the female parent which was previously emasculated. The 60 cross-pollinated flowers produced a total of 31 capsules (Cordea *et al*., 2019).

2.3.2. Reciprocal Hybridization

Usually, reciprocal crosses between species and varieties are difficult to achieve successfully. There can be structural, functional or genetic barriers which come into play during such crosses.

The failure of fruit development in many reciprocal crosses hints at the operation of a unidirectional incompatibility in orchids (Devi and Deka, 1992).

Reciprocal hybridization within the hybrids yielded progeny with remarkable variation in flower characters (Wallbrunn, 1988).

In a study, the phenotypic and breeding efficiency of *Phalaenopsis* reciprocal hybrids and their parents were studied. For reciprocal hybridization, *Phalaenopsis* 'KS Little Gem' and '1747' from Taiwan were used as parents. It was concluded that 'KS Little Gem' can be selected as the female parent during hybridization to generate *Phalaenopsis* with a longer shelf life than compared to the normal cross (Vo *et al*., 2015).

2.3.3. Backcrossing and Selfing

Backcrossing and selfing techniques are avoided nowadays as inbreeding can lead to reduction in vigour and produces inferior progeny. But, selfing and backcrossing techniques have been found immensely beneficial in breeding for superior hybrids especially in orchids.

Phalaenopsis Princess Kaiulani is developed from the cross between *P. violacea* and *P. amboinensis*. When it was backcrossed with *P. violacea,* the very variable *P. Princess Violet* was produced.

Introgressive hybridization played a major role in the development of orchid hybrids. The long continued inbreeding of *Phalaenopsis sanderiana* is a famous example leading eventually to superior clones (De *et al*., 2014).

2.4. Post Pollination Phenomena

Pollination shortens the life of the flowers and it induces phenomenal changes in morphology including anthocyanin formation, fading and wilting of sepal tips known as "dry-sepal" injury (Arditti *et al*., 1973).

A successful fertilization is indicated by the fading of the petals and greening and thickening of the ovary of the flower, which happens in a week. Shriveling and arching of the perianth lobes over the stigma indicates successful pollination. Time taken for capsule ripening differs in different genera and varies from 2 to 12 months. The fruit is a capsule. The seeds are very light and are dispersed by wind. All the seeds may not be viable and fertile.

Post pollination events include stigmatic closure, increase in fresh and dry weight of ovaries, production of hormones, synthesis and destruction of pigments, deresupination, nastic movements, new biochemical pathways and cessation of scent evolution (Arditti, 1977).

In orchids, ovules are not present or are poorly developed at the time of anthesis. A successful pollination event triggers ovule development within an ovary. Once the

ovules mature, double fertilization results in the formation of a zygote and a polar chalazal complex within the endosperm cavity of each fertilized ovule. The triggering of ovule development after a successful pollination event ensures the survival of the parent plant since little energy is channeled to the unpollinated ovaries. The polar chalazal complex fails to develop into an endosperm. Hence, an endosperm is absent from mature orchid seeds. Numerous seeds are produced within a single capsule. The seeds are very small; contain a simple embryo with no distinct tissue differentiation (Yeung *et al*., 1996).

In pollinated orchid flowers, dry matter is transported from the senescing perianth to sites of active metabolism such as the column and ovary. Protein and starch are lost from sepals and petals of *Cattleya labiata*, *Dendrobium nobile*, *Phalaenopsis amabilis* and *Rhynchostylis retusa* (Arditti and Flick, 1976).

The activity of ACC oxidase which catalyzes the conversion of ACC to ethylene was increased in the stigma after pollination (Nadeau *et al.,* 1993).

Guha *et al.* (2006) reported that successful production of seeds in orchids is preceded by complex sequences of physiological and morphological events initiated by pollination. According to him, the post pollination events serve three basic functions in orchids.

a) To protect the pollinia, ensure a close contact between the pollen and the stigmatic surface and provide a favourable environment for pollen germination and tube growth. This is attained by the swelling of the column and stigmatic closure.

b) To recycle substances from senescing organs into those that became the center of new activities.

c) To render the pollinated flowers no longer attractive to pollinators there by conserving pollinator- power and increasing the chance that unpollinated flowers will be visited by the pollinators.

The unpollinated flowers of *Cymbidium pendulum* and *C. aloifolium* attained senescence in 8 and 7 days, respectively, after pollination. The higher content of total

soluble sugars, reducing sugars and free amino acids was observed in all the floral organs of pollinated flowers than in unpollinated ones. Pollination also up-regulated the activity of hydrolytic enzymes like α-amylase, β-amylase and invertase as well as proteolytic enzymes like proteases in the floral organs (Attri *et al*., 2008).

The timing of pollen germination, progressive growth of pollen tubes in ovaries, and arrival of pollen tubes at embryo sacs in *Phalaenopsis aphrodite* was recorded in a study. The pollen germinated and pollen tubes entered the ovary 3 days after pollination. Pollen tubes continued to grow and filled the entire cavity of the ovary as the ovary elongated and ovules developed. Pollen tubes were found to enter the matured embryo sacs at approximately 60–65 days after pollination in an acropetal manner (Chen and Fang, 2016).

The molecular and biochemical changes during pollination-induced petal senescence in *Phalaenopsis* "Red Dragon" were studied. Petals appeared to be visibly wilting at 24 h after pollination, accompanied by the mass degradation of macromolecules and organelles during senescence. The mechanism regulating petal senescence in *Phalaenopsis* is complex and unclear. But the results point out that two processes, a counteraction against increased levels of reactive oxygen species (ROS) and the degradation of cellular constituents for maintaining nutrient recycling, are activated during pollination-induced petal senescence in *Phalaenopsis* (Chen *et al*., 2018).

2.5. Harvesting of Capsules for Culture

Harvesting of capsules should be done before its dehiscence. Browning or yellowing of the capsule is sign of maturity. Generally for orchids, it takes about 4-10 months for the capsules to mature and ripen. The time taken for maturity depends on the species and varieties involved in the cross.

Withner (1953) reported that very young as well as fully mature ovaries do not form good explants *in vitro* due to dormancy, pH, inhibitory and other metabolic factors.

Abraham and Vatsala (1981) suggested that harvesting the pods earlier prevented contamination of the seeds with fungal or bacterial spores and resulted in good germination of the seeds.

For dry seed culture, fully ripe pods or nearly ripe pods are used. Pods can be put in a sterile jar or paper cover and can be stored in the refrigerator, even up to 6 months without the loss of viability. Under natural conditions, germination of the mature orchid seeds is dependent upon the association of a mycorrhizal fungus. Since Knudson's discovery, orchid seeds can also germinate successfully in a culture medium in the absence of a mycorrhizal fungus. This method is known as asymbiotic germination, and is a useful propagation technique for most orchids (Yeung *et al*., 1996). The seeds can grow only in culture in the absence of mycorrhizal fungus and they must be sterilized carefully to avoid contamination and this may cause damage to some of the seeds. For green capsule culture, pods can be collected at 60-75 per cent pod maturity. At this stage, the green pods can be surface sterilized more easily during culture without damaging the seeds inside.

The harvest of seed capsules just a few days prior to the onset of dehiscence is the best stage at which the seeds will be fully mature and highly viable (Seaton, 1994).

In a study, it was observed that the seed germinability remained high (75–99.5%) throughout the harvesting period (90–216 DAP) in hand pollinated *Phalaenopsis* hybrids (Schwallier *et al.,* 2011).

Three *Phalaenopsis* orchid hybrids, namely, 'Athens', 'Moscow' and 'Lusaka' flowers were artificially self-pollinated. It was observed that seeds derived from 5-month mature capsules, invariably took least time to germinate than that of the 3-month or 7-month in all three hybrids, e.g., for 'Moscow' it was 13.9 days with a maximum of 90.3 % germination (Balilashaki *et al.,* 2015).

2.5.1. Capsule Maturity

Assessment of correct maturity stage is a major determining factor in green capsule culture.

Green capsules of *Paphiopedilum* harvested 4 months after pollination and that of *Cattleya*, *Cymbidium*, *Phalaenopsis* and *Eulophia* harvested 8-9 months after pollination exhibited germination satisfactorily (Rosa and Laneri, 1977).

The highest rate of germination in orchid genera such as *Cymbidium goeringii* and *Paphidiopedilum insigne* var. *sandrae,* was when the green capsules were harvested at 115-120 days and 195-200 days, respectively after pollination (Nagashima, 1982).

Hedge (1984) observed that the pods of *Dendrobium* species matured in about nine to seventeen months.

Capsules turning yellowish or brown were considered a symbol of capsule maturity (Yadav and Bose, 1989).

In *Cypripedium formosanum*, seeds collected from 90 to 105 days after pollination (DAP) are most suitable for in vitro culture (Lee *et al*., 2005).

The highest frequency (398 %) of seed germination was obtained with seeds harvested 70 days after pollination in *Cephalanthera falcata*. Minimal germination was observed with seeds harvested 100 d or later after pollination (Yamazaki and Miyoshi, 2006).

The effects of seed maturity were investigated through asymbiotic germination of *Dendrobium* hybrids. Capsules were harvested from two hybrids (Lucky Girl × Second Love 'Kirameki' and Lucky Girl × Hamana Lake 'Kumi') and one selfing of Second Love 'Kirameki' at 2, 3, 4, and 5 months after pollination and immature seeds were taken. Immature seeds from 4-month old capsules showed greatest germination rate of tested treatments, whereas 3-month old immature seeds showed the least germination (Udomdee *et al*., 2013).

The optimum germination percentage was obtained from immature seeds collected at 6 weeks after pollination in *Cypripedium macranthos*. At this time, the embryo reached the early globular stage. As seeds matured, the germination percentage declined sharply (Zhang *et al*., 2013).

The seeds derived from 5-month mature capsules, invariably took least time to germinate than that of the 3-month or 7-month old capsule in *Phalaenopsis* hybrids (Balilashaki *et al*., 2015).

2.5.2. Capsule Culture

Green capsule culture trials conducted on a wide range of orchids indicated that the success is possible only after fertilization (Valmayor and Sagawa, 1967).

When green capsules were harvested earlier, good seed germination could be obtained devoid of bacterial and fungal contamination (Abraham and Valsala, 1981).

Arditti *et al.* (1981) observed that both mature and immature capsules of *Epipactis* germinated asymbiotically. But, the seeds from immature capsules germinated well and rapidly.

Mitra (1986) observed that seeds obtained from unripe capsules germinated readily in several orchid species. Reduced germination at capsule maturity may be due dormancy factors and changes in enzyme compliments. He also suggested that the identification of critical stage at which dormancy sets in would be beneficial.

Green pod culture helps in reducing the time lapse between germination and sowing of seeds, saves them from exposure to sterilizing agents and allows production of large number of seedlings (Pathak *et al*., 1992).

Singh (1993) observed that the harvesting time between the dry seed culture process and the green pod culture process varied from 6-8 months depending on the genera. The reduction in harvesting time decreased the time required for flowering.

With progressing age, a decrease in the germination of fully mature *Vanda* seeds could be observed (Sharma, 1998).

Upon pollination in *Anoectochilus roxburghii,* the embryo and seed development is completed in 40 days. The seed of *A. roxburghii* matures rapidly (Li *et al*., 2019).

Seeds from 4-month-old hand-pollinated *Phalaenopsis amboinensis* orchids exhibited satisfactory seed germination and plantlet development (Utami and Hariyanto, 2019).

2.5.3. Seed/ Capsule Sterilization

Proper sterilization of seed/capsule is an important step toward the success of seed germination. The amount of time required and the concentration of disinfectants used depend on the sensitivity of the tissues toward the disinfectants and how difficult it is to remove the foreign organisms.

Sterilization before inoculation is necessary as orchid seeds are cultured under completely sterile conditions. Since mature seeds have tough seed coats, chemical treatments for sterilization can be safely practiced (Jordan, 1965).

For mature seeds from dehisced capsules, the seeds can be processed using small sterile filter paper packets or placed in nylon bags. The packets with seeds are placed directly into a sterilizing solution such as 5–10% bleach solution with a drop of surfactant, *i.e*., Tween 20, stirred gently for about 10 min. Inside a laminar air flow chamber, the paper packets are then washed several times with sterile distilled water before opening the packets and transferring seeds onto appropriate medium (Butcher and Marlow, 1989).

Seeds can also be decontaminated using the syringe method as reported by Ponert *et al*. (2011).

Jevsnik and Luthar (2015) introduced a protocol for seed decontamination. Seeds are disinfected in a solution of dichloroisocyanuric acid, sodium salt with a drop of Tween 20 for 8 min in a micro-centrifuge tube. The disinfectant and subsequent sterile water washes are removed by centrifugation.

Capsules obtained from plants of three *Phalaenopsis* cultivars (*viz*. 'Athens', 'Moscow' and 'Lusaka') were washed under running tap water, and surface sterilized by dipping into 70 % (v/v) ethanol for 30 s which was followed by agitation for 15 min in sodium hypochlorite (NaOCl) solution and Tween 20 under laminar air flow chamber. Then it is rinsed with sterile double distilled water three times each time after employing surface sterilizing agent (Balilashaki *et al*., 2015).

The most common disinfectant solutions are sodium hypochlorite (0.5–5%), calcium hypochlorite (9–10%), and commercial bleach solutions (10–20%). This can be enhanced by a short treatment in solutions of ethanol (70–90%), hydrogen peroxide (10%), and mercuric chloride (0.1–0.2%) (Yeung *et al*., 2018b).

If maturing capsules are still intact and have not yet split open, capsules can be sterilized using a strong 20–30% commercial bleach solution with a drop of wetting agent like Tween 20 for decontamination. The capsules can be stirred gently in the bleach solution for 15 min, rinsed in sterile water, then dipped in 70% ethanol for another minute, and washed with several changes of sterile water (Yeung *et al*., 2018b).

2.6. Seed Germination

Upon seed germination, the smaller cells at the chalazal end form the meristematic zone and the large cells at the micropylar end will enlarge so as to house the symbiont. In orchids, the surface layer of the embryo is specialized, taking on an epidermal cell characteristic. A surface cuticle layer has been reported in other flowering plant embryos and similar cuticular depositions can also be found in orchids (Rodkiewicz *et al*., 1994). In *Phalaenopsis amabilis,* a cuticle is present but it takes on a patchy appearance (Lee *et al*., 2008).

The success in asymbiotic germination using immature seeds is mostly due to the low levels of ABA at the end of the histodifferentiation phase.

Many endogenous factors like nutrient media and endogenous factors such as plant growth regulators and seed coat structures can influence seed germination and subsequent plantlet development (Arditti, 1967).

The removal of phenolic substances to improve germination can be achieved by treating with calcium hypochlorite solution and Tween 20 added as the wetting agent (Van Waes and Debergh, 1986).

For *in vitro* symbiotic and asymbiotic seed germination, culture conditions such as the quality and quantity of lights and temperature can also have important roles to play (Zettler and McInnis, 1994).

For in situ symbiotic germination, many additional factors are essential to the success of the process. The presence of compatible mycorrhizal fungi and environment factors play determining role in the success of seed germination (Lee *et al*., 2005).

The culture of immature seeds of epiphytic orchids usually results in a lower germination percentage than the culture of mature seeds (Mweeta *et al.,* 2008; Suzuki *et al*., 2012).

The addition of metabolizable sugars provides the added energy necessary to kick start the growth of the protocorm. In the absence of added sucrose in the medium, germination slows or fails (Yeung *et al*., 2018a).

The optimum germination percentage occurred when mature seeds of *Cypripedium debile* were collected and sowed on 1/4 Murashige and Skoog basal medium. The liquid culture also promoted germination of mature seeds (Hsu and Lee, 2012).

The highest germination percentage (46.6%) of *Phalaenopsis* Manchester was observed in 15% (v/v), 2 gl^{-1} peptone and modified VW medium. The study also showed that *Phalaenopsis* seeds cultured on a ½ MS medium containing CW and peptone can be used for clone propagation which gave about 27.9% of seed germination (Shekarriz *et al*., 2014).

2.6.1. Nature of Orchid Seed

In orchids, after a successful pollination and fertilization event, numerous seeds develop within a single capsule. Seeds are very small and embryos have no obvious histodifferentiation into distinct tissues, such as the root and shoot apical meristem, primary meristems, and organ, *i.e.*, cotyledon (Yam *et al.*, 2002).

The colour of the seeds of Orchidaceae can be white, cream, pale green, reddish orange or dark brown (Arditti, 1967). Shapes of the seeds are also various including filiform, fusiform, clavate, and ellipsoidal seeds (Molvray and Kores 1995).

