REGULATION OF FLOWERING IN *PHALAENOPSIS* ORCHIDS

By

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THESIS

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DECLARATION

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

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Certified that this thesis, entitled **"Regulation of flowering in *Phalaenopsis* orchids"** is a record of research work done independently by **Ms. Kaveriamma M.M.** 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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Introduction

1. INTRODUCTION

Orchids are the most pampered of plants and are undoubtedly the ornamental elite with their complex flowers and exquisite beauty. They are cosmopolitan, occurring in almost every habitat apart from the glaciers probably. The majority of cultivated orchids is native to the tropical countries and occurs in their greatest diversity in the humid tropical forests. The family Orchidaceae, is the largest in the plant kingdom with about 600-800 genera, over 25,000 species and more than a lakh and a half man-made hybrids. Despite their diversity, very few genera *viz*., *Dendrobium, Cattleya, Phalaenopsis, Cymbidium, Aranda, Vanda, Mokara, Aranthera, Oncidium* and a few others are commercially important. Because of their elegant, long lasting and enchanting floral variations, orchid cut flowers and pot plants fetch a high price. In the past couple of decades, orchids have occupied a coveted position in the international flower market, evolving into a multibillion dollar business. With the recent increase in the world floriculture trade, orchids have become the second most popular plants as cut flowers as well as pot plants with an annual growth rate of 10–20 per cent (Hossain, 2010).

In the West, for a layperson the name orchid is synonymous with phalaenopsis which is commonly referred to as 'phals'. The genus *Phalaenopsis* has around 80 species and over 40,000 man-made hybrids, i.e. over 25 per cent of orchid hybrids are contributed by this genus alone. The major countries growing phalaenopsis are Taiwan, Netherlands, USA, Japan, Germany and Thailand. In India, phalaenopsis cultivation is in infancy, confined to a few hobby growers in coastal Kerala and Karnataka. There exists a huge potential for commercial growing of phalaenopsis in India with the rise of an urban class with a higher disposable income.

Phalaenopsis 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. Phalaenopsis and its related hybrids are the most preferred among pot orchids worldwide and its cultivation is truly an international business. The mother plants are sourced from South East Asia, sphagnum moss which is used as media is from Chile, tissue culture and hardening is done in Holland, plants are grown in Taiwan and finally sold in U.K. and in the USA. An estimated 85–90 per cent of the potted orchids sold in the United States are *Phalaenopsis* and related genera (Nash, 2003). In 2009, 19.5 million potted orchids with a total wholesale value of $160 million were sold and orchids have become the most valued flowering potted crop in the United States (USDA, 2010). Taiwan, home to the orchid island (named for its abundance of phalaenopsis in the wild) produced more than 70 million pots of phalaenopsis and exported orchids valued at nearly $87 million in 2009. In the same year, the number of pot plants of phalaenopsis sold was 100 million to Europe, 20 million to Japan and 10 million to China (Yuan, 2011).

Constraints in phalaenopsis growing are many. The plants are expensive and need high investment though profits are manifold. They are slow growing plants, do not flower freely and are very much dependent on weather conditions. They need high humidity of above 50-60 per cent and perform well at a temperature range between 15°C and 35°C. Under humid tropics of Kerala, major setbacks in phalaenopsis growing are the slow growth of plants and the inability to induce blooms as per demand. Research on this aspect is limited. Hence it has become imperative to arrive at a package of practice suitable for phalaenopsis growing in Kerala. The experiments were designed with the following objectives.

1. To evaluate cut flower and pot plant varieties of *Phalaenopsis* under two microclimatic conditions.
2. To observe their flowering behaviour with respect to the weather elements.
3. To study the effect of cultural practices on flowering and floral characters.

Review of Literature

2. REVIEW OF LITERATURE

Orchids are one of the most beautiful and bewitching of all plants. They are often referred to as 'royals' in the plant kingdom. The family orchidaceae, represents a peak in the evolution of monocot plants. This is evident from the various adaptation mechanisms followed by the flower to attract pollinators. They have a cosmopolitan distribution and are found growing throughout the earth's surface except in snow covered areas and in the driest of deserts.

The family Orchidaceae is the largest among flowering plants in terms of species diversity with 600-800 genera, comprising of 25,000-30,000 species. In India, about 1300 species are distributed all over the north-eastern Himalayas (600 species), north-western Himalayas (300 species), Maharashtra (130 species), Andaman and Nicobar islands (70 species) and Western Ghats (200 species) (Rajeevan, 2007). Today there are over a lakh and a half man made hybrids.

Based on the nature of their growth, orchids can be classified as monopodials, sympodials and pseudomonopodials. Monopodials are characterised with stems, which lengthen indefinitely season after season and bear aerial roots almost to the top, though sometimes the roots are just at the base. The leaves may be flat, terete or intermediate. The inflorescence is lateral and produced at the leaf axils. A vast majority of orchids are characterised by the sympodial mode of growth, which includes all terrestrial orchids, both tropical and temperate. Growth of the stem eventually ceases, usually at the end of the growing season, and lateral shoots are produced in the following season. The inflorescence may either be lateral or terminal. Plants of this group produce pseudobulbs, which are swollen stems which store water and food. Pseudomonopodials are intermediate in nature.

Associated with the enormous number of Orchidaceae species is extraordinary floral diversification. So much variation is found in orchids that they can unquestionably be regarded as the most highly ecologically adapted flowering plants (Mehra and Vij, 1974). The extreme degree of morphological variability in orchids is attributed to genetic drift (Brieger *et al.,* 1975) and the final speciation in

these depends on mutation, cross over rates, exchange of gene pools, environmental diversities and pressures, and reproductive isolation of new adaptive forms (Sanford, 1974). The uniqueness of the Orchidaceae is reflected in its huge diversity (both floral and vegetative) coupled with peculiar pollination contrivances and wide natural hybridization. It is believed to be in an active state of evolutionary flux. Orchids can be epiphytes, terrestrial or saprophytes. Tropical species comprise 90–95 per cent of all species of the family Orchidaceae and 70–75 per cent of them are epiphytes (Tsavkelova *et al*., 2003).

Phalaenopsis orchid

Phalaenopsis, commonly called the moth orchids, is derived from the greek words 'Phalaena' and 'opsis' meaning 'resembling moth'. The inflorescence swaying in breeze resembles moths in flight and hence the name. In commerce, the flowers are referred to as 'Phals'. It is arguably the most important commercial genus of orchids. Species and hybrids in this genus are of high value in floriculture because of their beautiful and long-lasting flowers.

The genus *Phalaenopsis* comprises 60 to 80 species and has a wide geographic distribution. They are native to the jungles of tropical and sub-tropical Asia; extending from the eastern Himalayas through Taiwan and the Indonesian archipelago up to the South Pacific islands. The plants are mostly found in the humid tropical forests. They are epiphytes growing under the canopy of trees with bright filtered sunlight. They consist of a few leathery, obovate, deep green leaves, though some species have mottled and tessellated foliage. In the wild, the roots anchor to the tree trunk for support and derive nutrition from decaying bark, bird or insect droppings.

Phals are short stemmed monopodial orchids i.e. they exhibit indeterminate growth without lateral branching. Plants are slow growing and mature plants attain an average height of twelve to fifteen centimeters, although a few individuals may grow taller (Sahavacharin, 1981).

The plants in their native habitat are found growing on the trunks of trees where moisture stress is not uncommon during most parts of the year. The plants have no water storage organs like pseudobulb unlike in sympodial orchids. Instead, the leaves are thick and succulent and the aerial roots are fleshy, thus aiding in moisture conservation

The leaves of phalaenopsis exhibit CAM pathway of photosynthesis. The stomata have a mechanism of CO_2 absorption with a reduced loss of water due to transpiration. Stomata remain open during night and partly open during day break and at dusk. Throughout the day, when the temperature is high, the stomata remains closed so as to conserve moisture within the plant. *Phalaenopsis* plants have three kinds of roots *viz*., aerial, prostrate epiphytic and substrate roots. Aerial roots of epiphytes are covered with velamen, the hygroscopic tissue consisting of large, dead air-filled cells. It absorbs and retains water with dissolved mineral salts and nutrients; provides mechanical protection, reflects solar radiation, and is permeable to oxygen and carbon dioxide. Root tips have chlorophyll, thus contributing to photosynthesis as well. The prostrate epiphytic root tips may be either green or purple and, like leaf pigmentation, they appear to be governed by a simple, probably single-allele inheritance pattern. Substrate roots usually lack pigments at the root tips (Christenson, 2001).

Inflorescence of phalaenopsis is a long, arching raceme, with an indeterminate nature of growth. The inflorescence usually emerges from the 3rd or 4th node. It stays on plant for 2 to 4 months (Bose *et al*, 1999). But at times it stays for as long as 8 months, or more (Kaveriamma, 2007). But the inflorescence becomes unruly if left for longer periods on plant. The number of flowers per plant can vary from a few up to 30. Flowers are zygomorphic (bilaterally symmetric); a characteristic feature of the family orchidaceae. The three sepals are similar is shape, size and colour. Petals are three in number; two are similar and the third petal is modified into labellum or a lip. Labellum is trilobed with two lateral lobes which are similar; mid-lobe is triangular with two tendril like structures and a bifid callus. Labellum serves as a perfect landing pad for pollinators. The column like structure

'gynostemium' is positioned above the labellum. Gynostemium is a fusion of androecium and gynoecium, separated by a wall like structure called rostellum. It separates the male and female organs restricting self pollination.

For many orchids, 4–7 years are needed to complete the juvenile stage and to flower from the seed (Goh and Arditti, 1985). However, certain commercial hybrids can flower within 36 months from seeding (Hew and Yong, 1997). *P. pusilla*, a tropical epiphytic species, is especially noteworthy for flowering studies in orchids because of its rapid growth and relatively shorter time required to reach maturity.

In some species, *P. equestris, P. intermedia* and *P. schilleriana*, plantlets develop spontaneously at the tip of inflorescence after flowering. In other species (*P. lueddemannia*), plantlets form on nodes of the inflorescences. In the case of *P. stuartiana* and some other hybrids, plantlets may form on the roots.
All *Phalaenopsis* species have the same chromosome number (2n=2x = 38), but their genomes vary considerably in size (Arends, 1970).