One of the distinct features in a majority of orchid embryos is the absence of a cotyledon. However, Nishimura (1991) reported that a rudimentary cotyledon-like protrusion can be found in seven genera.

The absence of a cotyledon may represent a unique evolutionary trend within Orchidaceae (Arditti, 1992).

The seeds of orchids range from 0.15mm to 6mm in length and vary a great deal in width and in structural details. Aside from the size and shape of the seeds, the shape and size of the cells in the seed coat also varies taxonomically, especially in the structure of the cell walls (Dressler, 1993).

The series of events leading to the development of ovules in orchids is a post pollination event. The time interval from pollination to fertilization ranges from 10 days to 6 months (Valmayor and Sagawa, 1967).

Phalaenopsis orchid seeds have a small seed size, about 0.4 mm 0.08 mm and the long developmental time after pollination (Mweeta *et al.*, 2008).

In angiosperms, one of the key events at the time of embryo maturation is to develop desiccation tolerance and prepare for developmental arrest. In *Phalaenopsis amabilis*, desiccation tolerance is established at the last stages of seed maturation (Schwallier *et al.*, 2011).

The expression analysis of fertilization and early embryogenesis-associated gene in *Phalaenopsis* indicated that auxin and ethylene can play a role at the critical stages of seed formation (Chen *et al.,* 2016).

Orchid seed has a thin seed coat. It is developed mainly from the outer integument of the ovule after fertilization and no vascular tissue present. Even though orchid embryos are small, they have complex developmental programs similar to other flowering plants and the protocorms are designed to house their symbiont with the primary goal to form a functional shoot apical meristem (Yeung, 2017).

The structural organization of an orchid embryo mirrors the structure of a protocorm at the time of germination. In orchid, immature seeds can germinate asymbiotically to form protocorms, once they reach approximately half way into seed maturation prior to storage product synthesis. The ability to germinate precociously indicates that the protocorm body plan has already established. Precocious germination is only possible if histodifferentiation has occurred (Yeung *et al.,* 2018b)

2.6.2. Seed Germination and Development *in vitro*

In vitro germination techniques developed in the early 1900s, have resulted in more reliable germination and propagation of many orchid genera. The earliest *in vitro* orchid seed germination techniques utilized mycorrhizal fungi found in nature to stimulate germination and seedling development. In 1922, Lewis Knudson germinated orchid seeds *in vitro* by sowing seeds on sterile nutrient medium supplemented with sucrose.

Ernst and Arditti (1990) observed that *Phalaenopsis* seedlings germinated in the presence of many carbohydrate sources including glucose and maltoheptose. Germination percentage and seedling development was highest on glucose, with fewer seeds germinating on malto-oligosaccharides.

Requirements of photoperiod and light conditions vary in different orchid species. Several genera like *Paphiopedilum, Cymbidium* and *Phalaenopsis* are known to germinate and develop better on darkened media (Singh, 1992).

Inoculation of orchid seeds into a nutrient media under *in vitro* conditions improves the percentage of germination, as well as it reduces the time for differentiation of seeds, both biochemically and morphologically (Singh, 1993)

For the asymbiotic culture of *Paphiopedilum wardii*, hyponex N026 medium supplemented with 1.0 ppm NAA, 1.0 g l^{-1} peptone, 10% CW, and 1.0 g l^{-1} AC was suitable for the first sub-culture of plantlets (Zeng *et al.*, 2012).

High seed germination (94%) of *Phalaenopsis* orchids were obtained in MS medium supplemented with 100 mg/L coconut water or 1 g/L peptone and ½MS containing 1 or 2 g/L peptone. However, the longest seedling was found in MS or ½MS media supplemented with 100 mg/L coconut water or MS containing 1g/L peptone (Abbaszadeh *et al*., 2018).

2.6.3. Changes During Germination

The regulatory events in the embryo prior to seed maturity determined the fate of its proximal and distal parts during germination. Synthesis of DNA and cell division were confined to the proximal end, whereas cells at the distal end underwent enlargement (Raghavan and Goh, 1994).

The cells at the apical end of the protocorm become meristematic and are smaller in size when compared to the basal cells. Within the meristematic layers, some cells will differentiate and become shoot apical meristem (SAM) initials, eventually giving rise to a SAM. Once a shoot becomes functional, the protocorm begins to grow in size and turns into a plantlet. Depending on the species, the protocorm can degenerate or remain as part of the plantlet. The plantlets can take on varied forms. Adventitious roots will eventually form near the SAM (Veyret, 1974; Vinogradova and Andronova, 2002).

The accumulation of cuticular material may increase the hydrophobic characteristic of the seed coat that impedes the uptake of water and nutrients during seed germination in *Cypripedium formosanum* (Lee *et al*., 2005).

In *Phalaenopsis amabilis* var. *formosa,* the presence of lignin and cuticular material are discontinuous, accumulating mainly in the radial walls of the outermost layer of the seed coat. This feature allows water and nutrients access to the embryo for germination. Also, *in vitro* germination decreases sharply as the seeds approach maturity and this coincides with increasing ABA levels, and decreasing water content. ABA may regulate the processes of storage protein synthesis, acquisition of desiccation tolerance, and prevention of germination in orchid seeds (Lee *et al*., 2007).

A structural polarity is present in the embryo. A physiological polarity must be present within the embryo before seed germination, since the small and large cells have distinct cell fate upon seed germination. It is reported that for *Phalaenopsis amabilis,* a marked gradient of cell size exists within the embryo (Lee *et al.*, 2008).

The seed storage products are replaced by starch granules in the cytoplasm (Leroux *et al*., 1997).

Seeds from the crosses of *Phalaenopsis* cultivars, germinated maximum in half MS medium supplemented with BA, NAA and activated charcoal. Protocorms when sub cultured to half MS basal medium supplemented with BA and NAA exhibited leaf differentiation (Rahi, 2017).

It was observed that orchid seeds began to swell upon placement on a suitable germination medium and then transform into protocorms. Mitotic activity begins and this activity is confined mainly to the future shoot pole (Yeung *et al*., 2018b).

Utami and Hariyanto (2019) reported that the seed germination of *P. amboinensis* started with swelling embryo and rounding up at about 3 weeks after sowing (WAS) in a media.

2.7. Culture Media, Components and Media Supplements

Media and conditions used for cultures play an important role in the development of hybrids in *Phalaenopsis* orchids. Modifications are done to meet the requirements depending on the orchid genera used so as to suit the situations. The

suitability of the media is to be tested prior to developing the protocol for a particular species. Investigators can develop their own medium for a particular species and explant of interest. This will generate optimum and desirable growth responses, and help in getting a better understanding of how the seeds respond to the nutrient components of the medium.

2.7.1. Effect of Culture Media on Seed Germination

Some media like Knudson C, Vacin and Went and Mitra *et al*. media are broad spectrum and can be used for a variety of genera, while media like Burgeff N3f are exclusively used for *Paphiopedilum* seeds.

In vitro seed germination and morphogenesis studies in orchid genera including monopodials clearly indicated the superiority of MS medium over KC and VW media (Sangama, 1986).

Seeds of *Cymbidium elegans, Coelogyne prolifera, C. cristata, C porsecta, Aerides multiforum, Sarcanthus pellidus, Bulbophyllum cosmosus* and *Thunia alba* were inoculated in tubes containing KC or VW medium. Best germination for all the species was observed on KC medium and protocorms formed with root initials in 10-12 weeks (Sharma and Tandon, 1987).

Effect on seed germination and seedling growth of *Cymbidium* on different media and growth regulators was studied by Paek and Yeung (1991).

Bletilla striata seeds showed 99 percent germination on MS medium. Seed germination was achieved on KC medium but for seedling growth MS medium was found suitable (Stenberg and Kane, 1998).

Embryogenic callus was germinated from the explants of *Phalaenopsis* Wedding Promenade and *Phalaenopsis* Hanabonshi X *Phalaenopsis* Equestris 'Ilocos' by culturing them on New *Phalaenopsis* Medium (Reddy, 2008).

Phalaenopsis seeds were cultured on a modified ½MS medium devoid of growth regulator, after 2-3 months of culture, the seeds germinated into globular embryos and then protocorms with a high germination rate (Chen *et al*., 2000).

The different nutrient media used for orchid culture include Knudson C medium, Gamborg B5 medium, Fast medium, Harvais medium, Heller medium, Lindemann orchid medium, Malmgren orchid medium, Mitra orchid medium, Murashige and Skoog medium, New Dogashima medium, Schenk and Hildebrandt medium, Thomale-GD medium, Vacin and Went medium and Van Waes and Debergh medium (Park and Yeung, 2018).

The effect of medium composition on the asymbiotic germination and seedling development of five *Phalaenopsis* species were investigated. It was observed that the seeds germinated well on 1/10, 1/4 and 1/2 MS media. The optimum strength for protocorm survival of *P. aphrodite* subsp. *formosana* and *P. philippinensis* was 1/10 MS salts. For *P. appendiculata*, the protocorm survival was significantly higher in 1/2 MS medium than the others. For *P. lindenii* and *P. viridis*, the optimum strength was found in 1/4 and 1/10 MS media (Lee *et al*., 2010).

In an interspecific embryo culture study, it was observed that two-month-old capsules of *Phalaenopsis*, cultured in Vacin and Went and supplemented with kinetin and NAA, were found highly effective, producing 100% embryo development (Rungruchkanont and Promchot, 2017).

Embryo culture done with seeds from successful crosses among different varieties of *Phalaenopsis* like Winter Spot X Pink, Elegant Purple X Pink and Pink X Elegant Purple gave satisfactory germination in half strength MS media supplemented with BA, NAA and activated charcoal (Rahi, 2017).

It was observed that seeds from 4-month-old hand-pollinated *Phalaenopsis* orchids when sown on MS and VW culture media, the optimum seed germination of 90.7%, was achieved on VW medium (Utami and Hariyanto, 2019).

2.7.2. Effect of Growth Regulators on Seed Germination

Plant growth promoters are used to promote seed germination and seedling growth in orchid cultures. It was reported that the combined effect of 2, 4-D and kinetin or BAP promotes protocorm formation in some orchid species (Kusumoto, 1978).

The most commonly used auxins in orchid tissue culture media are the naturally occurring auxin, indole acetic acid (IAA), and the synthetic naphthalene acetic acid (NAA), indole butyric acid (IBA), and 2,4-dichlorophenoxyacetic acid (2,4-D). The synthetic kinetin (6-furfuryl amino purine), benzyl adenine (N6-benzyl amino purine, N6-benzyl adenine, BA, BAP), dimethyl amino purine (DMAP), thidiazuron (TDZ), and the naturally occurring zeatin are used most commonly in orchid culture media (Yam and Arditti, 2018).

The use of NAA at 1ppm enhanced seed germination in *Cypripedium* (Boesmann, 1962) and *Vanda* (Mathew and Rao, 1980). Similar effects were observed *Epidendrum radicans* seed germination and subsequent, but at higher concentrations it stimulated callusing growth (Nair, 1982).

1-Phenyl-3-(1, 2, 3-thiadiazol-5-yl)-urea (TDZ), 13.62 μM is found to promote direct embryo formation in *Phalaenopsis amabilis* var. f*ormosa* (Chen and Chang, 2004).

Cymbidium bicolor was germinated in ½ MS medium supplemented with BAP (0.5, 1.0, 2.0 and 3.0 ppm), NAA (0.5, 1.0, 2.0 and 3.0 ppm) and 2, 4-D (0.5, 1.0, 2.0 and 3.0 ppm) individually or in combinations, with or without $1.0 gl^{-1}$peptone. 1/2 MS medium supplemented with 1.0 ppm BAP and 2.0ppm 2, 4-D was found to be the best treatment (Mahendran and Bai, 2012).

The highest germination percentage of *Phalaenopsis amabilis* was observed with NAA (1.5 ppm) or combination of BAP (0.5 ppm) + NAA (0.5 ppm) (Bazand *et al*., 2014).

Seeds from the intervarietal crosses of *Phalaenopsis* germinated in half MS medium supplemented with 1 ppm BA, 0.1 ppm NAA and 0.5 per cent activated

charcoal and in Knudson-C medium supplemented with 3 ppm BA and 0.3 ppm NAA (Rahi, 2017).

The two-month-old capsules of *Phalaenopsis*, cultured in Vacin and Went and supplemented with 1 ppm Kinetin plus 0.1 ppm of NAA, were found highly effective, producing 100% capsule development (Rungruchkanont and Promchot, 2017).

2.7.3. Effect of Carbon Source on Seed Germination

Orchids require an external supply of carbohydrates for their growth and differentiation. Orchid seeds and seedlings have the ability to utilize various carbohydrates. However, different species have their own preference (Arditti, 1967).Orchid seeds utilize disaccharides such as sucrose for initial germination and subsequent growth. Some species of *Cymbidium* prefer glucose to sucrose, while *Phalaenopsis* prefers fructose to glucose.

Glucose, fructose, or oligosaccharides with these sugars could adequately satisfy the energy requirements of *Phalaenopsis* protocorms during its growth (Ernst *et al*., 1971).

Harrison and Arditti (1978) found that sucrose induced germination and enhanced development of chlorophyll in certain species that failed to germinate on sugar-free medium. Sucrose could be replaced by glucose.

It was reported that even though *Phalaenopsis* seeds and seedlings can utilize many sugars as carbon source, with the increased polymerization and increased molecular weight of the sugars used, seedling fresh weight and survival decreased (Ernst and Arditti, 1990).

Bhattacharjee *et al.* (1999) observed that sucrose at 20g/l was optimum for protocorm formation and development in *Phalaenopsis*.

It was reported that media containing sugar (glucose or sucrose) significantly increase both leaf size and root length of *Phalaenopsis gigantea* seedlings *in vitro* (Murdad *et al*., 2010).

It was reported that, 3% sucrose as carbohydrate source can be effectively used in *in vitro* culture of *Phalaenopsis* (Preetha and Shylaraj, 2015).

In *Phalaenopsis* hybrid 'Pink', the combination of 30 g L^{-1} sucrose and 10% carrot juice in MS medium resulted in the highest leaf length and total chlorophyll. The combination of 40 g L^{-1} sucrose and 10% carrot juice in VW medium resulted in the highest leaf length and total chlorophyll (Zahara *et al*., 2016).

Phalaenopsis hybrid 'Pink' cultured on the combination of sucrose (20 g L^{-1}) and carrot juice (10%) supplemented with either MS or VW media resulted in overall good plantlet growth (Zahara *et al*., 2017).

2.7.4. Effect of pH on Seed Germination

Rosa and Laneri (1977) observed that a pH of 5.2 for *Phalaenopsis* was satisfactory for germination.

Orchid seeds germinated well within a pH range of 4.8 to 5.2 with germination commencing at pH 3.6 and tapering off at 7.6 (Arditti, 1979).

In a study it was observed that, the browning rate was the lowest at pH, 6.5 while *in vitro* culture of variety 'R4'of *Phalaenopsis* (Zhao *et al*., 2006).

Prior to sterilization of media, the pH of the media needs to be adjusted to a range between 5 and 6. The acidity of a medium can influence ion uptake by the explants during *in vitro* culture (George and De Klerk, 2008).

The pH of the culture medium was adjusted to 5.7 with NaOH and HCl prior to autoclaving during asymbiotic seed germination in some hybrids of *Phalaenopsis* (Balilashaki *et al.*, 2015).

2.8. Genetic Analysis and Variability Studies

Genetic analysis of some *Phalaenopsis* hybrids at Sikkim Himalaya was done by De *et al*. (2019). High degree of genetic variance was recorded for various morphological characteristics including vegetative and floral traits.

In a study conducted to analyze the variability among *Phalaenopsis* varieties namely Elegant Yellow, Elegant Purple, Elegant Fairy, Pink, Violet and Winter Spot, with respect to morphological characters of leaf, shoot and flowers, floral biology and genetic relationship prior to hybridization. Among the morphological characters studied the varieties varied significantly with respect to total number of leaves, leaf area, and shoot height. Statistical analysis showed that varieties varied significantly for floral characters *i.e* number of days from first flower opening to last flower opening, days for wilting of the first flower, days for wilting of the last flower, flower size, spike length (Rahi, 2017).

2.9. Breeding of Hybrids

Important orchid genera like Cattleya, Cymbidium, Odontoglossum, Vanda, Dendrobium, *Phalaenopsis* etc. undergone various interspecific and intraspecific combinations to yield improved hybrids (Abraham and Vatsala, 1981).

While breeding with yellow *Phalaenopsis*, Singh (1982) observed that the farther from the species, the better becomes the flower shape, flower size and number of flowers per spike, but the lighter becomes the colour.

Takasaki (1989) reported that out the hybrids obtained by crossing the striped *Phalaenopsis* Kathleen Ali with the spotted *P*. Frisson, some had good spots while others had stripes with spots.

Harper (1993) emphasized the contribution made by *Phalaenopsis stuartiana* in the development of multiflora *Phalaenopsis* hybrids.

Moses (1994) discussed the progressive development of semi alba *Phalaenopsis* with their full, flat flowers and white sepals and petals contrasting with the deep red lips. Their breeding began in 1986 by crossing the lavender pink lipped *P. equestris* with the solid white *P. aphrodite*.

Chen *et al*. (1995) conducted an extensive varietal improvement programme in *Phalaenopsis* using 29 wild species and 873 varieties. They succeeded in releasing 35

new hybrid varieties. Studies on protoplast fusion, isoenzyme electrophoresis and DNA finger printing to assist in varietal identification were performed.

The majority of commercially grown orchid are bigeneric and multigeneric hybrids derived from sympodial and monopodial orchid genera such as Ar*achnis, Vanda, Renanthera, Ascocentrum, Cymbidium, Cattleya, Dendrobium, Oncidium, Phalaenopsis* and *Paphiopedilum* (Mercy and Dale, 1997).

Chen *et al*. (2000) observed the breeding behavior of *Phalaenopsis equestris* and reported that hybrids with compact, multiple branched, inflorescence grew faster compared to those with large flowers. They further reported that during the first ten years of the breeding programme till 1998, a total of 30 hybrids could be registered with the Royal Horticultural Society.

Yellow and red *Phalaenopsis* breeding initiated with a cross between *Phalaenopsis* 'Doris', and *P. manni* to produce *P*. 'Golden Louis'. Red *Phalaenopsis* improvement is generally accompanied by problems with fertility, flower size and low flower count. The first fertile and readily available red *Phalaenopsis* is 'Golden Buddha' (Ginsberg, 2000).

According to Thomas (2001) all modern white *Phalaenopsis* are progenies of *P. amabilis* and *P. aphrodite*. *Phalaenopsis* 'Doris' bred in 1940 is one of the prominent white hybrids. He reviewed the requirements for flower forms of commercial growers like strong self-supporting straight inflorescences, long lasting of blooms, plant compactness, wide temperature tolerance, disease resistance, firm substances and colour consistency.

Another shift in *Phalaenopsis* is breeding for blue flowers. The two species that contributed the basis of blue *Phalaenopsis* breeding are *Doritis pulcherrima* var. *coerulea* and *Phalaenopsis violacea* var. *coerulea* (Mountford, 2001).

Multi-generic hybrids have been developed by crossing *Phalaenopsis* with other genera like *Aerides, Arachnis, Doritis, Neofinetia, Rhyncostylis, Renanthera* and *Vanda* (Bhattacharjee and Das, 2008).

The long continued inbreeding of *Phalaenopsis sanderiana* lead to the development of superior clones (De *et al*., 2014).

Improvement of *Phalaenopsis* mainly evolved around the development of pure whites, pure pinks and white with pink lips. *P. amabilis, P. formosana* and *P. aphrodite* were extensively used for evolving of outstanding white hybrids. For development of pink coloured hybrids like the *P. lueddemanniana, P. sanderiana* and *P. schileriana* contributed prominently. White colours with pink lip was obtained in *P. intermedia* due to natural hybridization between *P.aphrodite* and *P. equestris* (De *et al*., 2019).

A *Phalaenopsis* cultivar, 'Yellow Green' that has small, deep-yellow flowers with red lip, and was introduced by artificial crossing of the *Phalaenopsis* cultivars 'Sogo Pride F-1016' and 'Tzu Ching Glint' (Vo *et al*., 2019).

Materials and Methods

3. MATERIALS AND METHODS

The research programme entitled 'Varietal evaluation and development of hybrids in *Phalaenopsis* orchids' was undertaken in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 2018-2020.

The parent materials for investigations were maintained in the green house and observations on vegetative and floral characters were taken. Statistical analysis including variability, heritability, genetic advance and correlation were also estimated. Hybridization and compatibility studies were also conducted on the 3 best varieties. Germination test was done in Plant Tissue Culture Laboratory of the department.

The major studies conducted are given below.

1. Evaluation of parent material
2. Hybridization and compatibility studies

3. 1. EVALUATION OF PARENT MATERIAL

3.1.1. Experimental Material

The experimental material comprised of ten *Phalaenopsis* orchid genotypes having good commercial value. The inflorescences of these parents, which are listed here in Table 1, are shown in Plate 1 and 2 respectively.

Table 1. List of Parents

Parental Genotypes	
P1	*Phalaenopsis* King Car Purple Queen
P2	*Phalaenopsis* Lianher Happy Song
P3	*Phalaenopsis* Fullers Rabbit
P4	*Phalaenopsis* Lianher Orange
P5	*Phalaenopsis* Fullers
P6	*Phalaenopsis* Taisuco Fire Bird x King Hisang Rose
P7	*Phalaenopsis* Reyoung Gold
P8	*Phalaenopsis* Jiuhbao Venus
P9	*Phalaenopsis* Wang Lin Rose
P10	*Phalaenopsis* Young Home Golden

The sources of parental genotypes used in the experiment are given in Table 2.

KING CAR PURPLE QUEEN

REYOUNG GOLD

FULLERS RABBIT

LIANHER HAPPY SONG

JIUHBAO VENUS

WANG LIN ROSE

YOUNG HOME GOLDEN

FULLERS

Plate 1: Single flowers of different genotypes of *Phalaenopsis* used in the study

TAISUCO FIRE BIRD x KING HISANG ROSE

LIANHER ORANGE

Plate 1: (continued) Single flower of the *Phalaenopsis* genotypes used in the study.

KING CAR PURPLE QUEEN

FULLERS

WANG LIN ROSE

LIANHER ORANGE

Plate 2: Inflorescences of the *Phalaenopsis* genotypes used in the study.

YOUNG HOME GOLDEN

LIANHER HAPPY SONG

REYOUNG GOLD

JIUHBAO VENUS

TAISUCO FIRE BIRD x KING HISANG ROSE

FULLERS RABBIT

Plate 2: (continued) Inflorescences of the *Phalaenopsis* genotypes used in the study.

Table 2. Sources of parental monopodial orchid genotypes

Parental orchid genotype	Source of planting material
Phalaenopsis King Car Purple Queen *Phalaenopsis* Lianher Happy Song *Phalaenopsis* Fullers Rabbit *Phalaenopsis* Lianher Orange *Phalaenopsis* Fullers *Phalaenopsis* Taisuco Fire Bird*KHR *Phalaenopsis* Reyoung Gold *Phalaenopsis* Jiuhbao Venus *Phalaenopsis* Wang Lin Rose	Rynco Orchids, Nedumangad, Thiruvananthapuram, Kerala

The planting material of these *Phalaenopsis* orchid parents consisted of meristem cultured clones in flowering stage. They were planted in plastic pots. Proper management practices were undertaken as per the Package of Practices Recommendations of Kerala Agricultural University (KAU, 2016).

Design : Completely Randomized Design (CRD)

Treatments : 10

Replications : 2

3.1.2. Experimental Methods

The selected materials were evaluated by recording observations on their vegetative and floral characters (both quantitative and qualitative).

3.1.2.1. Vegetative Characters

3.1.2.1.1. Quantitative Characters

3.1.2.1.1.1. Plant Height (cm)

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

3.1.2.1.1.2. Number of Leaves per Plant

Total number of laminate leaves per plant was recorded at maximum leaf stand.

3.1.2.1.1.3. Length of Leaf (cm)

The length of mature leaves was measured from the base to the tip from five leaves per plant and average was calculated and expressed in centimeters.

3.1.2.1.1.4. Width of Leaf (cm)

The width of mature leaves was measured at the widest region from five leaves per plant average was taken and expressed in centimeters.

3.1.2.1.1.5. Leaf Area (cm^2)

The leaf area was measured graphically from five leaves per plant and average was taken.

3.1.2.1.2. Qualitative Characters

3.1.2.1.2.1. Leaf Shape

The shape of the leaf is recorded as either oblong, narrow obovate or suborbicular.

3.1.2.1.2.2. Leaf Pigmentation

Anthocyanin pigmentation of the leaf is observed.

3.1.2.2. Floral Characters

3.1.2.2.1. Quantitative Characters

3.1.2.2.1.1. Days to First Flower Opening from Inflorescence Emergence

Number of days was counted from visible emergence of inflorescence to the opening of first flower and recorded.

3.1.2.2.1.2. Vase Life (days)

Vase life of the cut inflorescence in water as the holding solution was noted. Fading of the first flower was recorded as the end of vase life.

3.1.2.2.2. Number of Inflorescence

The number of inflorescence per plant is recorded.

3.1.2.2.3. Length of Inflorescence (cm)

The length of inflorescence was measured from the base of a fully opened inflorescence to the tip of the axis and expressed in centimeters.

3.1.2.2.3.2. Number of Flowers per Inflorescence

Total number of flowers produced per inflorescence was counted and recorded.

3.1.2.2.4. Internodal Length of Peduncle (cm)

Length of internode i.e., distance between two consecutive flowers was measured for the entire inflorescence and the mean was computed and expressed in centimeters.

3.1.2.2.5. Diameter of Peduncle (cm)

Diameter of peduncle was measured at the widest region using vernier calipers and expressed in centimeters.

3.1.2.2.6. Length of Flower (cm)

Flower length was measured at the widest region from the tip of the labellum to the tip of the odd sepal is recorded and expressed in centimeters.

3.1.2.2.7. Width of Flower (cm)

Flower width was recorded at the widest region and expressed in centimeters.

3.1.2.2.8. Flower Longevity on the Plant

The number of days the flowers remained in the plant is observed and noted.

3.1.2.2.2. Qualitative Characters

3.1.2.2.2.1. Inflorescence Type

The type of inflorescence is recorded as either solitary, raceme or panicle

3.1.2.2.2.2. Flower Colour

The colour of the flower and blending of colours were noted and recorded.

3.1.2.2.2.3. Flower Texture

The texture of the flower was noted as either smooth or rough.

3.1.2.2.2.4. Flower Fragrance

Whether fragrance is present or absent is recorded.

3.1.2.2.2.5. Arrangement of Petals

Arrangement of the petals were observed and recorded as open, touching or overlapping type.

3.1.2.2.2.6. Branching of Inflorescence

Branching of the inflorescence was observed and recorded.

3.1.2.2.2.7. **Season of Flowering**

Flowering time was observed and recorded as free flowering i.e., flowering all-round the year or seasonal i.e., flowering at specific seasons.

3.1.2.2.2.8. Colour Pattern of Petal

The colour blending and pattern of the petal was recorded as uniform, shaded, edged, stripped, netted, spotted or mixed.

3.1.2.2.2.9. Predominant Colour in Petal

The predominant colour present in the petal was carefully observed and noted.

3.1.2.2.2.10. Colour Pattern of Lip

The colour blending and pattern of the lip or labellum was recorded as uniform, shaded, edged, stripped, netted, spotted or mixed.

3.1.2.2.2.11. Predominant Colour of Lip

The predominant colour of the lip or labellum was carefully observed and noted.

3.1.3. Statistical Analysis

The collected data were subjected to the analysis of variance to test for significant difference among the ten *Phalaenopsis* orchid genotypes selected, following Panse and Sukhatme (1985). According to the statistical analysis three

varieties with best floral traits were selected for further studies. Genetic parameters *viz.*, variability, heritability, genetic advance and correlation were also estimated.

3.1.3.1. Analysis of Variance

ANOVA with two characters X and Y measured in 'g' genotypes raised in completely randomized design with 'r' replications is as follows

Source	Df	Mean square		
		X	Y	XY
Between genotypes	(g-1)	G_{xx}	G_{yy}	G_{xy}
Error	(r-1) (g-1)	E_{xx}	E_{yy}	E_{xy}

3.1.3.2. Coefficient of Variation

Genotypic and phenotypic coefficients of variation were estimated using the formula proposed by Singh and Chaudhary (1977). The phenotypic and genotypic coefficients of variation (PCV and GCV) for a trait X were estimated using the following formulae.

$$GCV = (\sigma_{gx} / M)*100$$

$$PCV = (\sigma_{px} / M)*100$$

Where,

σ_{gx} = genotypic standard deviation

σ_{px} = phenotypic standard deviation

M = mean of the character under study

3.1.3.3. Heritability and Genetic Advance

Heritability (H^2) in broad sense was estimated as the proportion of heritable component of variation. Broad sense heritability for each character was calculated as a percentage based on the formula given by Jain (1982).

Heritability coefficient (in broad sense), $H^2 = (\sigma_{gx}^2 / \sigma_{px}^2) * 100$

Where,

σ_{gx}^2 = genotypic variance of the character X

σ_{px}^2 = phenotypic variance of the character X

According to classification of Heritability by Allard (1960),

< 30 percent - Low heritability

30-60 percent - Medium heritability

>60 percent - High heritability

Genetic advance as percentage of mean (GA) = $K H^2 \sigma_{px} / M*100$

Where,

k = Selection differential whose value is 2.06 if 5% selection is to be practiced (Miller *et. al*., 1958).

H^2 = Heritability in broad sense

σ_{px} = Phenotypic standard deviation

M = Mean of the character over all varieties

Robinson *et al*. (1949) classified Genetic advance as percentage of mean into three categories i.e.

< 20 % - Low genetic advance

> 20 % - High genetic advance

3.1.3.4. Correlation Analysis

The correlation coefficients namely phenotypic, genotypic and environmental correlation coefficients between two characters denoted as 'x' and 'y' were worked out as follows:

Genotype correlation (r_{gxy}) $= \sigma_{gxy} / (\sigma_{gx} \times \sigma_{gy})$

Phenotypic correlation (r_{pxy}) $= \sigma_{pxy} / (\sigma_{px} \times \sigma_{py})$.

Environmental correlation (r_{exy}) $= \sigma_{exy} / (\sigma_{ex} \times \sigma_{ey})$.

Where,

σ_{gxy}, σ_{pxy} and σ_{exy} are the genotypic, phenotypic and environmental co-variances between the characters 'X' and 'Y'.

σ_{gx}, σ_{px} and σ_{ex} are the genotypic, phenotypic and environmental standard deviations for the character 'X'.

σ_{gy}, σ_{py} and σ_{ey} are the genotypic, phenotypic and environmental standard deviations for the character 'Y'.

3.2. HYBRIDIZATION AND COMPATIBILITY STUDIES

All possible self and cross combinations including reciprocals ($n^2 = 3^2 = 9$) were done among the best three varieties to conduct compatibility analysis. Following the conventional practice in orchid hybridization, the first and last flowers of each inflorescence were not used for crossing. Emasculation of flowers was not done as the pollinia are held within the clinandrium and covered by operculum which prevents self-pollination effectively. The parent plants used for crossing were protected by

insect-proof netting, to prevent pollination through insect pollinators. After pollination, flowers were tagged properly for identification.

3.2.1. Stage of Green Capsule Harvest

From the successful combinations green capsules were harvested at 70-90 per cent maturity. The best time to harvest a pod is when the tip of capsule starts to turn yellow.

3.2.2. Preparation and Cleaning of Capsule

The harvested green capsules were cleaned by removing the extra length of pedicel and adhering wilted perianth parts. They were then washed in running tap water. For proper cleaning soaking the intact capsules in one percent solution of laboline detergent in distilled water for 20 minutes was done. The capsules were then rinsed thoroughly three to four times with distilled water.

3.2.3. Surface Sterilization, Inoculation and Incubation

As the immature seeds cannot be stored, all seeds must be sown immediately after harvest. The pod is surface sterilized inside a laminar air flow chamber, first by immersing in 0.1 % mercuric chloride solution for 10 minutes. It is then washed in distilled water. Then it is immersed in 70 per cent ethyl alcohol for three to five minutes and then is 'flamed' by passing through the flame of a spirit lamp. If the pods are too much mature, it will split open at this time. Then it is cut open under sterile conditions. The immature embryos are scrapped out and sown in sterile flasks containing the culture medium. The capsules from all successful parental combination harvested at the green capsule stage are inoculated in half strength MS media supplemented with 0.1ppm NAA and 1ppm BA.