Phalaenopsis orchids are classified into subgenus *Phalenopsis*, *Polychilos*, *Parishinae* and *Aphyllae*. The challenge in growing phalaenopsis depends on identifying the plants, determining their habitat requirements and providing the necessary growing conditions. The species native to warm and humid habitats from the subgenus *Phalaenopsis* are easiest to grow and most commonly found in cultivation. They include *Phalaenopsis amabilis, P. aphrodite, P. philippinensis, P. sanderiana, P. schilleriana, P. stuartiana, P. equestris, P. lindenii* and *P. pulcherrima.* These plants form the basis for the majority of hybrids available today and are a boon to growers, as the hybrids adapt better and flower easily. The second group of species also found in cultivation is from the subgenus *Polychilos* and include *P. amboinensis, P. gigantean, P. luddemanniana, P. bellina, P. venosa* and *P. violaceae*. These plants are also native to warm and humid jungles. The flowers of these species are brightly coloured and have a waxy texture. The plants in the subgenus *Parishinae* and *Aphyllae* are difficult to grow and include *P. gibbosa, P.*

lobbii, P. parishii and *P. wilsonii*. These plants grow at higher elevations in seasonally cool and dry areas and are a challenge to cultivate (Fitch, 2004).*Phalaenopsis* species are used in hybridization programme and are available in a variety of colours, shapes and patterns. But species are not an option for commercial production because of their smaller sized flowers and for its inability to flower more than once a year. Distant hybridisation offers immense possible combination of traits like flower colour, pattern, shape of labellum, tepals etc. These hybrids adapt better to the newer environments, in addition to its ability to bloom throughout the year. Hybrids are resultants of intensive cross fertilization of distant and distinct species. They are more adaptable to wider environment variations than their botanical ancestors. Thus hybrids are commercially preferred for cultivation. Commercially phalaenopsis are categorised as grandiflora or cut flower types with large flowers and multiflora types or pot plant types with smaller sized flowers.

Factors affecting growth and flowering

The interaction of genotype, plant health and environment are critical in phalaenopsis growing. Genotype decides the expression of flower quality to a greater extent. The environmental factors, such as day temperature, night temperature, relative humidity, light period and intensity must be kept at an optimum range. The influence of temperature for blooming is around 60 per cent, light is at 30 per cent and other factors at 10 per cent. This ratio may be different for the setting values of greenhouse environment, different management techniques and various varieties (Chen, 2002). Typically a mature Phal growing in a house establishes a rhythm of blooming, producing a new leaf and after a leaf, a new flower spike. This takes about 10 to 14 months (Rogers, 2012).

Flower bud formation is one of the most important physiological processes for higher plants, and the flowering time is influenced by photoperiod, vernalization, drought stress, and so on. Chailakhyan (1936) demonstrated that flowering was regulated by the bioactive substances, which were produced in leaves that were subjected to favourable photoperiods, and the substances were transported to the

shoot apex to induce flower bud formation. He named the substances “flower-inducing hormone” or “florigen” (Hisamatsu *et al.,* 2006). In long day plants, *Sinapsis alba* and *Arabidopsis thaliana*, the C: N ratio of the phloem sap increased markedly and early during the inductive treatment, suggesting that an inequality in organic C and N supply to the apical meristem may be important for floral transition (Corbesier *et al*., 2002). Based on the study of orchid flowering regulation, various predominating pathways prevail for different species of phalaenopsis orchids. For example, autonomous factors, temperature (cool or ambient temperature) or hormone (cytokinin) pathways may play an important role for *P. aphrodite* subsp. *formosana* (Hsiao *et al*., 2011). The mature stage is different for each phalaenopsis variety. Some varieties can be induced to spike after a culture period of six or eight months post deflasking. Some varieties require fourteen months to reach mature stage.

Environmental signals, in particular, temperature and photoperiod, affect flower initiation and development in many species. The requirement for exposure to a particular photoperiod in order to affect flowering can be profoundly modified by temperature and vice versa (Vaz *et al*., 2004).

The principal environmental conditions that are known to affect flowering time are irradiance, light quality, day length, low temperature giving a vernalization effect, and ambient growth temperature. Also plant age and size are important and many plants flower once they reach a certain size/age regardless of the environmental stimuli (Bernier and Perilleux, 2005). Day length and temperature are the most important of environmental signals, providing seasonal cues that enable varieties of a species to become adapted to life at particular latitudes or altitudes (Giakountis and Coupland, 2008).

2.1 Performance evaluation

Lokesha and Vasudeva (1994) evaluated 746 Indian orchids and reported that those with large, showy flowers were the most vulnerable to commercial

exploitation and the most likely to be the endangered species. In rain-shelter greenhouse at KAU, Vellanikkara forty monopodial orchids were evaluated, of which eight were *Phalaenopsis*. Flowering season in *Phalaenopsis* was from July to February (Kaveriamma, 2007). Fifty *Dendrobium* varieties were evaluated and their vegetative and floral characters were recorded in rain-shelter greenhouse in Vellanikkara. In Barapani centre, 22 species of temperate orchids were evaluated for vegetative and flowering characters under 75 per cent shade net. Performance of nineteen different *Cymbidium* species and ten hybrids were evaluated and *Cymbidium* – Golden Girl, Red Star, Kennedy Wine, December Gold were reported best for cut flower production (AICFIP, 2011).

2.2 Induction of flowering

Flower forcing is an operation to induce flowering with a goal to produce flowers during the off-season or a specified date, for sale at higher prices than during normal blooming season. It has been hypothesised that, after reaching a ripeness-to-respond stage, and upon receiving proper stimuli (temperature and photoperiod), flowering hormones, known hypothetically as vernalin and florigen, are produced which stimulate the initiation of flower primordium (Chomchow, 2004).

The transition to reproductive growth is marked by formation of the inflorescence meristem (IM), which produces an elongated stem punctuated by narrow cauline leaves, secondary inflorescences, and flowers. Floral meristems are formed in a spiral pattern on the periphery of the IM and generate four types of floral organs in a characteristic whorled pattern: sepals, petals, stamens, and carpels (Hill and Lord, 1989). The Unusual Floral Organs (UFO) gene of *Arabidopsis* encodes an F-box protein required for the determination of floral-organ and floral-meristem identity (Hepworth *et al*., 2005). Axillary shoot apical meristems are established in the axils of the leaves produced by the primary shoot apical meristem and after forming only a few leaves, they can enter a dormant state. These dormant buds may be reactivated by endogenous or environmental signals, contributing to the enormous diversity of plant architectures observed in nature (Leyser, 2009).

For tropical regions, photoperiod and temperature do not vary drastically throughout the year, but certain species nevertheless are sensitive in their response to these slight changes. After several weeks of exposure to cool temperature, one of the latent axillary buds grows to a inflorescence. The start of flower induction is observed when the inflorescence length reaches about 1–4 cm (Nishimura , 1982). Exposure to a cold soak is necessary for flowering of many plants, including grasses, some fruits and ornamentals (Gordon, 1989).

The increase in the C: N ratio helps plants shift from vegetative to reproductive stage. The technique is to increase the temperature and light intensity in the greenhouse, decrease the N concentration and increase the P and other components (Chen, 2002). The pretreatment for spiking could be executed one month before cooling. However, inflorescence induction followed by temperature control is a reliable way to induce and control flowering time (Wu and Chang, 2011).

Reproductive development in the flowering shoot of phalaenopsis orchids begins with the transition of the dormant meristem from producing vegetative structures to producing inflorescence branches, floral bracts and finally flowers. Phalaenopsis orchids develop at least two undifferentiated bud primordia at each node, and then these buds will partially develop and become dormant and wait for proper conditions for flowering (Rotor, 1959). Usually, the inflorescence emerges from the fourth node below the apical leaf (Sakanishi *et al.*, 1980). When the weather becomes appropriate, the upper bud elongates and emerges through the epidermis of the stem and develops into an inflorescence (Wang, 1995).

2.2.1 Temperature

Phalaenopsis orchids have their origin in the tropical and subtropical areas of the South pacific islands and Asia, and thus have unique temperature and light requirements. In their native habitats, tropical conditions persist throughout the year with temperatures ranging from 82ºF to 95ºF (28ºC to 35ºC) during the day and 68 ºF to 75ºF (20ºC to 24ºC) at night.

Most of the commercial hybrids grow better and their leaves expand well at high temperatures of about 30°C (Kano, 2001). For the large flowered hybrids and clones of phalaenopsis, planting material generally does not flower uniformly until plants have an average leaf span of 25 cm or greater. Therefore plants are grown at a higher temperature to promote leaf development and inhibit flowering (Blanchard *et al*., 2007).

Temperature has been reported to control flowering in several orchid genera such as *Dendrobium* (Rotor, 1959), *Miltoniopsis* (Lopez and Runkle, 2006), *Phalaenopsis* and *Zygopetalum* (Blanchard and Runkle, 2006). The cooling temperature has a significant effect on spiking and it is variety specific. The spiking percentage is a function of the accumulated cooling temperature. The larger the difference between growing and cooling temperatures, the shorter is the spiking period (Chen, 2002). Uniform spiking can be achieved in phalaenopsis orchids through temperature control with 25°C/20°C day/night for 4–5 weeks. However, a cooler temperature is required for continuing the growth of inflorescence. The branch will become a vegetative shoot if it encounters high temperature (Hsiao *et al*., 2011).

For species that require low temperatures for flower induction (vernalization), one way to defer flowering is to expose plants to high temperatures (Sakanishi *et al*, 1980). Heating the greenhouse to ≥28°C can keep phalaenopsis from spiking. The spiking of phalaenopsis is induced by exposure to a temperature below 25°C for several weeks. During the low-temperature period, exposure to high light intensity is necessary to induce spiking (Wang, 1995). However, in temperate climates, this is an expensive cultural practice because of the large energy input. Since inflorescence induction of phalaenopsis is light dependent, an inexpensive alternative for inhibiting spiking in phalaenopsis is to alternate 5 days of heavy shading (darkness) with 2 days of light on weekly cycles (Wang *et al*., 2007).

Phalaenopsis develop at least two undifferentiated bud primordial at each node that partially develops and then become dormant (Rotor, 1959). Under

appropriate environmental and cultural conditions, the upper bud elongates and emerges through the epidermis of the stem and develops into an inflorescence (Wang, 1995). The number of flower buds in phalaenopsis was generally greater at cooler temperature treatments than with a higher average day temperature (Blanchard and Runkle, 2006).