3.2.4. Observations

3.2.4.1. Days to Green Capsule Harvest in Successful Crosses

Number of days from pollination to green capsule harvest in compatible crosses was observed and recorded.

3.2.4.2. Length of Capsule (cm)

Length of capsule from base to tip was measured and recorded.

3.2.4.3. Width of Capsule (cm)

Width of capsule at the widest region was noted and recorded.

3.2.4.4. Percentage of Capsule Set

Number of green capsules harvested to total number of pollinations made was recorded and the percentage was computed.

3.2.4.5. Percentage of Capsules with Germinating Seeds

Number of capsules with seeds that germinated on inoculation to the total number of capsules harvested was computed.

Results

4. RESULTS

The results of the investigations on 'Varietal evaluation and development of hybrids in *Phalaenopsis* orchids' carried out are presented below:

4.1.1 Evaluation of parent material

4.1.2 Estimation of variability components *i.e.,* PCV and GCV

4.1.3 Estimation of Heritability and Genetic Advance

4.1.4 Correlation Analysis

4.2. Hybridization and compatibility / incompatibility studies

4.1.1. EVALUATION OF PARENT MATERIAL

Ten *Phalaenopsis* orchid genotypes were evaluated in the greenhouse, being replicated two times. The vegetative and floral characters recorded among the genotypes were subjected to analysis of variance (Table 5) and significant variations were observed. The mean performances of the 10 genotypes for 33 (vegetative, floral and qualitative) characters studied are represented in Tables 3a, 3b, 4a, 4b and 4c.

4.1.1.1. Vegetative Characters

4.1.1.1.1. Quantitative characters

4.1.1.1.1.1. Plant Height (cm)

Plant height was significantly high for P_8 (57.45cm) followed by P_4 (55.73 cm) and P_6 (55.23 cm). The mean plant height was recorded the lowest for P_9 (41.00 cm) which was on par with P_7 (42.23 cm) and P_1 (42.65 cm) also.

4.1.1.1.1.2. Number of Leaves per Plant

The maximum number of leaves was recorded for P1 (8.50). For P2 (8.00) also comparatively higher number of leaves were recorded. This is

statistically on par with P4 (7.50) and P5 (7.50). The minimum values for this trait was for genotypes P8 (6.00) and P10 (6.00).

4.1.1.1.1.3. Length of Leaf (cm)

The length of leaves was found to be the highest for P10 (21.30) which were statistically on par with P2 (21.53) and P5 (21.23). The minimum value was recorded for P1 (18.28).

4.1.1.1.1.4. Width of Leaf (cm)

The values for width of leaves varied significantly among the genotypes. P2 (9.10) showed the highest value which was statistically on par with P6 (8.80), P7 (8.18) and P7 (8.15). The lowest value was observed in P10 (6.60) followed by P5 (6.55).

4.1.1.1.1.5. Leaf Area

Leaf area showed significant variations among the 10 genotypes. The highest leaf area was recorded for P6 (140.60) which was followed by P7 (125.00) and P9 (107.15). The lowest values were recorded for P5 (64.40) followed by P3 (70.90) and P1 (94.40).

4.1.1.1.2. Qualitative Characters

4.1.1.1.2.1. Leaf Shape

A wide variation was observed among the genotypes for the shape of the leaf. Oblong, narrow obovate and suborbicular shapes were observed among the plants.

4.1.1.1.2.2. Leaf Pigmentation

Anthocyanin pigmentation of the leaf was absent in all the genotypes.

4.1.1.2. Floral Characters

4.1.1.2.1. Quantitative Characters

4.1.1.2.1.1. Days to First Flower Opening from Inflorescence Emergence

The number of days was counted from visible emergence of inflorescence to the opening of first flower which varied significantly among the different genotypes (Table 4a). The highest value was recorded for P2 (41.5) which were statistically on par with P3 (38.25) and P5 (36.25). The lowest value was recorded for P8 (20.5) followed by P7 (24.25) and P10 (24.5).

4.1.1.2.1.2. Vase Life (days)

Vase life of the cut inflorescence was recorded and the highest vase life was observed for P8 (21.50) and P10 (21.25) which was statistically on par with P9 (18.00) followed by P7 (16.50). The lowest vase life was recorded for P3 (8.75) followed by P5 (12.00) and P2 (14.00).

4.1.1.2.1.3. Number of Inflorescence

The number of inflorescence showed no significant differences. The highest values were exhibited by P1 (1.50), P5 (1.50) and P7 (1.50) this was statistically on par with other genotypes.

4.1.1.2.1.4. Length of Inflorescence (cm)

The length of inflorescence was recorded to be the highest for P4 (18.43) followed by P6 (13.60) which was statistically on par with P6 (13.00) and P7 (12.63). The lowest valued was observed for P9 (4.33) followed by P3 (6.83) and P2 (9.30).

4.1.1.2.1.5. Number of Flowers per Inflorescence

The number of flowers produced per inflorescence was the highest for P1 (6.25) and P4 (6.25) which were statistically on par with P6 (6.00), P6 (5.50) and P8 (4.50). The lowest values were for P3 (3.00) and P9 (3.00), followed by P2 (3.50).

4.1.1.2.1.6. Internodal Length of Peduncle (cm)

Length of internode was the highest for P8 (4.20) which was statistically on par with P10 (4.15). The lowest value was for P1 (2.33) followed by P5 (2.43).

4.1.1.2.1.7. Diameter of Peduncle (cm)

Diameter of peduncle was found to be the highest for P6 (1.78) followed by P5 (1.75) which was statistically on par with the other genotypes. The lowest value recorded was for P10 (1.48).

4.1.1.2.1.8. Length of Flower (cm)

Flower length was the highest for P2 (9.50) which was statistically on par with P8 (9.23), P6 (9.15), P9 (9.13), P10 (9.05) followed by P3 (8.98) and P (8.75). The lowest value was recorded for P7 (7.30).

4.1.1.2.1.9. Width of Flower (cm)

Flower width was recorded the highest for P9 (10.90) followed by P8 (10.55) which was statistically on par with P10 (10.43). The lowest value was observed in P1 (7.40) followed by P7 (7.50).

4.1.1.2.1.10. Flower Longevity on the Plant

The number of days the flowers remained in the plant is noted to be the highest for P6 (100.75) followed by P10 (98.25) and P3 (96.00) and was statistically on par with P2 (95.00). The lowest value was recorded for P7 (70.75) followed by P9 (79.25) and P5 (86.00).

4.1.1.2.2. Qualitative Characters

4.1.1.2.2.1. Inflorescence Type

The type of inflorescence was racemose in almost all the genotypes except P7 which was a panicle.

4.1.1.2.2.2. Flower Colour

The colour of the flower and blending of colours shows wide variations among the different genotypes like white with purple dots, purple, white and dark magenta, pink, white and purple, violet, yellow, ivory, dark violet and light yellow.

4.1.1.2.2.3. Flower Texture

The genotypes had either smooth or rough flower texture. P1, P2, P4, P7, P9 and P10 exhibited smooth texture and P3, P5, P6 and P8 had rough texture.

4.1.1.2.2.4. Flower Fragrance

Almost all the genotypes had no fragrance except P2 which had a mild fragrance.

4.1.1.2.2.5. Arrangement of Petals

Arrangements of the petals were different for the genotypes under study. It was observed that the genotypes had open, touching or overlapping type of arrangement.

4.1.1.2.2.6. Branching of Inflorescence

The inflorescence was branching for only one genotype P7 other genotypes showed no branching.

4.1.1.2.2.7. Season of Flowering

Flowering time was observed and recorded as free flowering for genotypes P3 and P9 and seasonal flowering was exhibited by the other genotypes.

4.1.1.2.2.8. Colour Pattern of Petal

The colour blending and pattern of the petal showed a wide variation. PP7, P8 and P10 had uniform colour pattern, mixed colour pattern was exhibited by P2, P3, P4 and P5. P6 and P9 have shaded pattern whereas P1 had spotted colour pattern.

Table 3a. Mean Performance of 10 *Phalaenopsis* Orchid Parental Genotypes for Quantitative Vegetative Characters

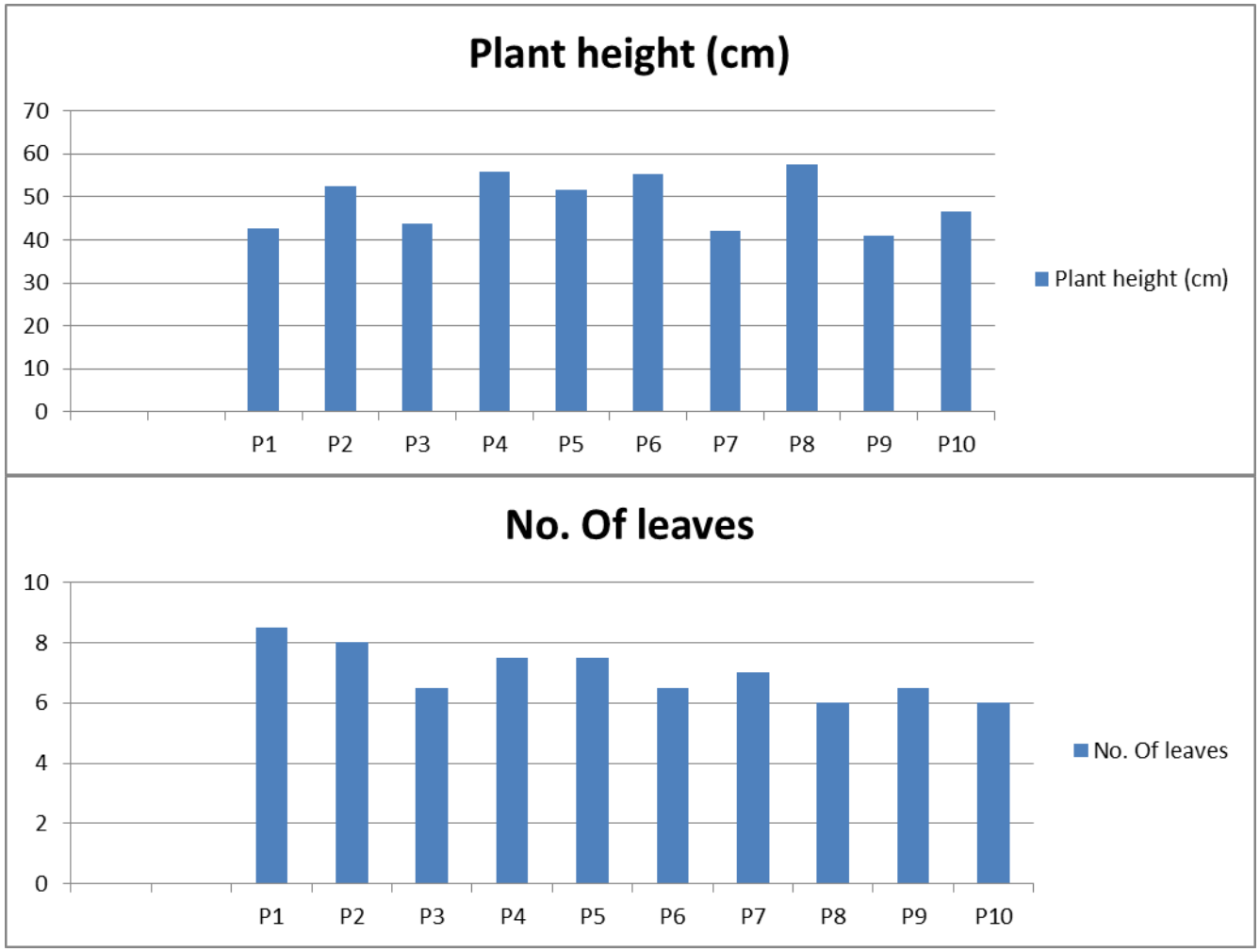
Parents	Plant height (cm)	No. Of leaves	Leaf width(cm)	Length of leaf (cm)	Leaf area
P_1	42.65	8.50	7.50	18.28	94.40
P_2	52.48	8.00	7.70	21.53	96.05
P_3	43.68	6.50	8.18	18.98	70.90
P_4	55.73	7.50	8.00	18.63	106.75
P_5	51.78	7.50	6.55	21.28	64.40
P_6	55.28	6.50	8.80	19.63	140.60
P_7	42.23	7.00	8.15	19.28	125.00
P_8	57.45	6.00	6.88	20.00	107.00
P_9	41.00	6.50	9.10	20.90	107.50
P_{10}	46.50	6.00	6.60	21.30	107.15
CD(0.05)	5.35	4.26	0.79	0.86	5.55
SE_m	1.68	0.50	0.25	1.28	1.74

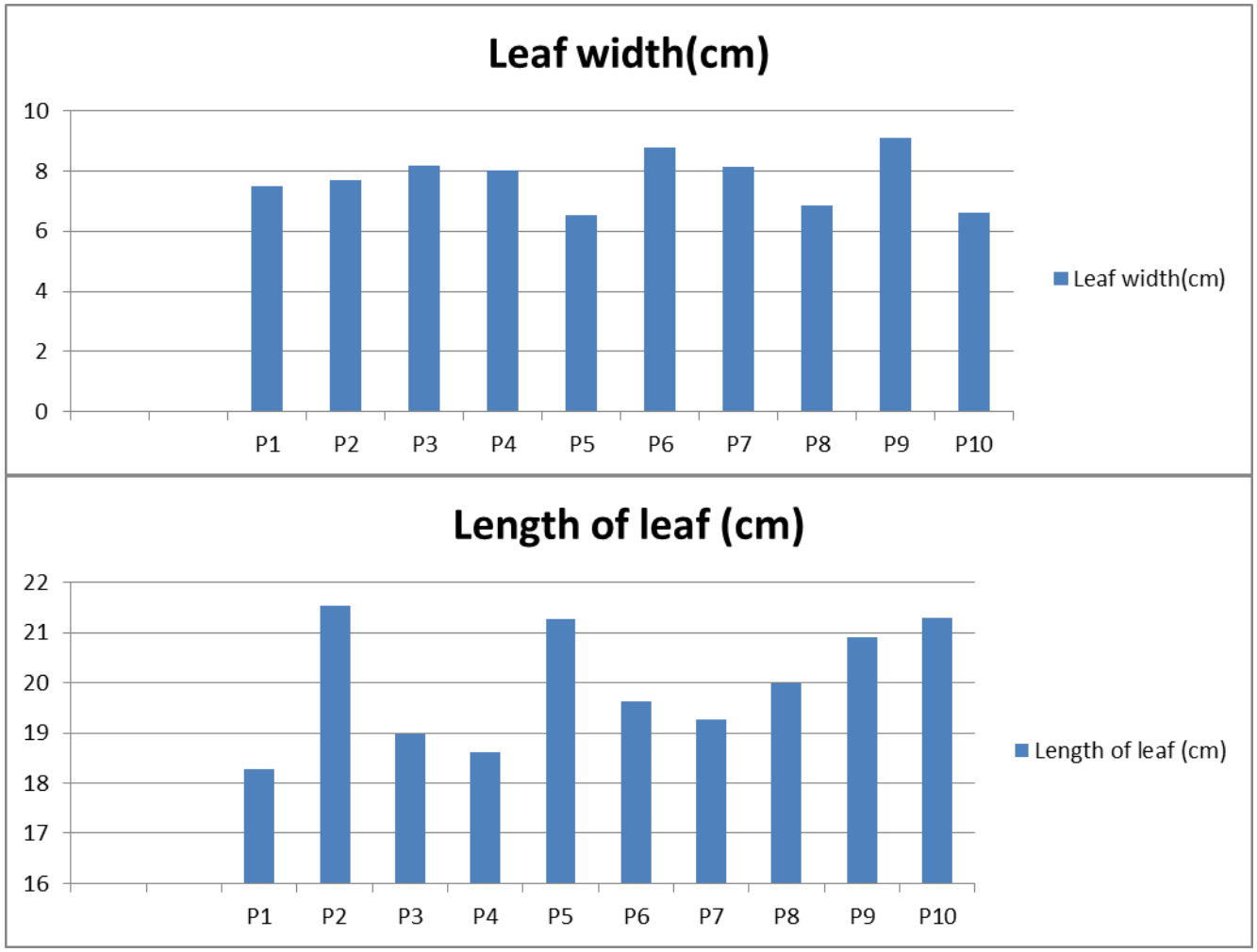
Table 3b. Qualitative Vegetative Characters

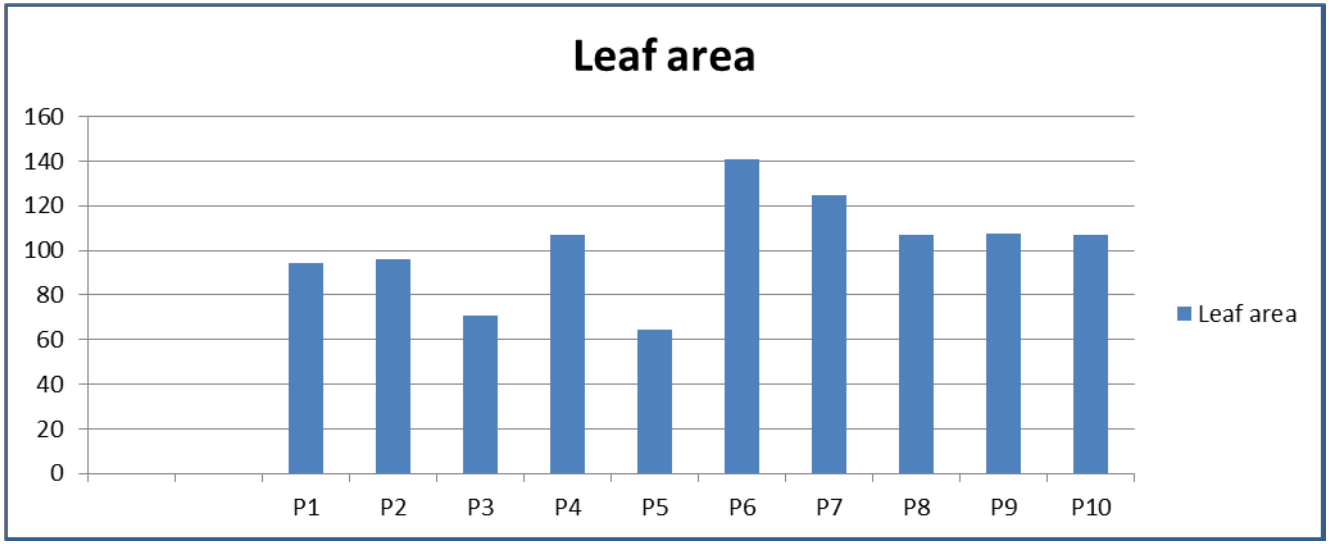
Genotypes	Leaf shape	Leaf pigmentation
P_1	oblong	Absent
P_2	oblong	Absent
P_3	suborbicular	Absent
P_4	oblong	Absent
P_5	narrow obovate	Absent
P_6	suborbicular	Absent
P_7	oblong	Absent
P_8	oblong	Absent
P_9	narrow obovate	Absent
P_{10}	oblong	Absent

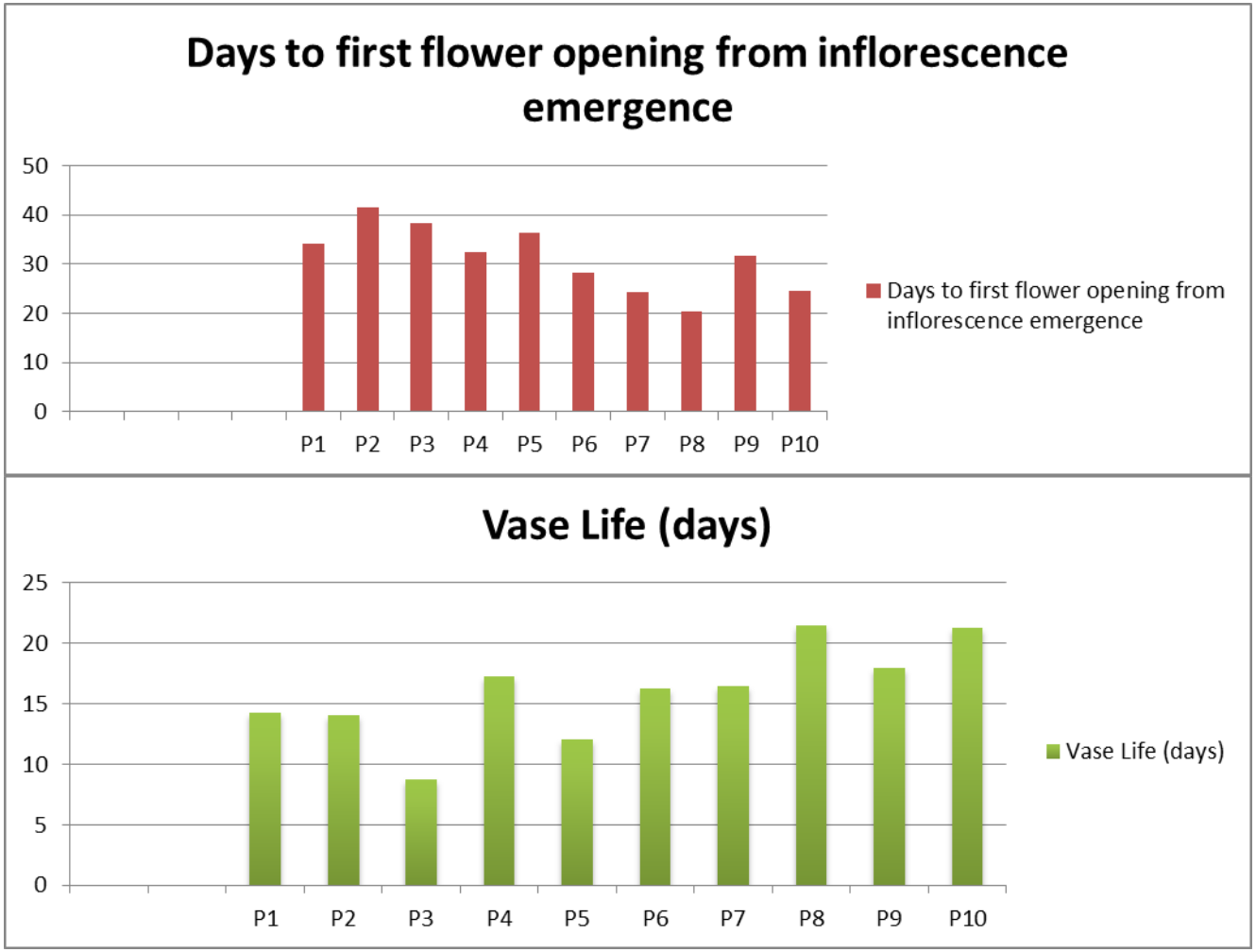
Table 4a. Mean Performance of 10 *Phalaenopsis* Orchid Parental Genotypes for Quantitative Floral Characters

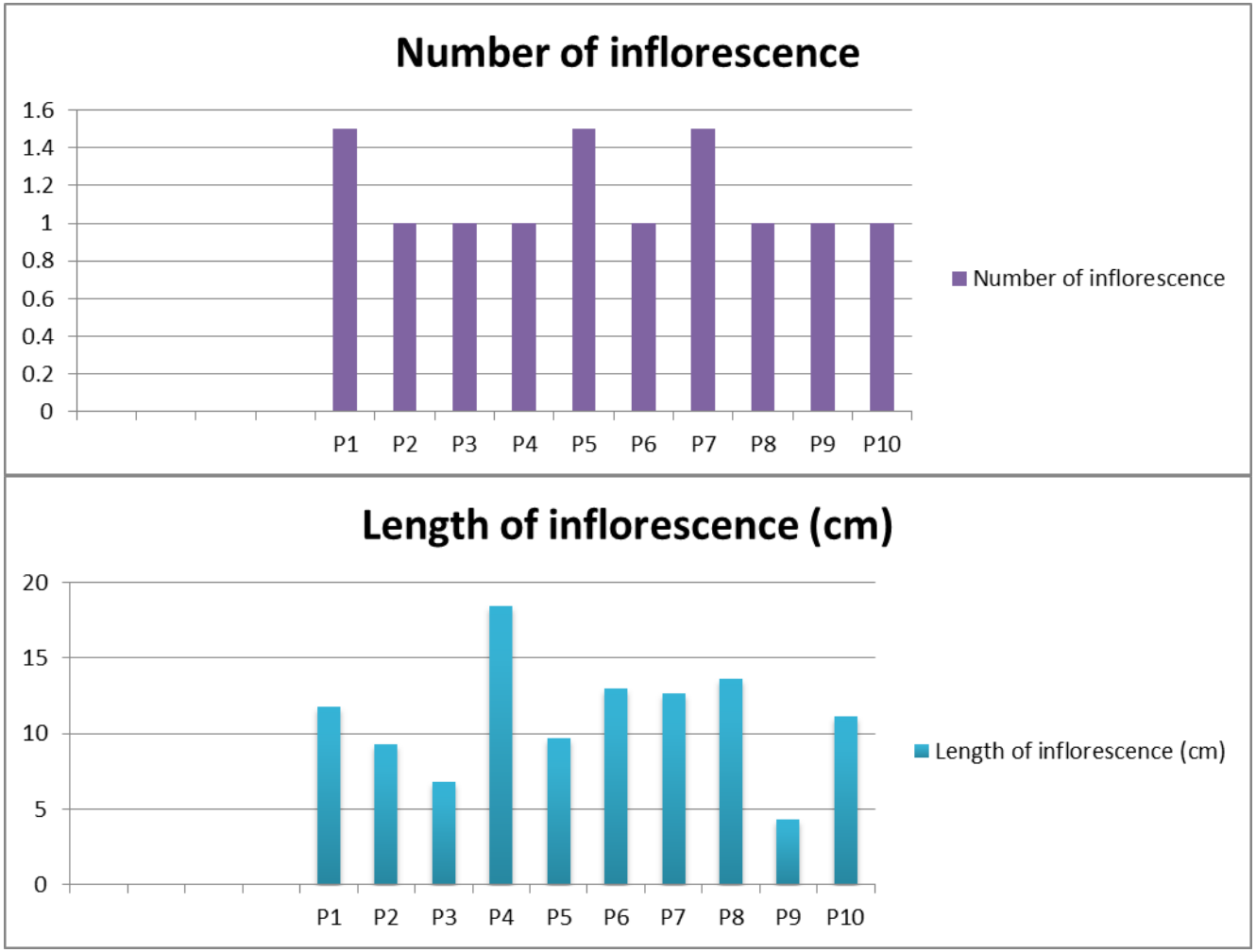
Parents	Days to first flower opening from inflorescence emergence	Vase Life (days)	Number of inflorescence	Length of inflorescence (cm)	Number of flowers per inflorescence	Internodal length of peduncle (cm)	Diameter of peduncle (cm)	Length of flower (cm)	Width of flower (cm)	Flower longevity on the plant
P_1	34.25	14.25	1.50	11.80	6.25	2.33	1.53	7.58	7.40	93.25
P_2	41.50	14.00	1.00	9.30	3.50	3.50	1.65	9.50	9.50	95.00
P_3	38.25	8.75	1.00	6.83	3.00	2.78	1.60	8.98	9.95	96.00
P_4	32.50	17.25	1.00	18.43	6.25	3.40	1.73	8.58	9.73	89.25
P_5	36.25	12.00	1.50	9.70	6.00	2.43	1.75	8.75	9.50	86.00
P_6	28.25	16.25	1.00	13.00	5.50	2.70	1.78	9.15	9.23	100.75
P_7	24.25	16.50	1.50	12.63	3.75	3.35	1.73	7.30	7.50	70.75
P_8	20.50	21.50	1.00	13.60	4.50	4.20	1.70	9.23	10.55	89.00
P_9	31.75	18.00	1.00	4.33	3.00	2.58	1.65	9.13	10.90	79.25
P_{10}	24.50	21.25	1.00	11.13	4.25	4.15	1.48	9.05	10.43	98.25
CD (0.05)	7.24	5.47	5.12	2.19	2.02	0.83	0.80	0.81	0.61	5.18
SE_m	2.27	1.71	0.27	0.69	0.63	0.26	0.09	0.25	0.19	1.62

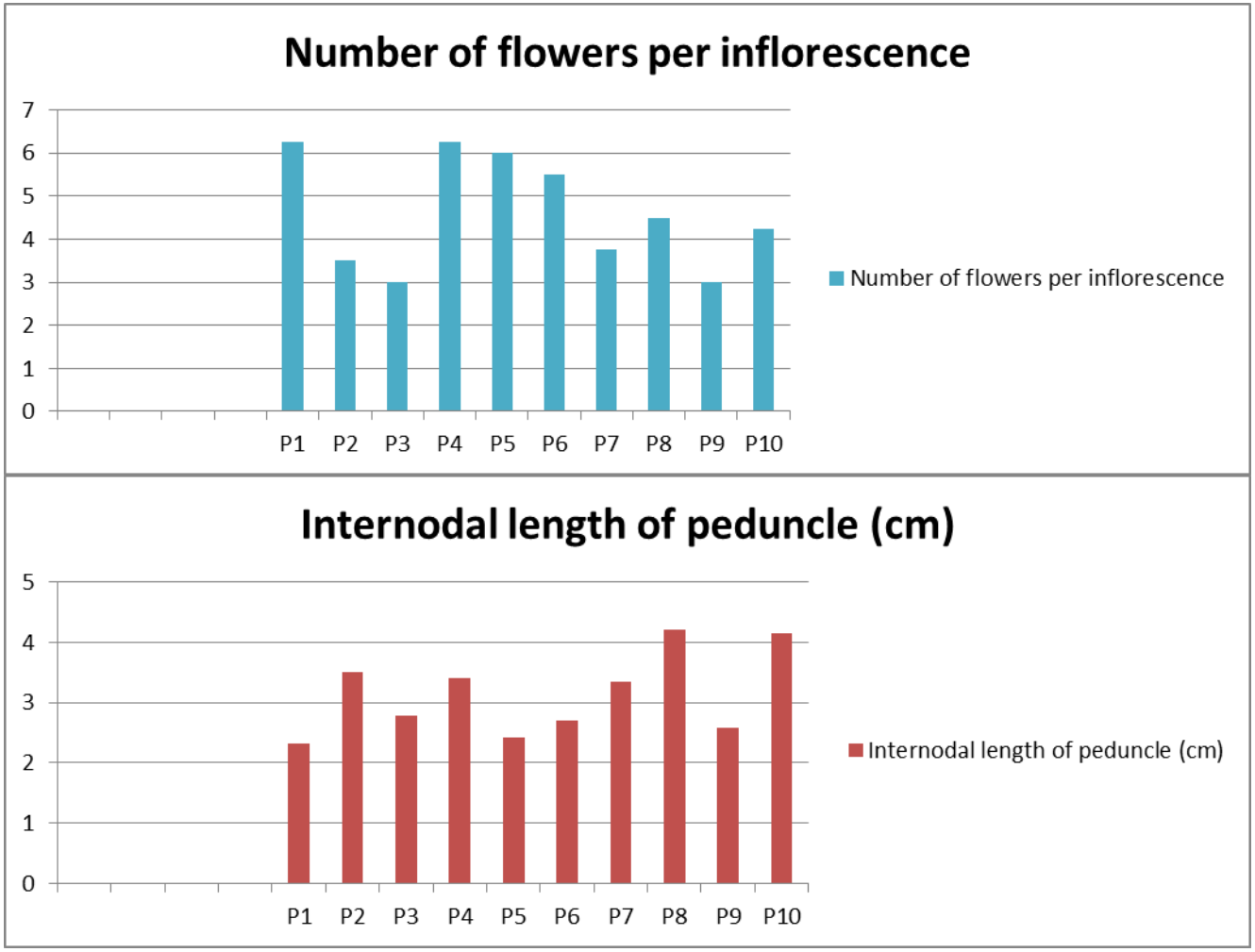

Plant height (cm)
70
60
50
40
30
20
10
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Plant height (cm)
No. Of leaves
10
8
6
4
2
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
No. Of leaves