Inflorescences may continue to elongate to several feet without producing flowers. In the plants grown under a high temperature (over 25°C), floral transition does not occur (Sakanishi *et al.,* 1980), and flower development is inhibited by the exposure to a high temperature when the inflorescence length is short (Chen *et al*., 1994). Elongation of inflorescence and rate of blooming, however requires higher temperature (25°C in the day and 17°C in the night) (Hew and Yong, 1997). If a plant with a young inflorescence (less than10 cm) is subsequently grown at 82°F (28°C) or higher, a inflorescence can form a vegetative air plantlet known as a "keiki" instead of flower buds, buds may abort or both. Flowering of many hybrids is increasingly delayed as the daily duration at 28°C increases and can be completely suppressed with extended high-temperature exposure (Runkle, 2010). Once an inflorescence has developed, time to open flower is a function of temperature and variety (Runkle, 2010).

The growth and flowering of *Phalaenopsis* have been studied under controlled environment using a phytotron in France. *P. amabilis* and *P. schilleriana* flowered when the night temperature varied between 12°C and 17°C and the day temperature does not exceed 27°C (Hew and Yong, 1997).

The adequate day and night temperature for vegetative stage is 28°C and 26°C. If the cooling temperature of day and night is kept at 25°C and 20°C, 100 per cent of the plants have single spike. When the cooling temperature during day and night is maintained at 23°C and 20°C, 40 per cent of the plants produce two spikes and 60 per cent produce single spike. On exposing to 20°C day temperature and 18°C night temperature, 100 per cent plants are with two spikes. Some varieties can be induced to spike at the growing regions (day temperature of 28°C and night temperature of 26°C). Some varieties cannot be made to induce spikes at the cooling

regions (day temperature 20°C, night temperature 18°C). The reason for these results can be explained by the diversity of *Phalaenopsis* varieties (Chen, 2002).

2.1.1.1.1 Altitude

The fact that flowering can be induced by low temperature has contributed significantly to the large scale production of *Phalaenopsis* cut flowers and potted plants in Taiwan and Japan. Phal hybrids are first cultivated in lowlands. When they reach a certain stage of development, they are transported to cooler places in highlands during summer for flower induction. The age of plant, timing of transfer to highlands and their eventual return to the warmer lowlands are critical for flower induction and development (Hew and Yong, 1997).

2.1.1.1.2 Cold room

Flower induction in *Phalenopsis* hybrids grown under greenhouses can be achieved by cooling the greenhouses, but the high energy requirement makes it economically unattractive (Hew and Yong, 1997). The required cooling day temperature is 25°C for *P. amabilis* and 20°C for *P.* Fortune Saltzman. The endurance of the low temperature needs to be considered. Some varieties are sensitive to lower temperature. If the cooling temperature is too low for the variety, these plants are easily damaged by the treatment (Chen, 2002). Inflorescence initiation (sometimes referred to as spiking) occurs in mature plants after at least 2 to 4 weeks of temperatures below 26 °C under otherwise favorable conditions (Runkle, 2010).

2.2 Nutrients

Phalaenopsis does not require high amount of fertilizer because of its slow growth probably under less than optimum growing conditions (Batchelor, 1983). Varying the concentrations of N, P, and K did not produce significant changes in the number of expanded leaves in *Odontoglossum* (Yoneda *et al*, 1999)

Phalaenopsis has a long juvenile period. High fertilizer concentrations in general accelerate vegetative growth. In most bark mixes, fertilizers providing 200 mgL^{-1}nitrogen promote growth over lower concentrations. High fertilizer concentrations ensure high flower count but do not always increase flower size (Wang, 2005).

Plants respond to higher N rate by producing larger leaves. Plant growth at 100 mg N per litre during a seven months period was exceptional by commercial standards. One leaf was produced at an average once every 40 days. Heavy fertilizer was found to have better vegetative growth and more flower count in phalaenopsis (Wang and Gregg, 1994). *Phalaenopsis* needs at least 50 per cent of the N in the nitrate form for improved growth and flowering, regardless of being planted in a bark mix or sphagnum moss (Wang, 2005).

Fertilizing Phals weekly with half strength 20-10-10 of NPK and changing to 0-10-10 of NPK as blooming fertilizer when a new flower spike first appears can result in larger and usually more flowers (Rogers, 2012).

Leaves and substrate roots of orchids differ significantly with respect to macro and micro-nutrient accumulation; leaves accumulate more nitrogen, phosphorus, potassium, calcium and manganese, while roots accumulate more magnesium, iron and zinc (Trelka *et al*., 2010).

2.3 Plant growth regulators (PGRs)

In addition to ambient temperature, plant growth regulators (PGRs) play an important role in inflorescence induction. Plant growth regulators (PGRs) control physiological processes at extremely low concentrations. A wide range of PGRs, including gibberellins, auxins, cytokinins and ABA, affect flowering in orchids. However, different experimental conditions and types of orchids may have various effects.

Phytohormones regulate diverse processes in plants, such as development the response to biotic and abiotic stress. For integrating environmental and intrinsic signals, an appropriate perception system for the hormone and a signaling cascade leading to the accordant output are needed. Cytokinin is one of those phytohormones. Upon cytokinin binding to membrane-located receptor histidine kinases, a signal is transduced to the nucleus via consecutive phosphorylation of the receptor itself, histidine phosphotransfer proteins and finally signal type-B response regulators which function as transcription factors (Hwang and Sheen, 2001)

For phalaenopsis, cytokinins [benzylaminopurine (BA)] stimulate flowering, and auxin suppresses the BA effect; gibberellin is not effective when applied alone but when added in combination with BA seems to accelerate the BA effect slightly (Hew and Clifford, 1993). In addition, cytokinins such as benzyladenine can increase inflorescence number of *Phalaenopsis* when applied at the onset of exposure to inductive temperatures. However, in some hybrids, spikes can develop abnormally if cytokinins are applied at an excessive rate or after flower initiation (Runkle, 2010). Treating whole plants with 200 mg L^{-1} Kinetin also increased the flower spikes per plant (Wu and Chang, 2011).

Gibberellins are biochemically described as tetracyclic diterpene acids and are associated with flower induction in several species. When exogenously applied, these plant growth regulators lead to petal growth and flower induction in long-day plants under conditions of short days. The opposite can occur in some exceptions (Cardoso *et al.*, 2010). In *Bc.* Marcella Koss, the application of 250 mg L^{-1} GA_3 combined with decreased irrigation frequency induced flowering in around 83 per cent plants. By using the same GA_3 concentration witht frequent irrigation, only 17 per cent plants could be induced to flower. The number and size of flowers increased after application of higher GA_3 concentrations (Cardoso *et al*., 2010).

Exogenous application of gibberellic acid (GA) can be used to increase inflorescence length, but more commonly, chemicals that inhibit the biosynthesis of active GA are used to suppress inflorescence elongation (Runkle, 2010). Although

gibberellin is not effective at inducing flowering, it increases spike length and flower size (Wu and Chang, 2011).

When *Phalaenopsis amabilis* is grown under high temperature (30/25°C, day/night), flowering is blocked, and this can be reversed by gibberellin A_3 (GA_3) treatment. Associated with GA_3 treatment under high temperature are increases in sucrose, glucose and fructose as compared with warm-treated plants (Runkle, 2010). Spraying with sucrose solution alone caused leaf epinasty in plants grown under high temperature. Epinasty was released by about 9 days of GA_3 treatment. In GA_3-treated plants under high temperatures, sucrose application to the source leaves led to an increase in sugar content in both leaves and inflorescence. In contrast, although in warm-treated plants sucrose application to the source leaves increased sugar content in the leaves, it did not increase sucrose content in the inflorescence. These results corroborate the hypothesis that in *Phalaenopsis,* GA_3 stimulates sink activity in the apical meristem and promotes the translocation of sucrose from source leaves to the apex of the inflorescence, where it accumulates. Treatment with GA_3 led to an increase in sucrose synthase activity and had no effect on invertase activity (Chen *et al*., 2008).

Hormones play a central role in shoot branching control. Auxin inhibits bud outgrowth whereas cytokinin promotes the same (Sachs and Thimann, 1967). These hormones can efficiently regulate shoot branching, but their application also affects flowering, leaf development, and height, reducing their utility for the horticultural industry (Liang *et al*., 2010).

The application of hormones is not a good idea. The concentration of hormones is not easy to control. If the concentration is too high, the shape of flower could be changed unpredictably (Chen, 2002). Use of hormones has a small effect on spiking. However, the application of hormones with the cooling stress could increase the spiking percentage and shorten the cooling period. The adequate hormone levels are influenced by the characteristics of varieties (Chen, 2002).

Qualitative and quantitative changes in gibberellin-like subtance(s) were observed in lateral buds (potential flower buds) but not in leaves or terminal buds (potential vegetative buds) sampled from olive trees at intervals during the winter and spring. At least two types of gibberellin-like substances were found in extracts of lateral buds; their levels increased progressively during the low temperature induction period, reaching a maximum shortly before floral initiation (Badr *et al*., 1970).

2.4 Inflorescence pruning

To conserve the energy and to force the plant to rest in preparation for a good presentation of flowers in the following blooming season, the inflorescence is cut off at its lowest point with a sterilized tool. This prevents an enzyme produced in the nodes and tip of the inflorescence (which keeps the plant in the reproductive mode) from entering the plant, thus allowing the plant to devote all its energies to growth following a brief rest.

Rose plants usually respond to pruning by creating a large number of metabolic sinks. The main effect of pruning has been studied on carbohydrate movement to storage regions in lower parts of the plant (Zieslin *et al*., 1975).

The contents of endogenous hormones (including GA_3, GA_{4+7}, IAA, ABA, ZR and iPA) in grapes were analyzed using ELISA in different buds located in different nodes of two-year-old Kyoho grape seedlings. The results revealed that the reproductive phase appeared at about 20th node. The contents of GA_3 and GA_{4+7} decreased, the ABA content increased sharply during the process of phase change, but the content of ABA was so low that it was hardly detected at the nodes 31 - 35th; the ratio of ABA /GA_3 and ABA/GA_{4+7} varied slightly at the nodes 16 - 20th , but rose thereafter. So it was concluded that the ratio obviously reflected the relationship between variations in hormones and phase change (Yaqin *et al*., 2006).

Many plant species respond to stress by inducing flower buds. The short-day plants *Pharbitis nil* and *Perilla frutescens* var. crispa flower under long days in

response to the stress of poor nutrition or low-intensity light. Grafting experiments using two varieties of *P. nil* revealed that a transmissible flowering stimulus is involved in stress-induced flowering (Wada and Takeno, 2010).

2.5 Growing media

In their natural habitats phalaenopsis species are epiphytes. Their roots are exposed to air movement and they absorb moisture from the humid air, as well as from rains and dews. While growing phalaenopsis in containers filled with an artificial medium for our convenience, we must consider aeration, capillary action, water and nutrient-holding capacities, stability and weight of the medium components, as well as cost and consistency (Wang *et al*., 2007).

Plant growth vastly improved in a medium consisting of 20 per cent coarse sphagnum peat and 80 per cent fir bark, compared with fir bark alone (Wang *et al*., 2007).

Managing the root zone of potted orchids can be one of the most critical aspects to growing a healthy crop. If one can grow plants with healthy roots, making them flower is a much easier task (Wang *et al*., 2007).

Pure sphagnum moss is probably the single best material for growing young phalaenopsis in warm (tropical and subtropical) conditions (Wang *et al*., 2007). Moisture tension of the tightly packed sphagnum moss remains at or higher than -20 kPa until 90 per cent of the water is lost, compared to 55 per cent water loss in a peat/diatomite mix; thereby, providing higher amount of available water to roots. The pH of the sphagnum moss in growing containers is often between 3.0 and 3.5. Yet, the low pH does not seem to negatively affect plant growth or cause apparent toxicity from the accumulation of certain micronutrients in plants (Wang *et al*., 2007).

Microbial association

Little is known about the composition and functional activity of orchid-associated bacteria. Rhizobacteria are recognized to have a great and often favorable impact upon plant development. The endophytic bacteria from the roots of terrestrial orchid *Pterostylis vittata* were studied by Dileep *et al,* in 1998. Associative bacteria populated the aerial roots 30 times more densely than its substrate roots. Thus, the aerial roots may serve as a favorable habitat for bacteria due to the presence of spongy velamen (Tsavkelova *et al*., 2005).

Leaf surfaces (phyllospheres) have been shown to provide appropriate conditions for colonization by microorganisms including diazotrophic bacteria that are able to fix atmospheric nitrogen (Furnkranz *et al*., 2008). Various bacteria belonging to very different phylogenetic groups share the ability to reduce atmospheric N_2 to ammonium via the enzyme nitrogenase. N_2-fixing bacteria are found in diverse habitats, however, in undisturbed terrestrial systems such as tropical rain-forests biological nitrogen fixation (BNF) is considered particularly important for the maintenance of ecosystem nitrogen pools (Vitousek, 2002). Besides the provision of N_2, plant-associated bacteria are important for supporting growth, health and stress resistance in plants (Lugtenberg *et al*., 1991).

The composition of the bacterial community on the plant roots depends on the condition of plant growth. Under conditions simulating the climate of moist tropical forests, the aerial roots proved to be populated with phototrophic microorganisms, among which cyanobacteria predominated. Interlaced fungal hyphae and filamentous cyanobacteria formed a sheath on the surface of the aerial roots (Tsavkelova, 2003).

Abundance of heterotrophic and phototrophic bacteria are present in the roots of cultivated tropical orchids of *Calanthe*, *Acampe* and *Dendrobium* genera (Tsavkelova *et al*., 2004). Functional role of the isolated strains was proved by high nitrogen-fixing activity of the orchid-associated cyanobacterial community, and indole-3-acetic acid (IAA, auxin) production by heterotrophic bacteria (Tsavkelova

et al., 2005). IAA is responsible for division, enlargement and differentiation of plant cells and tissues; it plays a major role in xylem- and root formation (Davies, 1995). Auxin biosynthesis is also widespread among soil and plant associated bacteria. Moreover, its production is a determinant trait both for plant growth promoting rhizobacteria (PGPR) and plant pathogens (Patten and Glick, 2002).

Pseudomonadaceae, Enterobacteriaceae, Flavobacteriaceae, Burkhol-deriaceae, Xanthomonadaceae, and Bacillaceae families are well known plant-associated bacteria. The prevalence of *Pseudomonas* and *Bacillus* endosymbionts was also shown for Australian terrestrial orchids (Wilkinson *et al*., 1994). Nevertheless, the limited specificity in selection of the bacterial partners allows the plants to host diverse microbial populations. Microbial complex of greenhouse terrestrial *Calanthe vestita* Lindl. var. rubro-oculata also differed from that of two greenhouse epyphites (*Aeolidia papillosa* and *Dendrobium moschatum* (Tsavkelova *et al*., 2004). Therefore, orchid bacterial communities vary depending on the species or the root type. This diversity may be due to the composition of the root exudates.

Orchids are known to produce various phenolic compounds and phytoalexins, which were shown to suppress a number of different microorganisms (Stoessl and Arditti, 1984). Plant exudates also supply the rhizosphere with tryptophan which is the main precursor in microbial Indole acetic acid (IAA) biosynthesis IAA producing bacteria transform it into auxin, increasing its exogenous level (Kravchenko *et al*., 2004).

Bacteria colonizing plant roots are in many cases beneficial for plant growth, development, and productivity. Promotion of the processes of xylem and root formation is the most pronounced among the diverse effects of this phytohormone (Dorffling, 1982). The synthesis of phytohormones (auxins, gibberellins, and cytokinins) by such associated microorganisms is believed to be one of the major forms of host plant–microbial interactions (Tsavkelova *et al*., 2004). Bacteria associated with the roots of greenhouse tropical orchids were shown to produce indole-3- acetic acid (IAA) and to excrete it into the culture liquid (Tsavkelova *et al*., 2004).

The utility of spraying some known N_2-fixing microorganisms on rice leaves grown both in N-less sand culture and under field conditions was examined. The effect was compared with that of spraying a phyllosphere N_2-fixing isolate of *Klebsiella*, $KUPBR_2$, and application of nitrogenous fertilizers. All the growth parameters studied including dry weight and N-content were enhanced. Under field conditions number of tillers was increased by 26 per cent with *Klebsiella pneumoniae* M5al and by 65 per cent with Aphanothece. The dry weight of the plants was enhanced by 61–119 per cent. (Nandi and Sen, 1981).

2.6 Post harvest studies

Orchid flowers are extremely sensitive to ethylene, even at very low levels. Premature fading of *Cymbidium* flowers was observed when exposed to as little as 0.0002 ppm of ethylene for 24 hours (Davidson, 1949).

After pollination, senescence is greatly accelerated. This was observed at a slightly slower rate following emasculation. Both pollination and emasculation stimulate ethylene production (Burg and Dijkma, 1967). The physiological and biochemical processes associated with pollination (post pollination phenomenon) have been extensively studied. The stigma and rostellum are the primary sites for ethylene production (Chadwik *et al*., 1980). Depending on the orchid species, unpollinated orchid flowers may remain fresh for weeks or even months (Arditti, 1992). Accelerated flower wilting as a result of ethylene evolution following pollination in *Cymbidium*, *Doritaenopsis*, *Dendrobium*, and *Phalaenopsis* orchids is induced by a loss of water from cells of the upper layer of the petals, leading to their in-folding and water-soaked appearance (Lee and Lin, 1992; Porat, 1994).

2.7 Physiology of phalaenopsis

Phalaenopsis is a CAM (Crassulacean Acid Metabolism) plant. Unlike most other plants, CAM plants only absorb CO_2 during the night and store in the vacuoles. CO_2 is released during daytime which enters calvin cycle and is then used for photosynthesis. CAM idling is considered as a form of very strong CAM, while CAM cycling is weak CAM. In the epiphytic Gesneriaceae *Codonanthe crassifolia,*

Guralnick *et al.* (1986) observed CAM cycling in well-watered plants and CAM idling in drought-stressed plants.

After being acclimated to constant warm (28°C day/28°C night) and cool-night temperature (28°C day/20°C night) regimes in growth chambers for 2 weeks, the two groups of mature *Phalaenopsis aphrodite* subsp. *formosana* plants both clearly exhibited a diurnal oscillation of stomatal conductance, net CO_2 uptake rate, malate and starch levels, and the phosphoenolpyruvate carboxylase activities. Hence, *P. aphrodite* is an obligate crassulacean acid metabolism plant. Nevertheless, different night temperature greatly affected both the stomatal conductance and the contribution of ambient and respiratory CO_2 to the nocturnal accumulation of malate. However, the amounts of nocturnal accumulated malate and daily deposited starch appeared to have no significant difference between the two groups. These results demonstrate that *P. aphrodite* is congruent with the characteristics of CAM plants having great flexibility and plasticity in response to changes in environmental conditions. In addition, the formation of reproductive stem, *viz*. inflorescence, was noticeably inhibited by a constant warm temperature, but induced by a fluctuating warm day and cool night condition (Chen *et al*, 2008).

2.7.1 Stomata

Stomata are found on most aerial parts of plants, including the epidermis, stems and flowers. Stomata control the loss of water and the exchange of gas and thus control water relations and metabolism. Transpiration via stomata supplies water and minerals to the entire plant system. When a plant encounters adverse environmental conditions, such as drought, a plant hormone called abscisic acid triggers stomata to shut tightly in order to prevent plants from dehydration and wilting. The stomata of *Arachnis* cv. Maggie Oei, *Aranda* cv. Deborah, *Arundina graminifolia*, *Bromheadia finlaysoniana, Cattleya browringlana x C. forbesii* and *Spathoglottis plicata* occur only on the lower epidermis of the leaves and are located within hyperstomatic chambers formed by cuticular ledges extending from guard cells (Hew *et al*., 2005).

2.7.1.1 Stomatal density

Stomata play a key role in the acclimation and adaptation of plants to their environment. Stomatal distribution per unit area indicated significant variations amongst apple rootstocks (Pathak *et al*., 1976), chestnut types (Sahin and Soylu, 1991) and Sarılop clones. Stomatal frequency can change more than two fold in response to radiation, water status or according to developmental stages (Jones, 1987).

Stomata are found on most aerial surface of plants often including the adaxial epidermis, stems and flowers (Esau, 1977). Stomata are present in all orchid flowers. The density of stomata in floral parts was considerably lower than in the leaves. Also, the distribution of stomata in various floral parts varied. Floral stomata were incapable of photosynthesis although they contained starch (Hew *et al*., 2005).

2.7.1.2 Stomatal conductance

Stomatal conductance and transpiration were not affected by changes in CO_2 concentration, irradiance, relative humidity and abscisic acid treatment. Water vapour conductance in flowers was low and comparable to that of upper epidermis of the leaves which in all cases was devoid of stomata. The transpiration of orchid flowers in light and in dark was identical. This, together with other evidence indicates that stomata in orchid flowers do not function in the control of water loss (Hew *et al*., 2005). Diurnal fluctuations of acidity in orchids were first reported nearly 125 years ago by Warburg in 1886.