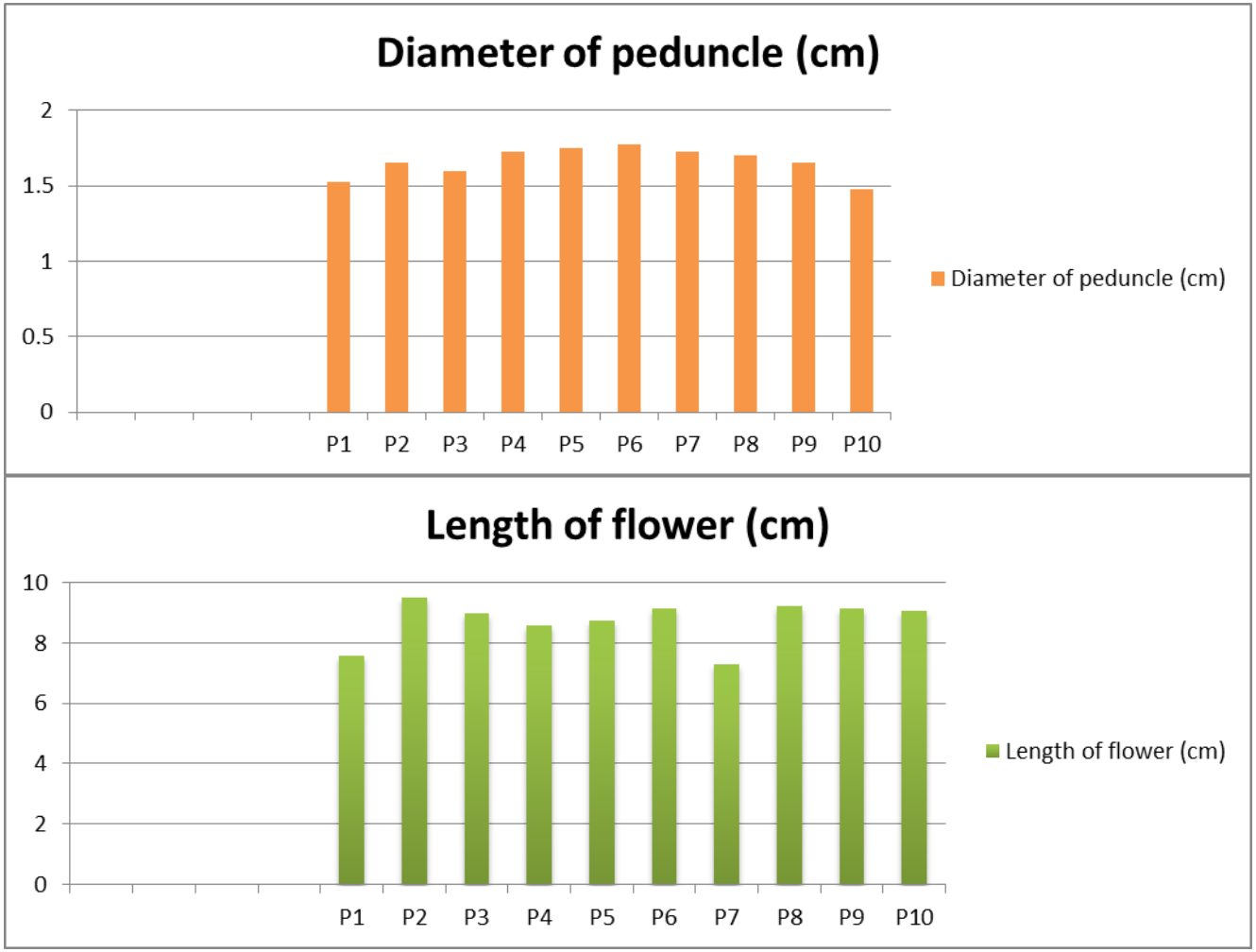

Leaf width(cm)
10
8
6
4
2
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Leaf width(cm)
Length of leaf (cm)
22
21
20
19
18
17
16
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Length of leaf (cm)


Leaf area
160
140
120
100
80
60
40
20
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Leaf area


Days to first flower opening from inflorescence emergence
50
40
30
20
10
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Days to first flower opening from inflorescence emergence
Vase Life (days)
25
20
15
10
5
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Vase Life (days)


Number of inflorescence
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Number of inflorescence
Length of inflorescence (cm)
20
15
10
5
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Length of inflorescence (cm)


Number of flowers per inflorescence
7
6
5
4
3
2
1
0
P1 P2 P3 P4 P5 P6 P7 P8 P9 P10
Number of flowers per inflorescence
Internodal length of peduncle (cm)
5
4
3
2
1
0
P1 P2 P3 P4 P5 P6 P7 P8 P9 P10
Internodal length of peduncle (cm)


Diameter of peduncle (cm)
2
1.5
1
0.5
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Diameter of peduncle (cm)
Length of flower (cm)
10
8
6
4
2
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Length of flower (cm)

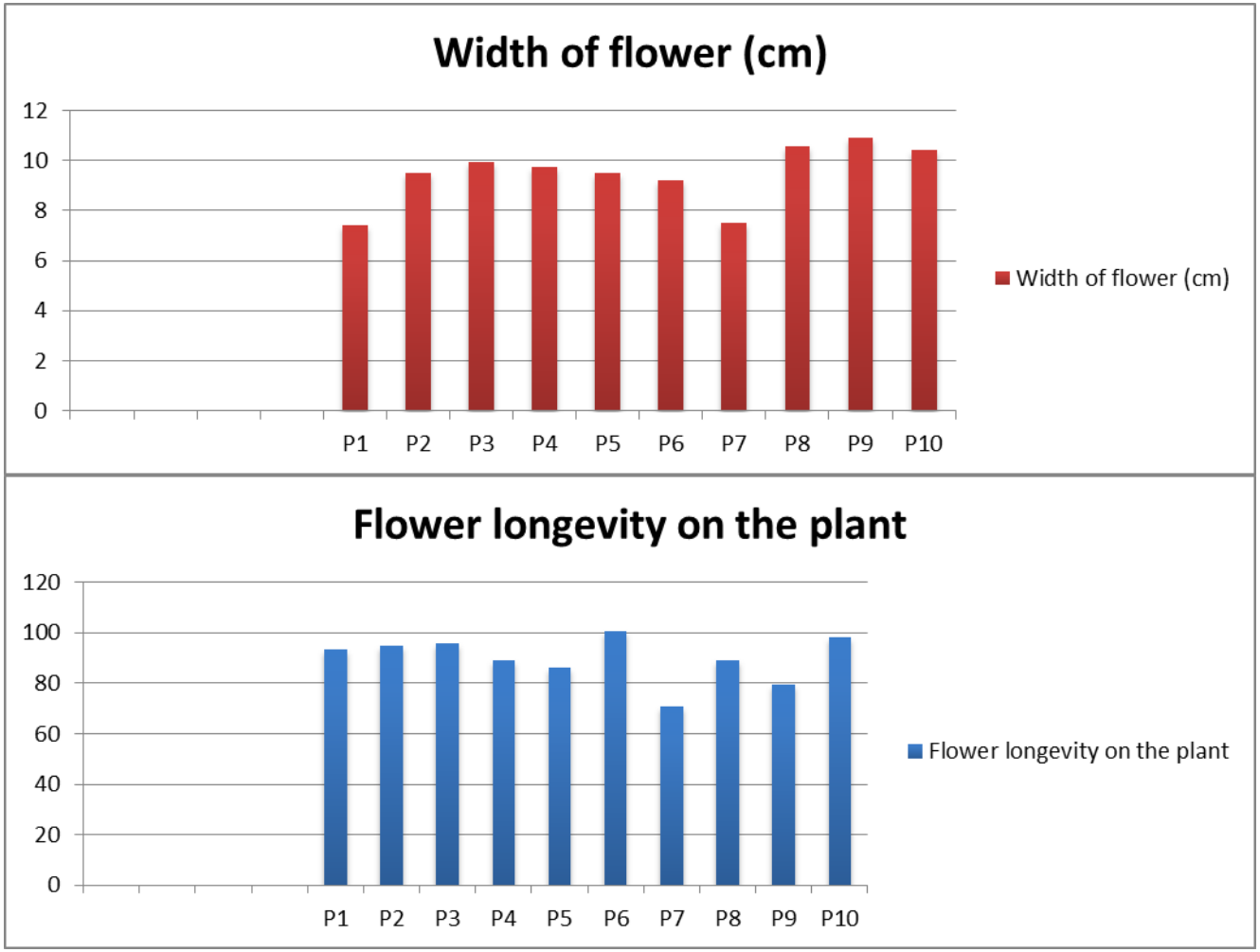

Width of flower (cm)
12
10
8
6
4
2
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Width of flower (cm)
Flower longevity on the plant
120
100
80
60
40
20
0
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
Flower longevity on the plant

Table 4b. Qualitative Floral Characters

Genotypes	Inflorescence type	Flower colour	Flower texture	Flower fragrance	Branching of inflorescence
P_1	Racemose	White with purple dots	Smooth	Absent	Absent
P_2	Racemose	Purple	Smooth	Present	Absent
P_3	Racemose	White and dark magenta	Rough	Absent	Absent
P_4	Racemose	Pink	Smooth	Absent	Absent
P_5	Racemose	White and purple	Rough	Absent	Absent
P_6	Racemose	Violet	Rough	Absent	Absent
P_7	Panicle	Yellow	Smooth	Absent	Present
P_8	Racemose	Ivory	Rough	Absent	Absent
P_9	Racemose	Dark violet	Smooth	Absent	Absent
P_{10}	Racemose	Light yellow	Smooth	Absent	Absent

Table 4c. Qualitative Floral Characters (cont.)

Genotypes	Colour pattern of petal	Predominant colour in petal	Colour pattern of lip	Predominant colour of lip	Arrangement of petals	Season of flowering
P_1	spotted	white	edged	white	open	Seasonal
P_2	mixed (shaded and spotted)	purple	shaded	yellow	open	Seasonal
P_3	mixed (shaded and spotted)	white	mixed (shaded and spotted)	purple	overlapping	Free flowering
P_4	mixed (shaded and netted)	pink	uniform	violet red	open	Seasonal
P_5	mixed (shaded and spotted)	purple	mixed (shaded and spotted)	pink	overlapping	Seasonal
P_6	shaded	violet	mixed (shaded and spotted)	violet	open	Seasonal
P_7	uniform	yellow	shaded	orange	open	Seasonal
P_8	uniform	ivory	mixed (shaded and stripped)	reddish orange	touching	Seasonal
P_9	shaded	dark violet	uniform	violet	open	Free flowering
P_{10}	uniform	light yellow	uniform	violet	open	Seasonal

4.1.1.2.2.9. Predominant Colour in Petal

The predominant colour present in the petal showed variation like white, purple, pink, purple, violet, yellow, ivory, dark violet and light yellow.

4.1.1.2.2.10. Colour Pattern of Lip

The colour blending and pattern of the lip or labellum was recorded as uniform for P4, P9 and P10, P2 and P7 showed shaded colour pattern, P1 exhibited edged pattern and others mixed.

4.1.1.2.2.11. Predominant Colour of Lip

The predominant colour of the lip or labellum was recorded as white, yellow, purple, violet red, pink, violet, orange and reddish orange.

4.1.2. Estimation of Variability Components

The genotypic, phenotypic and environmental variances and coefficients of variation at genotypic and phenotypic levels were estimated for the 10 parental *Phalaenopsis* genotypes (Table 6a and 6b).

The vegetative characters such as leaf area, plant height, number of leaves leaf width and length of leaf in the decreasing order exhibited the highest estimates of variance at both genotypic and phenotypic levels.

Among the floral traits, length of inflorescence, number of flowers per inflorescence, vase life, internodal length of the peduncle and days to first flower opening from inflorescence emergence showed highest genotypic and phenotypic variances in the decreasing order.

4.1.3. Estimation of Heritability and Genetic Advance

Estimates of heritability in broad sense and genetic advance (% mean) in the parental genotypes were calculated (Table 6b).

Heritability per cent was categorized as suggested by Allard (1960) as low (<30), moderate (30-70) and high (>70). On that account floral characters like length of inflorescence, flower longevity on the plant, days to first flower opening from

inflorescence emergence, length of flower, width of the flower and internodal length of peduncle exhibited high heritability (>70%). Vegetative characters like plant height, leaf width and leaf area showed high heritability. Moderate heritability was exhibited by the floral characters like vase life and number of flowers per inflorescence.

A wide range of values were observed for the characters under study for genetic advance. According to Robinson *et al.* (1949), characters with values >20% were considered to have high genetic advance. Majority of the characters exhibited high genetic advance. The highest value was observed for days to first flower emergence from inflorescence opening (88.85%) followed by flower longevity on the plant (58.63%).

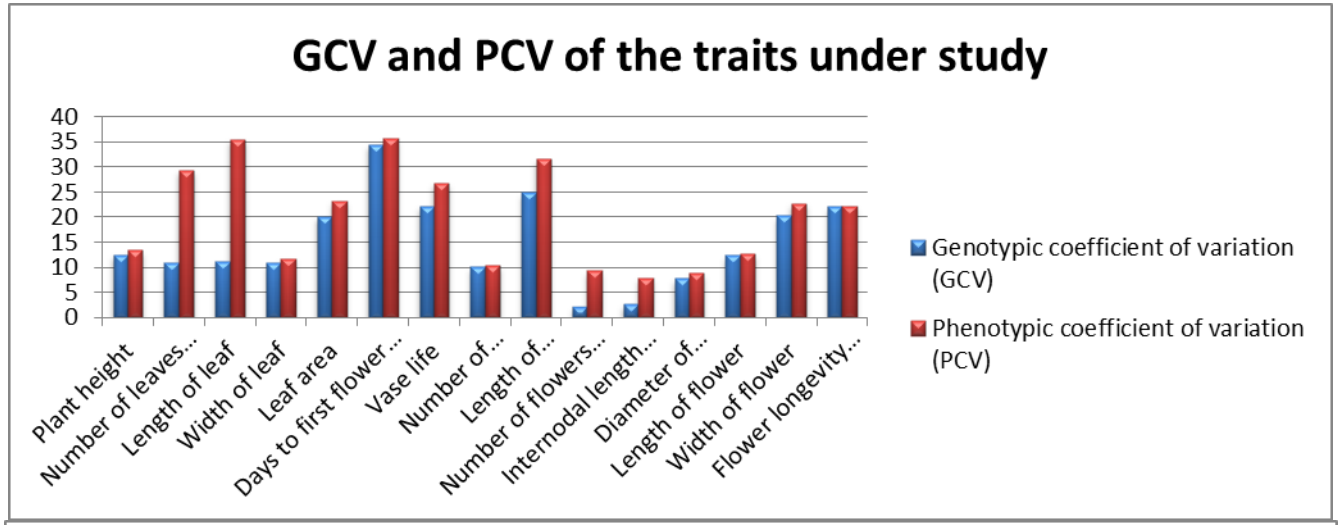
High heritability (>70%) combined with high genetic advance (>20%) was exhibited by majority of the characters studied like days to first flower opening from inflorescence emergence, flower longevity on the plant and vase life . Moderate heritability with high genetic advance was observed for plant height, leaf area, length of flower and width of flower.

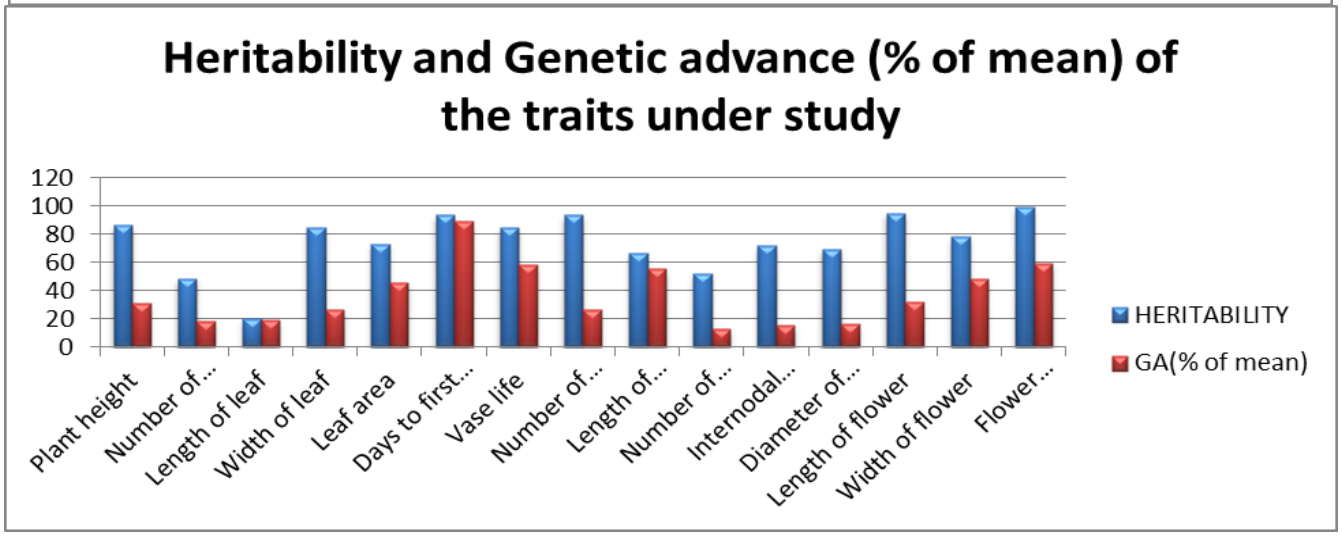
Table 6a. GCV and PCV of the Characters under Study.