2.7.1.3 Stomatal rhythm

Arachnis, *Aranda* and *Cattleya* have thick leaves which exhibit Crassulacean acid metabolism, and their stomata open when acidity levels are lowest, or shortly thereafter. *Aranda* and *Arachnis* require higher light intensities for sufficient de-acidification to permit stomatal opening than *Cattleya*. Stomata of the thin leaved

Arundina, *Bromheadia* and *Spathoglottis* open during the day. The stomatal rhythms, morphology and distribution, as well as the pathways of carbon fixation and light requirements for deacidification reflect the natural habitat of each species (Goh *et al*., 1977).

2.7.2 Net CO_2 uptake

In phalaenopsis, the optimum temperature for photosynthesis measure by CO_2 absorption was 15-25 °C and this temperature range is almost the same as the optimum temperature for floral transition. Therefore, a larger amount of CO_2 absorbed at 15–25 °C (low temperature) may be used in other than the growth of leaves (Kano, 2001).

2.7.3 Chlorophyll

Ling and Subramaniam (2007), studied leaves of 12 *Phalaenopsis violacea* orchids and found that the concentration of chlorophyll-a was higher than the concentration of chlorophyll-b by about 100%. At the same time, they showed that orchid cultivars differ by chlorophyll a/b ratio. A low ratio (< 2) of a/b chlorophyll is characteristic of orchids grown in shadowed areas and chlorophyll-b concentration may increase under stressful conditions. Leaves of *Phalaenopsis* contain more chlorophyll-a and chlorophyll-b than roots, however, growing media did not affect chlorophyll concentration in leaves and roots (Trelka *et al*., 2010). The natural color for inflorescences is green. It contains chlorophyll and has the photosynthesis ability same as that of leaves and roots.

2.7.4 Malic acid

For the estimation of malic acid content in *Phalaenopsis* leaves, the distribution of malic acid within the leaf and the relationship between malic acid content and pH of hot water leaf extracts were studied. Kuboto et al.,(1997) reported

that at the end of the dark period, malic acid content was at a constant level of about 150 mmol m^{-2} from the leaf tip to the central part of the leaf with the exception of the leaf edges. Both at 7 hrs after the onset of lighting and at the end of the light period, the contents were 15 to 25 mmol m^{-2} within the leaf. There was a close correlation between malic acid contents in the leaf and pH of hot water extracts ($r^2 = 0.93$). The pH indicated a value from 4.0 to 4.5 when malic acid accumulated at the end of the dark period, and the value rose from 5.5 to 6.0 with decomposition of malic acid at the end of the light period. It was concluded that malic acid metabolism could be estimated by measuring the pH of leaf-disk extracts both at the end of light and at the end of darkness in the plants.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Regulation of flowering in *Phalaenopsis* orchids" was carried out in the *Phalaenopsis* orchidariums *viz*., top ventilated rain-shelter and fan and pad greenhouse of the All India Co-ordinated Floriculture Improvement Project at the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara from November 2010 to May 2012. The studies were conducted with the objectives to evaluate the varieties for their performance, to enhance the vegetative growth of the plants and to improve flowering. It was done through nutrient and growth regulator application, temperature control and the use of growing media. The materials used and methodology adopted for the investigations are dealt with in this chapter.

3.1 Location

All the studies except the trial on altitude effect on flower induction were conducted at Vellanikkara. The site is situated at a latitude of 10°31' N and longitude of 76°13' E. The area lies 22.25m above MSL. The trial on altitude effect on flower induction was conducted at Nelliampathy, Palakkad district. Nelliampathy is located at 1050 m above MSL. It is situated at latitude of 10°53' N and 76°68' E longitude.

3.2 Climate

The experimental site enjoyed a humid tropical climate with maximum and minimum average temperatures of 36.59°C and 21.30°C during the period of investigation. The mean relative humidity varied from 46.86 per cent to 78.70 per cent. The light intensities varied between 6000 and 11000 Lux. Weather conditions were controlled in the 'fan and pad' greenhouse. Details are furnished in Appendix 1. Nelliampathy on the other is bestowed with a subtropical weather with average maximum temperature of 28°C and minimum temperature of 18°C during the period

of study. The weather parameters recorded during the period are presented in Appendix 2.

3.3 Planting material

Phalaenopsis is a short-stemmed monopodial orchid with an indeterminate growth and without lateral branching. Leaves are large, varying in colour, shape and orientation with a CAM pathway of photosynthesis. Aerial roots are fleshy with velamen tissues at the tip that aid in absorption of moisture. The varieties used for the various experiments are listed below.

1. Roxanne
2. Chin Shang Stripe
3. Kathleen Ai
4. Magic Kiss
5. Medium Pink
6. Taisuco Confidence
7. Lin Jessica
8. Carlotta

3.4 Experiments

3.4.1 Performance evaluation

The experiment was carried out with an objective of evaluating the performance of two types of phalaenospsis, *viz*., cut flower type and the pot plant type under two different growing systems, *viz*., fan and pad system of greenhouse and top ventilated rain-shelter. Temperature in the fan and pad remained lower by about 5 to 6ºC during the day time throughout the period of study than in the rain-shelter (Appendix 2). Two varieties, each, were taken under cut flower and pot plant types.Eighteen months old tissue culture plants of varieties Chin Shang Stripe, Kathleen Ai were used as cut flower types and Magic Kiss and Medium Pink were used as pot plant types.

3.4.2 Induction of flowering by low temperature treatment

3.4.2.1 Higher altitude

Ten mature plants, each, of phalaenopsis var. Roxanne were kept in the shade house at the Orange and Vegetable farm, Nelliampathy for 2, 3 and 4 weeks, respectively. Control set of plants were kept in the rain-shelter at Vellanikkara. Plants were brought back and half of them were kept in rain-shelter greenhouse and the remaining half was kept in the 'fan and pad' greenhouse to compare the performance under the two systems.

3.4.2.2 Cold room conditions

Six mature plants, each, of phalaenopsis var. Roxanne were kept in cold room (AC room) for 2 and 3 weeks. The temperature was set at 23°C which recorded a maximum of 24.45°C and a minimum of 20.62°C during the period of study. Set of plants taken as control was kept in rain-shelter greenhouse where temperature during the period of study varied between 33.59°C and 25.60°C. It is presented in Appendix 3.

3.4.3 Nutrient-growth regulator combination

Three different combinations of the major nutrients *viz*., NPK 10:10:10, 20:10:10 and 10:20:10 were tried at 0.1 per cent concentration and at fortnightly intervals. Chemicals used for the preparation of the nutrient solution were ammonium nitrate, orthophosphoric acid and potassium nitrate. Growth regulators and their concentrations used in combination treatments were GA_3 at 1 ppm and 2 ppm and Kinetin at 100 ppm and 200 ppm. The 15 treatments thus imposed are listed below.

i. NPK 10:10:10 - 0.1%
ii. NPK 20:10:10 - 0.1%
iii. NPK 10:20:10 - 0.1%

Plate 1. Performance evaluation studies of *Phalaenopsis* under two growing systems

iv. NPK 10:10:10 - 0.1% + GA_3 1 ppm
v. NPK 10:10:10 - 0.1% + GA_3 2 ppm
vi. NPK 20:10:10 - 0.1% + GA_3 1 ppm
vii. NPK 20:10:10 - 0.1% + GA_3 2 ppm
viii. NPK 10:20:10 - 0.1% + GA_3 1 ppm
ix. NPK 10:20:10 - 0.1% + GA_3 2 ppm
x. NPK 10:10:10 - 0.1% + Kinetin 100 ppm
xi. NPK 10:10:10 - 0.1% + Kinetin 200 ppm
xii. NPK 20:10:10 - 0.1% + Kinetin 100 ppm
xiii. NPK 20:10:10 - 0.1% + Kinetin 200 ppm
xiv. NPK 10:20:10 - 0.1% + Kinetin 100 ppm
xv. NPK 10:20:10 - 0.1% + Kinetin 200 ppm

In the above experiment, cow dung (1:100 dilution) with liquid *Pseudomonas* (5ml L^{-1}) was sprayed uniformly to all the plants on alternate weeks.

3.4.4 Inflorescence pruning

Spent inflorescences of cut flower variety Roxanne and pot pant variety Carlotta were pruned at three different levels *viz*., first node, second node and third node along with control wherein spent inflorescence was retained without pruning. Five plants were taken per treatment.

3.4.5 Growing media studies

Eighteen months old plants of variety Magic Kiss were used to assess the influence of three different types of growing media, namely coconut husk chips, coconut husk bits and sphagnum moss. Fifteen plants were included under each treatment. The plants were grown in the respective media along with charcoal and tile bits.

Plate 2. Inside view of the two growing systems

3.4.6 Post harvest studies

Post harvest studies involved five varieties of phalaenopsis. Inflorescences of varieties Roxanne, Taisuco Confidence, Magic Kiss, Goldie and Mimi were studied. The inflorescences were harvested when two third of the flowers were open. Standard inflorescences having 2-3 open florets and 1-2 buds were used for the study. A slanting cut was given at the base of the flower stalk to help better water absorption.

3.4.7 Physiological studies

Physiological studies were conducted at the Department of Plant Physiology and stomatal studies were taken up at the Department of Entomology, College of Horticulture, Vellanikkara.

3.4.7.1 Stomatal density

Five different varieties of *Phalaenopsis* viz., Roxanne, Magic Kiss, Medium Pink, Chin Shang Stripe and Kathleen Ai were chosen for the study. Stomatal impressions were taken at three different areas using glue (quick fix). These impressions were photographed at a magnification of 10 x using phase contrast microscope. The number of stomata was recorded and expressed as density per square millimetre.

3.4.7.2 Stomatal conductance

Five different varieties of *Phalaenopsis* viz., Roxanne, Magic Kiss, Pink Magic, Chin Shang Stripe and Kathleen Ai were chosen for the study. Stomatal conductance was studied by using an open system portable infra red gas analyzer, IRGA (model LI-6400, LI-COR Inc., Lincoln, NE, USA) at an interval of 2 hours for a 24 hour cycle and expressed as cm s^{-1}. The observation was recorded on January

Plate 3. Cold room (air conditioned room) exposure of plants

23rd 2012 when the ambient air temperature was 32/21 °C and relative humidity was 64 per cent.

3.4.7.3 Stomatal rhythm

Five different varieties of *Phalaenopsis* viz., Roxanne, Magic Kiss, Pink Magic, Chin Shang Stripe and Kathleen Ai were chosen for the study. Stomatal rhythm was studied based on stomatal conductance observed using portable IRGA (model LI-6400, LI-COR Inc., Lincoln, NE, USA) at an interval of 2 hours for a 24 hour cycle. The rhythmic opening and closing was explained based on stomatal conductance. The observation was recorded on January 23rd 2012 during which the ambient air temperature was 32/21 °C and relative humidity was 64 per cent.

3.4.7.4 Net CO_2 uptake

Five different varieties of *Phalaenopsis* viz., Roxanne, Magic Kiss, Pink Magic, Chin Shang Stripe and Kathleen Ai were chosen for the study. Net CO_2 uptake was studied by placing fully expanded leaves in the leaf chamber of open portable infra red gas analyzer, IRGA (model LI-6400, LI-COR Inc., Lincoln, NE, USA) and CO2 uptake rate was measured as change of CO_2 concentration which was expressed in μ mol $CO_2/m^2/s$. The observation was recorded at an interval of 2 hours for a 24 hour cycle on January 23rd 2012 during which the ambient air temperature was 32/21 °C and relative humidity was 64 per cent.

3.4.7.5 Chlorophyll estimation

The chlorophyll content in fully expanded leaves was estimated according to the method described by Arnon (1949). 100 mg fresh leaves were blended and then extracted with 10 ml of 80 per cent acetone. This was centrifuged at 4000 rpm for 10 minutes and the supernatant was collected and volume made upto 25ml with acetone. The absorbance was read at 645 and 663 nm using a spectrophotometer. The

calculation of chlorophyll a, chlorophyll b and total chlorophyll were done using the formula given below and pigment content expressed as mgg^{-1} fresh weight.

Chlorophyll content was estimated using Arnon's equation:

Chl a = $(12.7 \times A_{663} - 2.69 \times A_{645}) \times v/1000 \times 1000/w$

Chl b = $[(22.9 \times A_{645}) - (4.68 \times A_{663}) \times v/1000 \times 1000/w$

Total Chl = $[(8.02 \times A_{663}) + (20.2 \times A_{645}) \times v/1000 \times 1000/w$

Chlorophyll a/b ratio was derived by dividing chlorophyll a by chlorophyll b for each observation.

3.4.7.6 Malic acid estimate as titrable acidity

The malic acid content was estimated by measuring titrable acidity at the nd of light period (6pm) and at the end of dark period (6am) in the month of January 2012. The method described by Osmond and Avadhani (1970) was followed. The leaf samples were collected in ice box and transferred at this low temperature to the lab. Two grams of the leaf sample was boiled in distilled water (10ml) for 15 minutes. After cooling, the volume made up to 50 ml and titrated with 0.1 N NaOH using phenolphthalein as indicator.

Malic acid content as titrable acidity was calculated and expressed as mg per gram fresh weight of plant tissue.

3.3.8 Nutrient analysis

Micro and macronutrients were analysed in the leaf and root samples in plants grown in sphagnum moss, coconut husk chips and coconut husk bits. Growing media *viz*., sphagnum moss coconut husk chips and coconut husk bits were analysed as well. Nitrogen was analysed using kjedahl method. Potassium and micronutients like zinc, copper, sulphur, manganese, magnesium, iron were analysed using atomic absorption spectrophotometer.

3.3.9 Bacterial colonies

Microbial load was analyzed in media, in phyllosphere and rhizosphere for plants grown in sphagnum moss and without moss (coconut husk/chip bits). It was done using serial dilution technique. Various bacteria were identified by microbiological, biochemical and biotechnological tests at the Department of Microbiology, KAU.

3.4 General management

Phalaenopsis plants were planted in transparent plastic pots with preferred media of husk, sphagnum moss, tiles and charcoal bits. Plants were watered using fogger thus providing adequate moisture, but with plenty of good drainage and aeration for the roots. Snail and disease occurrence were higher in fan and pad greenhouse. Snails were the major pest and were removed manually. Major disease were caused by *Fusarium* (collar rot), *Erwinia* (soft rot) and *Sclerotium*. Affected plants were secluded and treated with Bavistin 0.1%. Chemicals were not very effective at a later stages of growth. Prophylactic measures were useful *viz*., cow dung slurry along with liquid *Pseudomonas* was sprayed at fortnightly interval. 0.1 per cent micronutrient spray was given at monthly intervals.

3.5 Design of the experiment

A completely randomized block design with three replications was laid out.

3.6 Observations

Biometric observations were recorded at monthly interval for an increase in vegetative growth and daily observations were taken for floral characters.

A. PLANT CHARACTERS

1. Increase in plant height

Increase in height of the plant was measured from the base to the growing apex for a period of one year and the difference recorded was expressed in centimetres.

2. Increase in leaf length

Increase in leaf length was recorded for a period of one year and expressed in centimetres.

3. Increase in leaf breadth

Increase in leaf breadth was measured for a period of one year and expressed in centimetres.

4. Increase in leaf area

Dot method (Bleasdale, 1973) was used to measure the increase in leaf area and was expressed in square centimetres.

5. Interval of leaf production

The interval between the production of two successive leaves was taken as the interval of leaf production and expressed in days.

B. FLORAL CHARACTERS

The following flower characters were observed and recorded during the period of study.

1. Time taken for emergence of inflorescence

Days taken for the appearance of the inflorescence from the date of initiation of the experiment.

2. Time taken for emergence of first flower bud

Days taken from inflorescence emergence to emergence of first flower bud was recorded in days.

3. Time taken for opening of the first flower bud

Days taken for the opening of first flower after inflorescence emergence was recorded in days.

4. Flowering duration

Flowering duration was measured as the number of days from the opening of first flower to wilting of the last flower and recorded in days.

5. Number of inflorescences

Number of inflorescences produced on each plant was noted for the treatments.

6. Length of inflorescence

The total length of the inflorescence in each plant was recorded in centimetre.

7. Number of florets per inflorescence

The number of florets per inflorescence in each plant was recorded and the mean values were expressed as the number of florets per inflorescence.

8. Flower size

Size of individual floret was recorded as the length (vertically) and width (across) of the flower and expressed in centimetre.

9. Branching of inflorescence

Qualitative character like presence or absence of branching in inflorescence was recorded.

10. Season of flowering

Flowering season were recorded for the chosen varieties.

3.7 STATISTICAL ANALYSIS:

The generated data from the study were subjected to analysis of variance suggested by Panse and Sukhatme (1985). Treatment means were compared using DMRT wherever necessary. MSTATC and SPSS software were also made use of.

Results

4. RESULTS

Results of the experiment titled 'Regulation of flowering in *Phalaenopsis* orchids' are presented in this chapter.

4.1 PERFORMANCE EVALUATION

Data generated from the trial involving performance of two varieties each of pot plant and cut flower types of phalaenopsis are presented in Table 1 (vegetative characters) and Table 2 (flowering characters). They were evaluated under two different growing systems (Plate 1 and Plate 2), namely top ventilated rain-shelter and fan and pad system of greenhouse, for a period of 12 months.

4.1.1 Vegetative Characters

4.1.1.1 Plant height

The data pertaining to the effect of different growing conditions on an increase in plant height is presented in Table 1. Among pot plants, significant differences with regard to growth were observed in the variety Magic Kiss. In rain-shelter, an increase in height of 1.09 cm was recorded against 0.47 cm in fan and pad. No significant increase in plant height between growing systems was found in variety Pink Magic and cut flower types Chin Shang Stripe and Kathleen Ai.

4.1.1.2 Leaf length, leaf breadth and leaf area

Leaf characters were superior in rain-shelter compared to fan and pad in all varieties. An increase in leaf length, breadth and leaf area of 6.46 cm, 1.54 cm and 10.50 cm^2, respectively, were recorded in variety Magic Kiss grown in rain-shelter greenhouse which was significantly superior compared to values of 1.71 cm, 0.72 cm, and 1.23 cm^2 in fan and pad. No significant differences were observed in variety Medium Pink and the cut flower varieties Chin Shang Stripe and Kathleen Ai.

Table1. Performance evaluation of vegetative characters in phalaenopsis varieties under two growing systems

Variety	Increase in plant height		Increase in leaf length		Increase in leaf breadth		Increase in leaf area		Interval of leaf production	
	GROWING SYSTEMS									
	RS	FP	RS	FP	RS	FP	RS	FP	RS	FP
Magic Kiss	1.09	0.47	6.46	1.71	1.54	0.72	10.50	1.23	97.92	151.61
Medium Pink	0.99	0.97	1.30	1.28	0.58	0.51	0.80	0.72	152.00	164.53
CD(0.05)	.04		1.75		0.46		1.50		15.35	
Chin Chang Stripe	0.48	0.26	1.88	0.41	0.66	0.58	1.32	0.22	251.50	287.25
Kathleen Ai	0.73	0.58	1.60	1.08	0.79	0.44	1.38	0.68	153.20	194.30
CD(0.05)	0.03		0.06		0.03		0.25		37.49	

*RS: Rain-shelter (top ventilated)

*FP: Fan and pad greenhouse

4.1.1.3 Interval of production of leaves

Leaves produced at a significantly shorter interval of 97.92 days in variety Magic Kiss grown under rain-shelter compared to fan and pad (151.61 days). It was not significant in variety Medium Pink. Among cut flower types, interval of production between growing systems was not significant in Chin Shang Stripe. But notable differences existed in variety Kathleen Ai wherein leaves were produced at an interval of 153.20 days in rain-shelter against 194.30 days in fan and pad.

4.1.2 Flowering Characters

4.1.2.1 Time taken for emergence of first flower bud

Among pot plants, in the variety Magic Kiss, the number of days taken for emergence of flower bud was minimum (32.08 days) in rain-shelter. The duration was significantly longer (38.92 days) in fan and pad. In variety medium pink, the first bud emerged early (30.69 days) in rain-shelter but in fan and pad, bud emerged after 33.92 days. Among cut flower types, in variety Chin Shang Stripe, the days taken for the emergence of bud were on par with 35.08 days in rain-shelter and 36.77 days in fan and pad. In Kathleen Ai, the values were on par with 33.85 days for fan and pad and 34.85 days for rain-shelter.