Sl. No.	Characters	Genotypic coefficient of variation (GCV)	Phenotypic coefficient of variation (PCV)
1.	Plant height	12.49	13.39
2.	Number of leaves per plant	10.86	29.37
3.	Length of leaf	11.17	35.48
4.	Width of leaf	10.87	11.76
5.	Leaf area	20.15	23.28
6.	Days to first flower opening from inflorescence emergence	34.52	35.61
7.	Vase life	22.17	26.86
8	Number of inflorescence	10.07	10.39
9.	Length of inflorescence	24.89	31.59
10.	Number of flowers per inflorescence	2.12	9.27
11.	Internodal length of peduncle	2.70	7.87
12.	Diameter of peduncle	7.79	8.81
13.	Length of flower	12.36	12.68
14.	Width of flower	20.35	22.79
15.	Flower longevity on the plant	22.09	22.23

Table 6b . Variances, Heritability and Genetic Advance of the Characters under Study.

Sl. No.	Characters	Genotypic variance	Phenotypic variance	Environmental variance	Heritability	Genetic advance (% of mean)
1.	Plant height	37.24	42.86	5.62	86.90	30.87
2.	Number of leaves per plant	0.58	4.23	3.65	13.67	18.20
3.	Length of leaf	0.02	0.17	0.15	19.91	18.86
4.	Width of leaf	0.71	0.83	0.12	85.41	26.49
5.	Leaf area	0.40	0.53	0.13	74.93	45.63
6.	Days to first flower opening from inflorescence emergence	14.61	15.55	0.94	93.94	88.85
7.	Vase life	12.55	18.41	5.86	68.15	57.82
8	Number of inflorescence	81.61	86.89	5.28	93.93	25.93
9.	Length of inflorescence	1.31	2.11	0.80	62.10	55.32
10.	Number of flowers per inflorescence	0.18	3.43	3.25	52.20	12.92
11.	Internodal length of peduncle	0.002	0.02	0.022	71.36	14.99
12.	Diameter of peduncle	0.46	0.59	0.13	78.31	16.18
13.	Length of flower	1.37	1.44	0.07	94.94	32.06
14.	Width of flower	40.30	50.58	10.28	79.69	47.71
15.	Flower longevity on the plant	507.80	513.85	6.05	98.82	58.63


GCV and PCV of the traits under study
40
35
30
25
20
15
10
5
0
Plant height
Number of leaves...
Length of leaf
Width of leaf
Leaf area
Days to first flower...
Vase life
Number of...
Length of...
Number of flowers...
Internodal length...
Diameter of...
Length of flower
Width of flower
Flower longevity...
Genotypic coefficient of variation (GCV)
Phenotypic coefficient of variation (PCV)


Heritability and Genetic advance (% of mean) of the traits under study
120
100
80
60
40
20
0
Plant height
Number of...
Length of leaf
Width of leaf
Leaf area
Days to first...
Vase life
Number of...
Length of...
Number of...
Internodal...
Diameter of...
Length of flower
Width of flower
Flower...
HERITABILITY
GA(% of mean)

4.1.4. Correlation Analysis

Among the different characters studied, eleven characters were selected for the genotypic phenotypic and environmental correlation studies. The eleven characters included were plant height, width of leaf, leaf area, days to first flower opening from inflorescence emergence, vase life, number of inflorescence, length of inflorescence, number of flowers per inflorescence, diameter of peduncle, length of flower and flower longevity on the plant. The significance of the genotypic and phenotypic correlation was tested and presented in the Tables (7a and 7b). Environmental correlation is presented in Table 7c.

High positive correlations at genotypic and phenotypic levels were observed in many vegetative and floral traits. High significant positive correlations were exhibited by length of the flower with number of flowers per inflorescence (r_g= 0.90, r_p= 0.81), length of inflorescence with number of inflorescence (r_g = 0.78, r_p= 0.60), number of flowers per inflorescence with vase life (r_g = 0.71, r_p = 0.67). There was a positive correlation between flower longevity and diameter of the peduncle (r_g = 0.65, r_p = 0.47). Vase life was correlated positively with leaf area (r_g = 0.65, r_p = 0.59). Significant positive correlation was observed in number of inflorescence and plant height (r_g = 0.63, r_p = 0.58) and diameter of peduncle and plant height (r_g = 0.51, r_p = 0.47).

Highly significant negative correlation was observed between days to first flower opening from inflorescence emergence and vase life (r_g = -0.93, r_p= - 0.71), leaf area (r_g = -0.65, r_p = 0.59) and number of flowers per inflorescence (r_g = -0.68, r_p = -0.47).

Significant negative environmental correlation was observed between flower longevity with number of flowers per inflorescence (r_e = -0.86) and vase life (r_e = -0.68). High significant positive environmental correlation was observed between plant height and length of inflorescence (r_e = 0.88) as well as with length of flower (r_e = 0.62). The environmental correlation was found to be low in comparison with the genotypic and phenotypic correlation for most of the character combinations.

Table 7a. Genotypic Correlation of the Selected Traits under Study.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
X1	1.00										
X2	-0.27^{NS}	1.00									
X3	0.17^{NS}	0.49*	1.00								
X4	-0.19^{NS}	0.19^{NS}	-0.65**	1.00							
X5	0.24^{NS}	-0.15^{NS}	0.65**	-0.93**	1.00						
X6	0.63**	-0.21^{NS}	0.40^{NS}	-0.45*	0.40^{NS}	1.00					
X7	0.40^{NS}	-0.31^{NS}	0.02^{NS}	-0.05^{NS}	-0.03^{NS}	0.78**	1.00				
X8	0.40^{NS}	-0.39^{NS}	0.30^{NS}	-0.68**	0.70**	0.40^{NS}	-0.36^{NS}	1.00			
X9	0.51*	-0.04^{NS}	-0.12^{NS}	0.22^{NS}	0.06^{NS}	-0.28^{NS}	-0.46*	0.33^{NS}	1.00		
X10	0.28^{NS}	-0.05^{NS}	-0.15^{NS}	-0.06^{NS}	0.33^{NS}	-0.29^{NS}	-0.46*	0.40^{NS}	0.90**	1.00	
X11	0.43^{NS}	-0.24^{NS}	-0.08^{NS}	0.29^{NS}	-0.08^{NS}	0.11^{NS}	0.29^{NS}	0.15^{NS}	0.65**	0.24^{NS}	1.00

X1-Plant height,X2- Width of leaf, X3-Leaf area, X4- Days to first flower opening from inflorescence emergence, X5-Vase life, X6-Number of inflorescence, X7-Length of inflorescence, X8-Number of flowers per inflorescence, X9-Diameter of peduncle, X10-Length of flower, X11-Flower longevity on the plant.
(** significant at 0.05 and * significant at 0.01 levels).

Table 7b. Phenotypic Correlation of the Selected Traits under Study.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
X1	1.00										
X2	-0.29^{NS}	1.00									
X3	0.13^{NS}	0.43^{NS}	1.00								
X4	-0.11^{NS}	0.12^{NS}	-0.59**	1.00							
X5	0.25^{NS}	-0.17^{NS}	0.59**	-0.71**	1.00						
X6	0.58**	-0.25^{NS}	0.38^{NS}	-0.34^{NS}	0.33^{NS}	1.00					
X7	0.49*	-0.34^{NS}	0.01^{NS}	-0.02^{NS}	0.08^{NS}	0.60**	1.00				
X8	0.38^{NS}	-0.45*	0.27^{NS}	-0.47*	0.67**	0.38^{NS}	-0.14^{NS}	1.00			
X9	0.47*	-0.01^{NS}	-0.11^{NS}	0.16^{NS}	0.15^{NS}	-0.30^{NS}	-0.17^{NS}	0.21^{NS}	1.00		
X10	0.30^{NS}	-0.04^{NS}	-0.15^{NS}	-0.08^{NS}	0.30^{NS}	-0.30^{NS}	-0.28^{NS}	0.31^{NS}	0.81**	1.00	
X11	0.35^{NS}	-0.14^{NS}	-0.08^{NS}	0.20^{NS}	-0.14^{NS}	0.08^{NS}	0.17^{NS}	0.01^{NS}	0.47*	0.23^{NS}	1.00

X1-Plant height,X2- Width of leaf, X3-Leaf area, X4- Days to first flower opening from inflorescence emergence, X5-Vase life, X6-Number of inflorescence, X7-Length of inflorescence, X8-Number of flowers per inflorescence, X9-Diameter of peduncle, X10-Length of flower, X11-Flower longevity on the plant.
(** significant at 0.05 and * significant at 0.01 levels).

Table 7c. Environmental Correlation of the Selected Characters under Study.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
X1	1.00										
X2	-0.42^{NS}	1.00									
X3	-0.50*	-0.28^{NS}	1.00								
X4	0.03^{NS}	-0.19^{NS}	-0.23^{NS}	1.00							
X5	0.32^{NS}	-0.28^{NS}	0.09^{NS}	0.27^{NS}	1.00						
X6	0.16^{NS}	-0.63**	-0.19^{NS}	0.38^{NS}	-0.25^{NS}	1.00					
X7	0.88**	-0.44^{NS}	-0.19^{NS}	0.05^{NS}	0.46*	-0.10^{NS}	1.00				
X8	0.33^{NS}	-0.69**	0.26^{NS}	0.18^{NS}	0.60**	0.34^{NS}	0.36^{NS}	1.00			
X9	0.33^{NS}	0.08^{NS}	-0.07^{NS}	-0.02^{NS}	0.49*	-0.52*	0.45*	-0.08^{NS}	1.00		
X10	0.62**	0.01^{NS}	-0.15^{NS}	-0.21^{NS}	0.07^{NS}	-0.33^{NS}	0.61**	-0.17^{NS}	0.61**	1.00	
X11	-0.34^{NS}	0.67**	-0.14^{NS}	-0.36^{NS}	-0.68**	-0.32^{NS}	-0.40^{NS}	-0.86**	-0.32^{NS}	0.06^{NS}	1.00

X1-Plant height,X2- Width of leaf, X3-Leaf area, X4- Days to first flower opening from inflorescence emergence, X5-Vase life, X6-Number of inflorescence, X7-Length of inflorescence, X8-Number of flowers per inflorescence, X9-Diameter of peduncle, X10-Length of flower, X11-Flower longevity on the plant.
(** significant at 0.05 and * significant at 0.01 levels).

4.2. HYBRIDIZATION AND COMPATIBILITY / INCOMPATIBILITY STUDIES

Intercrossing in all possible combinations involving three best parental genotypes of *Phalaenopsis* selected based on inflorescence characters were done. P8, P10 and P4 were selected for intercrossing. The compatibility / incompatibility relationships between the selected genotypes were thus studied.

4.2.1. Crossings Attempted among the Selected *Phalaenopsis* Genotypes

All the 9 possible combinations were attempted. These 9 combinations included 3 selfs and 6 crosses (Table 8).

Out of the 9 crosses attempted, 4 combinations, P8 x P10, P8 x P4, P10 x P8, P10 x P4 succeeded in producing harvestable green capsules which included only the cross combinations. The total estimate of success was 44.44% and the relative success of crosses was estimated to be 66.66%.

All the 4 combinations inoculated *in vitro*, germinated successfully.

4.2.2. Analysis of Compatibility and Incompatibility

Combinations involving P8 and P10 as female parents produced harvestable capsules. None of the crosses with P4 as female parent produced capsules. Also none of the self-crosses produced any capsules.

Pollination was attempted, but the flowers abscised before the onset of any visible post pollination change in the self-crosses. For the crosses P4 x P8 and P4 x P10, the pollinated flowers with swelling ovaries abscised during the third and fourth weeks. The strength of incompatibility was of the highest degree with P4 as the female parent.

Table 8. Details of the Cross Combinations Attempted.

Cross combinations	Result	Remarks
P8*P8	-	Flowers abscised before any post pollination changes
P8*P10	Capsule developed	-
P8*P4	Capsule developed	-
P10*P10	-	Flowers abscised before any post pollination changes
P10*P8	Capsule developed	-
P10*P4	Capsule developed	-
P4*P4	-	Flowers abscised before any post pollination changes
P4*P8	-	Swelled ovaries withered off after 3-4 weeks
P4*P10	-	Swelled ovaries withered off after 3-4 weeks

4.2.3. Observations

The observations taken on the harvested green capsules from the successful cross combinations are presented in Table 9.

4.2.3.1. Days to Green Capsule Harvest in Successful Crosses

Time taken for the harvest of green capsules in compatible combinations (4/9) was analyzed.

Duration of green capsule harvest ranged from 97.50 days in P8 x P10 to 139.00 days in P10 x P4. The time taken when P10 was used as the female parent was higher than that when P8 was used as the female parent.

4.2.3.2. Length of Capsule (cm)

There is no significant variation in the length of harvested capsules. The highest value was recorded for P10 x P4 (8.10) and the lowest value was for P8 x P10 (7.30).

4.2.3.3. Width of Capsule (cm)

No significant variation was observed among the capsules harvested from the different crosses. The highest value was exhibited by P8 x P4 (3.80) and the lowest value for P10 x P4 (3.15).

4.2.3.4. Percentage of Capsule Set

Number of green capsules harvested to total number of pollinations made was recorded and the percentage was the highest for P10 x P8 (63.00%) followed by P8 x P10 (61.00%). The lowest capsule set was observed in P10 x P4 (50.15%).

4.2.3.5. Percentage of Capsules with Germinating Seeds

Number of capsules with seeds that germinated on inoculation to the total number of capsules harvested was computed. The values ranged from 49.65% in P10 x P4 to 100 % in P8 x P10.

Table 9. Observations on Harvested Green Capsules.

Cross combination	Days to green capsule harvest in successful combination	Length of capsule	Width of capsule	Percentage capsule set (%)	Percentage of capsule with germinating seeds (%)
P8*P10	97.50	7.30	3.25	61.00	100.00
P8*P4	111.50	7.70	3.80	59.35	96.80
P10*P8	132.00	7.95	3.40	63.00	57.75
P10*P4	139.00	8.10	3.15	50.15	49.65
C.D.	14.33	0.43	0.93	8.05	3.24
SE(m)	3.55	0.14	0.15	1.99	0.80

Plate 3: General view of the greenhouse.

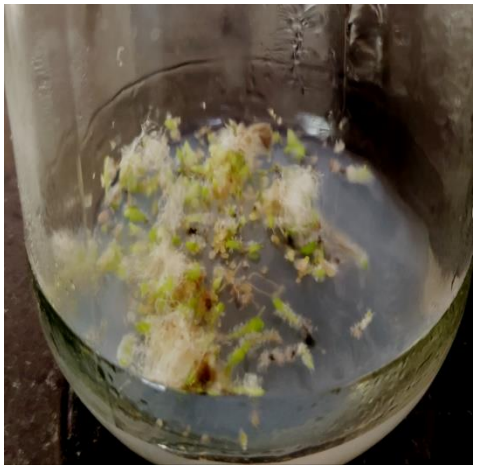
P10 x P8

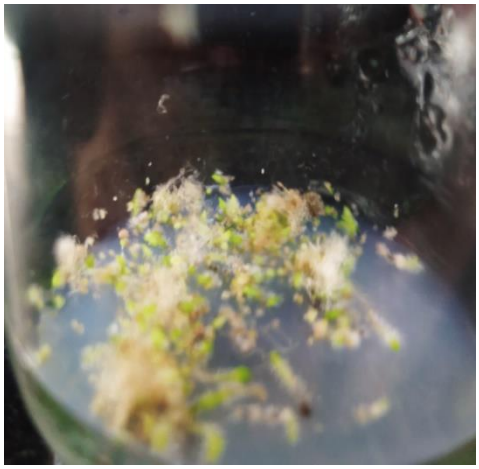
P10 x P4

Plate 4: Green capsules harvested from successful crosses.

P8 x P4 **P8 x P10**

Plate 4: (cont.) Green capsules harvested from successful crosses.





P10 x P8 **P10 x P4**

P8 x P4 **P8 x P10**

Plate 5: Germination of seeds *in vitro* obtained from the successful cross combinations.

Discussion

5. DISCUSSION

Orchids are a spectacular group of plants which possess long lasting charm, diverse flower shapes, colours and sizes and have been admired for their horticultural and therapeutic properties in the national and international markets. They are famous around the globe and represents royalty and aristocracy.

The *Phalaenopsis* or the 'moth orchids' represent one of the most astonishing, unique and commercially important group of orchids. The cultivation of *Phalaenopsis* is gaining popularity due to the ease in cultural practices, diverse flower colour, shape, size and delicacy (De *et al*., 2019). The genus *Phalaenopsis* consists of about 60 species and over 40,000 man-made hybrids. Nowadays the creation of novel orchid hybrids for quality cut flowers has become a business rather than a hobby to earn riches in the international market.

The present research programme was conducted for developing newer hybrids in *Phalaenopsis* through hybridization. The study resulted in obtaining 4 hybrid combinations which germinated successfully in *in vitro* conditions and requires further study to fully develop the hybrid plantlets. Salient findings of the research are discussed below.

5.1. COMPARISON BETWEEN PARENTS BASED ON VEGETATIVE CHARACTERS

A detailed study regarding the vegetative and floral characters is significant for understanding the diversity found in the genus *Phalaenopsis* and for selecting the parents for a successful hybridization programme. McDonald (1991) stated that vigorous hybrids result in bigger, better blooms and more floriferous nature with greater flower substance thus emphasizing the importance of vegetative vigour. General health and superior vegetative characters are important while selecting parents for a hybridization programme. Hence a brief comparative analysis of the vegetative traits of the genotypes utilized in the present study is presented below.

The ten *Phalaenopsis* genotypes used in the present study were evaluated with respect to vegetative characters prior to hybridization. A wide range of variation for

vegetative characters was observed among the genotypes. The range was significant for most of the vegetative characters like plant height (41.00 to 57.45 cm) and leaf area (64.4 to 140.6 cm^2). The wide range of variations may be because of the fact that the genotypes employed in the study are higher order monogeneric, bigeneric or multigeneric hybrids. This was in conformity with the findings of Rahi (2017). High phenotypic and genotypic coefficients of variation were observed for leaf area and indicating high variability for these characters which is similar in several monopodial orchid genera (Thomas and Lekha Rani , 2017).

Hurst (1898) had reported that in orchids higher order multigeneric hybrids showed a wider range of character variation as compared to lower order primary hybrids. Similarly, McConnel and Kamemoto (1983) found that even reciprocal crossings in multigeneric hybrids yielded offsprings differing in vegetative traits and flower yield, pointing out to their highly heterozygous nature. This wide range of variations is a result of their diverse genetic makeup. A wide range of variants will be produced when these genotypes are hybridized.

The characters like number of leaves per plant, length of leaf and width of leaf was recorded to have lesser variations among the genotypes. This may be due to the fact that most of the parental genotypes used in the present study are elite hybrids and have standardized vegetative traits but have improved floral traits as an ornamental according to the market preferences.

5.2. COMPARISON BETWEEN PARENTS BASED ON FLORAL CHARACTERS

A clear understanding on the floral biology is of great importance in orchid breeding due to its structural and functional complications. The important floral characters are length of inflorescence, number of flowers per inflorescence, length of flower, diameter of the peduncle and flower longevity in the plant. These characters studied exhibited a significant variation in quantitative floral characters which was in conformity with the reports of De *et al*. (2019).

Lekha Rani (2002) reported that the length of inflorescence and its rate of growth decide the days to first flower opening from inflorescence emergence. There

was a positive correlation between the number of flowers per inflorescence and days to first flower opening from inflorescence emergence. A similar trend was observed by Ninitha (2003).

McDonald (1991) pointed out that length of inflorescence is a trait of prime importance in orchid breeding. Length of internode must be optimum to prevent overcrowding of flowers and for the proper display of flowers.

A majority of the genotypes used in the present study also exhibited increased number of flowers. Number of flowers per inflorescence is a trait of utmost importance in orchid hybridization, as had been detailed by Kamemoto (1983), McConnel and Kamemoto (1983), Singh (1986) and McDonald (1991). Singh (1982) pointed out that higher order orchids showed increased number of flowers per inflorescence. High genotypic and phenotypic coefficients of variation were observed among the genotypes studied for number of flowers per inflorescence and vase life which was found in conformity with the results of Thomas (2008) in monopodial orchids.

Tippit (1997) suggested that the number of flowers per inflorescence in a hybrid was the geometric mean of the two genetically dissimilar parents involved in the cross combination. Length of the flower also showed a significant variation as well as high genotypic and phenotypic coefficients of variation among the genotypes used in the study and this was in conformity with the findings of Rahi (2017) and De *et al*. (2019).

Most of the characters exhibited high heritability and genetic advance in the present study which was in conformity with the reports in several monopodial orchids (Thomas and Lekha Rani, 2017).

Flowering in orchids are either seasonal or free flowering (throughout the year). Ninitha (2003) concluded that out of twelve monopodial orchids under study, five were of free flowering nature and the rest were seasonal. Similarly in the present study, two of the genotypes exhibited free flowering nature and eight genotypes

exhibited seasonal flowering. Rahi (2017) also observed free flowering and seasonal flowering among *Phalaenopsis* genotypes.

From the preliminary analysis, it was evident that a wide range of variation exists among the ten genotypes of *Phalaenopsis* studied, for most of the floral characters evaluated and the best among them was selected as parents in hybridization programme.

5.3. COMPATIBILITY ANALYSIS

Even though intermingling of the genomes is a characteristic feature of Orchidaceae, several cases of incompatibility have been reported, especially in monopodials. There are mainly two types of incompatibility systems in orchids as detailed below (Leonhardt, 1977).

a) Exogenous barriers which include geographical isolation, pollinator specificity and seasonal flowering habit.

b) Endogenous barriers which are genic or chromosomal in nature.

Exogenous barriers can be repressed easily by hybridization under controlled conditions but endogenous barriers can be permanent in nature. The inability of the pollen to germinate on the stigmatic surface or the inability of the pollen tube to grow and reach the ovules may be associated with endogenous barriers of genic origin. In a comparatively milder form of incompatibility, fertilization is prevented and it occurs in the ovary. All these forms of incompatibility reactions are manifested externally as early flower drop following pollination.

In the present study, pollinated flowers abscised without any visible changes in 3 combinations. After initial swelling and ovary development, the pods withered off after 3-4 weeks in the case of 2 cross combinations. This ovary drop can be attributed to the genic incompatibility described above. Only 4 combinations produced harvestable capsules out of the 9 cross combinations attempted.

Ninitha (2003) also suggested that high ovary drop after initial swelling following pollination may be due to genic incompatibility. Devi and Deka (1994), Varghese (1995), Sobhana (2000) and Lekha Rani (2002) have also reported high initial ovary drop in orchids indicating genic incompatibility.

Leonhardt (1977) in *Cymbidium* reported that a total of 2466 pollinations were made with 265 (10.75 %) fruits harvested of which 182 (68.68 %) contained an average of 31.30 per cent seeds with apparently viable embryos. Of these, seeds from 142 fruits (53.58 %) germinated, producing seedlings.

In the present study, 4 out of the 9 combinations attempted produced harvestable green capsules (77%) which germinated *in vitro*. Thomas (2008) reported that, out of the 225 combinations attempted in monopodial orchids, 70 (31.11 %) succeeded in producing harvestable green capsules. Out of these, 55 (24.44 %) combinations which contained filled seeds were inoculated *in vitro.* Seeds from 43 (19.11%) combinations germinated *in vitro.* Similar results were observed by Ninitha (2003) in monopodials and by Rahi (2017) in *Phalaenopsis* and in another study in *Phalaenopsis* by Cordea *et al*. (2019).

In the present study, P4 did not produce any capsules when used as a female parent but was comparatively a good male combiner. Here the strongest incompatibility was observed in P4 as pollinated flowers in the combinations attempted, abscised without any changes or 3-4 weeks after pollination. The extent of incompatibility reaction denotes the number of cross combinations in which the incompatibility reaction is presented when a particular parent is used, at any stage. It can be concluded that, the more the number of crosses exhibiting incompatibility reaction, the greater the extent of incompatibility.

Summary

6. SUMMARY

The research programme entitled 'Varietal evaluation and development of hybrids in *Phalaenopsis* orchids' was undertaken in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 2018-2020. The objective of the study was to assess the genetic variability of *Phalaenopsis* orchids and to improve them through intervarietal hybridization.

- Ten genotypes of *Phalaenopsis* having good market value and cut flower quality were evaluated in the green house using completely randomized design with 2 replications.

- Observations were recorded for vegetative characters and floral characters from the parental genotypes.

- Analysis of variance gave out significant differences among the parental genotypes with respect to the majority 15 (five vegetative + ten floral) biometric characters studied.

- The vegetative characters such as leaf area, plant height, number of leaves and leaf width exhibited the highest estimates of variance at both genotypic and phenotypic levels. Among the floral traits, length of inflorescence, number of flowers per inflorescence, vase life, internodal length of the peduncle and days to first flower opening from inflorescence emergence showed highest genotypic and phenotypic variances.

- On that account floral characters like length of inflorescence, flower longevity on the plant, days to first flower opening from inflorescence emergence, length of flower, width of the flower and internodal length of peduncle exhibited high heritability (>70%). Vegetative characters like plant height, leaf width and leaf area showed high heritability. Moderate heritability was exhibited by the floral characters like vase life and number of flowers per inflorescence.

- Genetic advance (% mean) was studied for all the characters. Majority of the characters exhibited high genetic advance (> 20%). The highest value was

observed for days to first flower opening from inflorescence emergence followed by flower longevity in the plant.

- High heritability (>70%) combined with high genetic advance (>20%) was exhibited by majority of the characters studied like days to first flower opening from inflorescence emergence, flower longevity on the plant and vase life . Moderate heritability with high genetic advance was observed for plant height, leaf area, length of flower and width of flower.

- The genotypic, phenotypic and environmental correlations of the ten parental genotypes were studied for eleven biometric characters. High positive correlation at genotypic and phenotypic levels was observed between most of the vegetative and floral characters studied.

- High significant positive correlations were exhibited by length of the flower with number of flowers per inflorescence, length of inflorescence with number of inflorescence and number of flowers per inflorescence with vase life. There was a positive correlation between flower longevity and diameter of the peduncle. Vase life was correlated positively with leaf area. Significant positive correlation was observed in number of inflorescence and plant height and diameter of peduncle and plant height.

- Highly significant negative correlation was observed between days to first flower opening from inflorescence emergence and vase life, leaf area and number of flowers per inflorescence.

- Significant negative environmental correlation was observed between flower longevity with number of flowers per inflorescence and vase life. High significant positive environmental correlation was observed between plant height and length of inflorescence as well as with length of flower. But these values were found to be low in comparison with the genotypic and phenotypic correlation for most of the character combinations.

- Qualitative characters for the ten parental genotypes were also analysed with respect to the leaf shape, leaf pigmentation, inflorescence type, flower colour, flower texture, flower fragrance, arrangement of petals, branching of inflorescence, season of flowering, and colour pattern of petal, predominant colour in petal, colour pattern of lip and predominant colour of lip.
- P3 and P9 exhibited free flowering nature whereas all the other genotypes showed seasonal flowering.
- Intercrossing in all possible combinations involving three best parental genotypes of *Phalaenopsis* selected based on inflorescence characters were done. P8, P10 and P4 were selected for intercrossing.
- All the 9 possible combinations were attempted. These 9 combinations included 3 selfs and 3 crosses and 3 reciprocals.
- Out of the 9 crosses attempted, 4 combinations, P8 x P10, P8 x P4, P10 x P8, and P10 x P4 succeeded in producing harvestable green capsules.
- The total estimate of success was 44.44% and the relative success of crosses was estimated to be 66.66%.
- Combinations involving P8 and P10 as female parents produced harvestable capsules. None of the crosses with P4 as female parent produced capsules.
- None of the self-crosses produced any capsules.
- The flowers abscised before the onset of any visible post pollination change in self-crosses. For the crosses P4 x P8 and P4 x P10, the pollinated flowers with swelling ovaries abscised during the third and fourth weeks. The strength of incompatibility was of the highest degree with P4 as the female parent.
- The percentage of capsule set was the highest for P10 x P8 followed by P8 x P10. The lowest capsule set was observed in P10 x P4.
- The percentage of capsules with germinating seeds was analyzed and the values ranged from 49.65% in P10 x P4 to 100 % in P8 x P10.
- Germination *in vitro* was observed for the 4 combinations from which the green capsules were successfully harvested.

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Appendix

Appendix 1. Media composition for half strength Modified Murashige and Skoog medium

Chemical composition	Quantity in mgl^{-1}
KNO_3	950.00
$NH_4\ NO_3$	200.00
$MgSO_4.7H_2O$	185.00
KH_2PO_4	85.00
$CaCl_2.2H_2O$	220.00
$FeSO_4.7H_2O$	13.90
Na_2 EDTA.$2H_2O$	18.65
$MnSO_4.4H_2O$	11.15
$ZnSO_4.7H_2O$	4.30
H_3BO_3	3.10
KI	0.415
$Na_2MoO_4.2H_2O$	0.125
$CuSO_4.5H_2O$	0.0125
$CoCl_2.6H_2O$	0.0125
Nicotinic acid	0.25
Pyridoxine HCl	0.25
Thiamine HCl	0.05
Glycine	1.00
Sucrose	30000.00

VARIETAL EVALUATION AND DEVELOPMENT OF HYBRIDS IN *PHALAENOPSIS* ORCHIDS

by

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ABSTRACT

The research programme entitled "Varietal evaluation and development of hybrids in *Phalaenopsis* orchids" was undertaken in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 2018-2020. The objective of the study was to assess the genetic variability of *Phalaenopsis* orchids and to improve them through intervarietal hybridization.

The first experiment was varietal evaluation wherein ten genotypes of *Phalaenopsis*, King Car Purple Queen, Lianher Happy Song, Fullers Rabbit, Lianher Orange, Fullers, Taisuco Fire Bird x King Hisang Rose, Reyoung Gold, Jiuhbao Venus, Wang Lin Rose and Young Home Golden having good market value and cut flower qualities were evaluated in the green house using completely randomized design. The analysis of variance revealed that significant difference exists among the parental genotypes with respect to the majority of biometric characters studied. Vegetative characters such as leaf area, plant height, number of leaves and leaf width exhibited the highest estimates of variance at both genotypic and phenotypic levels. Among the floral traits, length of inflorescence, number of flowers per inflorescence, vase life, internodal length of the peduncle and days to first flower opening from inflorescence emergence showed the highest genotypic and phenotypic variances.

Floral characters like length of inflorescence, flower longevity on the plant, days to first flower opening from inflorescence emergence, length of flower, width of the flower and internodal length of peduncle as well as vegetative characters like plant height, leaf width and leaf area showed high heritability. Many of the characters exhibited high genetic advance (> 20%); the highest value was observed for length of the inflorescence followed by leaf area. High heritability (>70%) combined with high genetic advance (>20%) was exhibited by majority of the characters under study like days to first flower opening from inflorescence emergence, flower longevity on the plant and vase life.

The genotypic, phenotypic and environmental correlations of the ten parental genotypes were studied for eleven biometric characters. High positive correlation at

genotypic and phenotypic levels was observed between most of the vegetative and floral characters studied. Highly significant positive correlations were exhibited by length of the flower with number of flowers per inflorescence, length of inflorescence with number of inflorescence and number of flowers per inflorescence with vase life.

The second experiment was hybridization and compatibility analysis. Intercrossing in all possible combinations involving the three best parental genotypes of *Phalaenopsis* selected based on inflorescence characters was done. Jiuhbao Venus, Young Home Golden and Lianher Orange were selected for intercrossing. All nine possible combinations including three selfs, three crosses and three reciprocals were attempted.

Out of the nine crosses, four combinations succeeded in producing harvestable green capsules. They are Jiuhbao Venus x Young Home Golden, Jiuhbao Venus x Lianher Orange, Young Home Golden x Jiuhbao Venus and Young Home Golden x Lianher Orange. The highest strength of incompatibility was exhibited by Lianher Orange. Germination *in vitro* was observed for the four combinations from which the green capsules were successfully harvested. These hybrid combinations can be utilized for further crop improvement programmes.