4.1.2.2 Time taken for opening of first flower bud

In pot plant types, in variety Magic Kiss, days taken for opening of 1st bud was on par in rain-shelter (58.69 days) and in fan and pad (60.46 days). For variety Medium Pink, the values were on par in both the systems, recording 58.54 days in rain-shelter and 59.92 days in fan and pad.

Between the cut flower types, in variety Chin Shang Stripe the duration for opening of flower buds in rain-shelter as well fan and pad was on par with 60.31.

days and 62.62 days respectively. In Kathleen Ai, days for opening of the first flower bud was 62.46 days in rain-shelter and 64.08 days in fan and pad.

4.1.2.3 Flowering duration

Significant differences were found in different growing systems with regard to flowering duration (Fig.1). Among pot plant varieties, Magic Kiss recorded maximum duration of 158.85 days when grown in fan and pad compared to 114.31 days in rain-shelter. In the variety Medium Pink, flowering duration was significantly higher (222.54 days) in fan and pad and it was minimum (125.08 days) in rain-shelter.

Among cut flower types, significant differences were found between growing systems in both the varieties. Chin Shang Stripe stayed in bloom for a maximum period of 180. 85 days in fan and pad while in rain-shelter it remained for 133.00 days. In variety Kathleen Ai, flowering lasted for 149.46 days in fan and pad and for 82.00 days in rain-shelter.

4.1.2.4 Number of inflorescences

Among pot plants, inflorescence count was not significantly different between the two growing systems. In cut flower types, inflorescence count was significantly higher (1.31) in variety Chin Shang Stripe, compared to 1.0 in rain-shelter. In Kathleen Ai, the values were not significant with 1.0 in rain-shelter and 1.15 in fan and pad.

4.1.2.5 Length of inflorescence

Among pot plants, Magic Kiss recorded significantly higher inflorescence length (33.27 cm) when grown in rain-shelter compared to 28.09 cm in fan and pad. In variety Medium Pink, inflorescence length was significantly superior (38.55 cm) when grown in fan and pad compared to 24.29 cm in rain-shelter.

Top ventilated rain-shelter

Plate 4. The growing systems in regular flowering season (August – October)

Fan and pad greenhouse

Table.2. Performance evaluation of flowering and floral characters of phalaenopsis varieties under two growing systems

Variety	Emergence of first flower bud (days)		Opening of first flower bud (days)			Length of inflorescence (cm)		Inflorescence/ year (no)		Florets/ inflorescence (no)		Flower size length (cm)xbreadth (cm)		Branching of inflorescence		Season	
						GROWING SYSTEMS											
	RS	FP	RS	FP	RS	FP	RS	FP	RS	FP	RS	FP	RS	FP	RS	FP	RS	FP
PP: Magic Kiss	32.08	38.92	58.69	60.5	114.3	158.85	33.27	28.09	0.92	0.85	8.62	8.77	5.92x5.8	5.76x5.77	Not branched	Branched	Sept-Dec	Aug-Mar
PP: Medium Pink	30.69	33.92	58.54	59.9	125.1	222.54	24.29	38.55	1.00	0.80	8.54	16.69	5.70x5.43	5.96x5.78	Rarely branch	Branched	Sept-Jan	Sept-May
CD(.05)	0.863		1.86		13.912		4.05		0.253		2.28		NS	0.25				
Significance	S		NS		S		S		S		NS/S							
CF: Chin Shang Stripe	35.08	36.77	60.31	62.6	133.00	180.85	34.04	28.85	1.00	1.31	7.38	9.31	7.05x6.62	7.55x6.79	Not branched	Branched	Sept-Jan	Sept-Apr
Kathleen Ai	34.85	33.85	62.46	64.1	82.00	149.46	26.42	28.69	1.00	1.15	6.00	9.77	7.37x6.83	7.79x6.90	Not branched	Branched	Sept-Dec	Sept-Mar
CD(.05)	0.467		1.99		24.70		3.58		0.308		2.53		0.513					
Significance	S		NS		NS		S		NS		S		NS					

***RS: Rain-shelter (top ventilated)** *** S: Significant**

*** FP:Fan and Pad greenhouse** ***NS: Not significant**

Fig. 1 Flowering duration in different varieties of phalaenopsis as influenced by growing systems

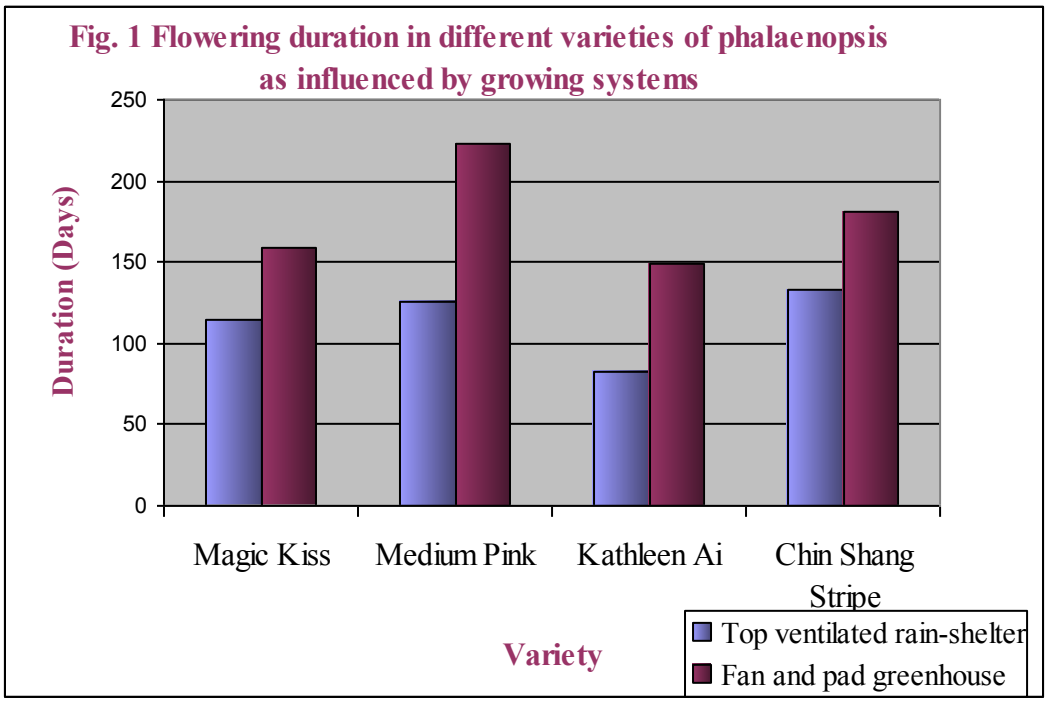
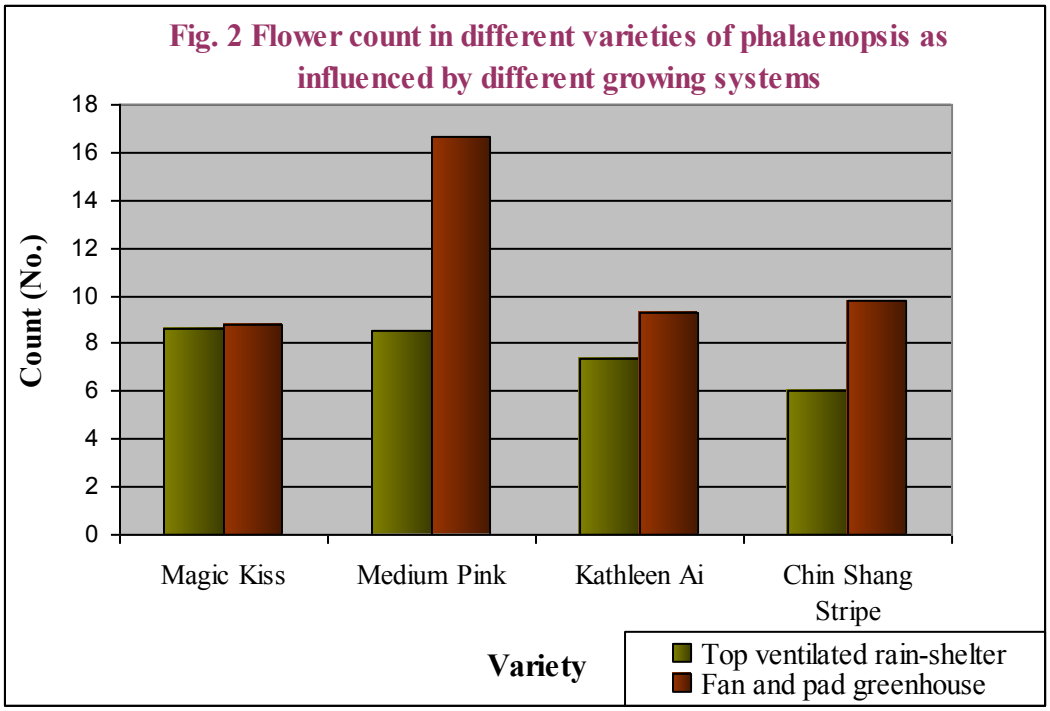


Fig. 2 Flower count in different varieties of phalaenopsis as influenced by different growing systems



Cut flower variety Chin Shang Stripe measured maximum inflorescence length (34.04 cm) in rain-shelter and it was lower (28.85 cm) in fan and pad. Kathleen Ai recorded inflorescence length which was on par in fan and pad (28.69 cm) and rain-shelter (26.42 cm).

4.1.2.6 Number of florets per inflorescence

Among pot plants, the flower count varied across varieties under both the growing systems (Fig.2). Variety Magic Kiss did not show significant differences with respect to flower count. It was 8.62 in rain-shelter and 8.77 in fan and pad. In variety Medium Pink, significantly higher flower count (16.69) was noted in fan and pad compared to rain-shelter (8.54).

In the cut flower category, differences were not significant between growing systems in Chin Shang Stripe. It recorded a value of 9.31 in fan and pad and 7.38 in rain-shelter. Kathleen Ai had a significantly higher flower count of 9.77 in fan and pad against 6.00 in rain-shelter.

4.1.2.7 Flower size

Difference in flower size was not significant between the two growing systems.

4.1.2.8. Branching of inflorescence

Branching was observed in all the varieties grown in fan and pad. But those in the rain-shelter did not branch except in variety Medium Pink which showed occasional branching.

Top ventilated rain-shelter

Plate 5. The growing systems in off season (Apr – May)

Fan and pad greenhouse

4.1.2.8 Season of flowering

For all the varieties, flowering season prolonged in fan and pad. Plants bloomed in September and continued up to April-May compared to rain-shelter where the season ended by December- January.

4.2 Induction of flowering by low temperature treatment

Data pertaining to induction of flowering in *Phalaenopsis* variety Roxanne by exposure to low temperature is presented in Table 3, Table 4 and also Plate 4.

4.2.1 Higher altitude

Data generated by exposing the plants of phalaenopsis var. Roxanne to low temperature (by keeping plants at higher altitude) for a period of 2 weeks, 3 weeks and 4 weeks along with control kept at lower altitude (rain-shelter and fan and pad) are presented here. After the period of exposure, plants were kept in top ventilated rain-shelter as well as in fan and pad. Details are furnished in Table 3, Table 4, Fig.3 and Fig. 4.

4.2.1.1 Time for emergence of inflorescence

In plants brought down after two weeks of exposure to cold temperature, inflorescences emerged after 28.67 days (Fig.3). In plants that were exposed for 3 and 4 weeks respectively, inflorescences had emerged by the time they reached back to Vellanikkara. In plants kept under fan and pad, plants exposed for two weeks produced inflorescence after 26.67 days. But the other two treatments had inflorescence even before arriving (Fig.4).

Plate 6. Flower induction in phalaenopsis var. Roxenne by low temperature treatment (Higher altitude)

Treatment pants kept in rain-shelter

Treatment plants kept in fan and pad

Table. 3 Flowering characters in phalaenopsis plants exposed to low temperature (higher altitude) and grown in rain-shelter

Sl.No	Duration (Weeks)	Emergence of inflorescence (days)	Emergence of first flower bud (days)	Opening of first flower bud (days)	Flowering duration (days)	Length of inflorescence (cm)	Florets/ inflorescence (no)	Flower size length (cm)x breadth(cm)
1	0	80.33 (8.96^{b})*	27.70^{a}	51.97^{a}	65.17	28.57^{a}	5.30^{a}	10.51^{a}x8.70^{a}
2	2	28.67 (4.74^{a})*	30.33^{a}	56.33^{a}	70.83	33.20^{a}	5.75^{a}	10.37^{a}x8.61^{a}
3	3	18.00 (4.24^{a})*	29.30^{a}	61.33^{ab}	71.17	33.53^{a}	5.84^{a}	10.18^{a}x8.57^{a}
4	4	18.27 (4.27^{a})	31.73^{a}	63.73^{ab}	74.33	34.70^{a}	5.30^{a}	9.87^{a}x8.37^{a}

*Square root transformation value

Treatments with even alphabets (a, b) as superscripts form one homogeneous group

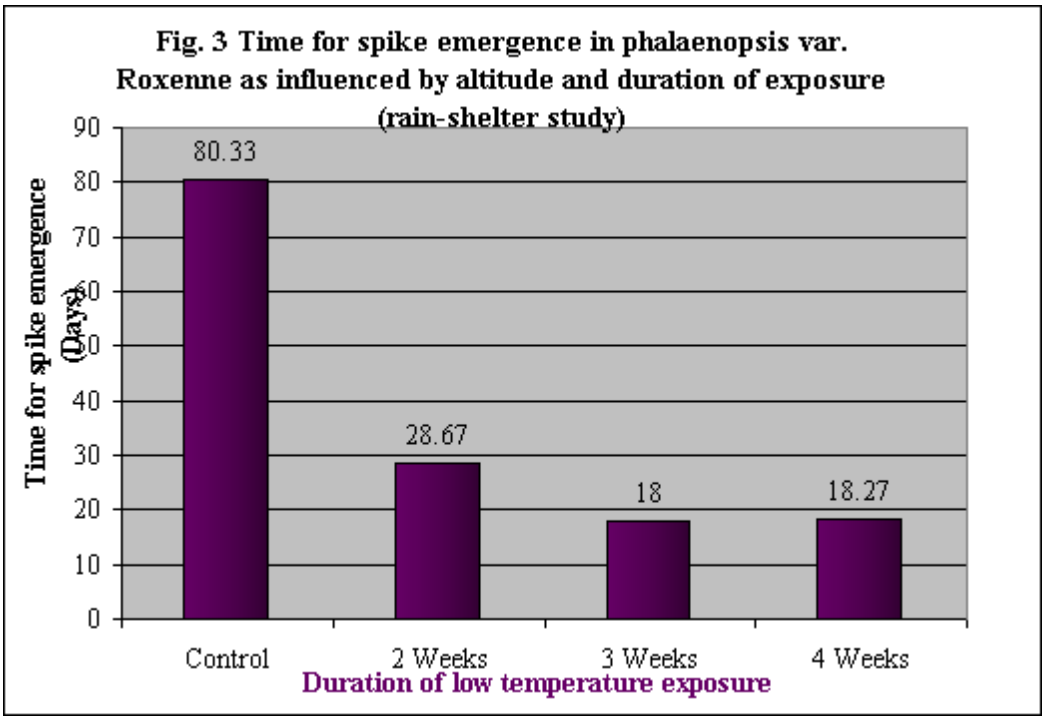


Fig. 3 Time for spike emergence in phalaenopsis var. Roxenne as influenced by altitude and duration of exposure (rain-shelter study)
Time for spike emergence (Days)
90
80
70
60
50
40
30
20
10
0
80.33
28.67
18
18.27
Control
2 Weeks
3 Weeks
4 Weeks
Duration of low temperature exposure

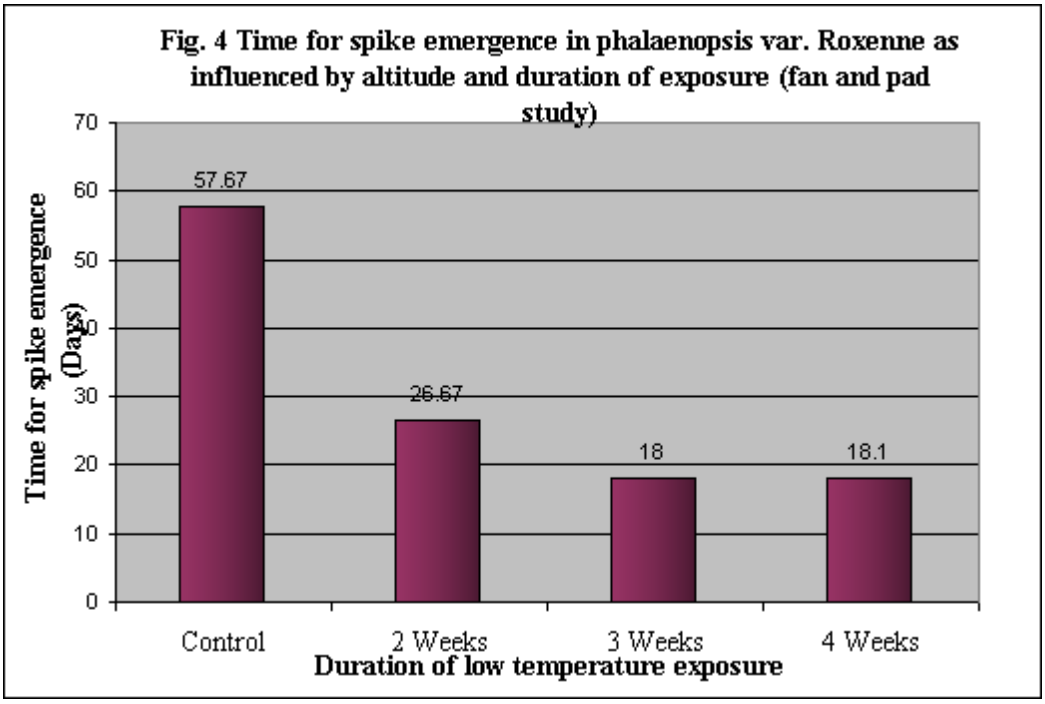


Fig. 4 Time for spike emergence in phalaenopsis var. Roxenne as influenced by altitude and duration of exposure (fan and pad study)
Time for spike emergence (Days)
70
60
50
40
30
20
10
0
57.67
26.67
18
18.1
Control
2 Weeks
3 Weeks
4 Weeks
Duration of low temperature exposure

Table. 4 Flowering characters in phalaenopsis plants exposed to low temperature (higher altitude) and grown in fan and pad

Sl.No.	Duration (Weeks)	Time for emergence of spike (days)	Time for bud emergence (days)	Time for opening of first bud(days)	Flowering duration (days)	Spike length (cm)	Flower count (no)	Flower size length(cm) x breadth (cm)
1	0	57.67 (7.5^{b})*	32.33^{a}	55.66^{a}	183.17^{b}	39.63^{a}	9.67^{b}	9.96^{a} x 8.77^{a}
2	2	26.67 (4.88^{a})*	30.66^{a}	60.63^{a}	229.83^{a}	48.53^{a}	16.33^{a}	10.20^{a}x 8.57^{a}
3	3	18.00 (4.24^{a})*	30.00^{a}	59.00^{a}	226.83^{a}	48.27^{a}	15.33^{a}	10.13^{a} x 8.43^{b}
4	4	19.30 (4.39^{a})	30.37^{a}	59.37^{a}	219.00^{a}	45.33^{a}	13.33^{a}	9.90^{a} x 8.77^{a}

*Square root transformation value

Treatments with even alphabets (a, b) as superscripts form one homogenous group

4.2.1.2 Time taken for emergence of first flower bud

In rain-shelter, emergence of the first bud recorded post inflorescence emergence was earliest (27.70 days) in control. It was on par with the other treatments. Under Fan and pad system, emergence of bud was on par among treatments.

4.2.1.3 Time for opening of first flower bud

Days taken for opening of the first bud was minimum (51.97 days) in plants kept in rain-shelter as control. However the value was on par in case of plants exposed to low temperature for 2 weeks (56.33 days), 3 weeks (61.33 days) and 4 weeks (63.73 days). In fan and pad, days taken for opening of flower bud was minimum (55.66 days) in control. The plants exposed to higher altitude were on par with that of control.

4.2.1.4 Flowering duration

In rain-shelter studies, flowering duration was higher (74.33 days) in plants kept for 4 weeks at higher altitude. It was on par with 3 weeks (71.17 days) and 2 weeks (70.83 days). Plants kept in fan and pad had significantly longer flowering duration compared to rain-shelter. Among treatment plants, plants exposed for 2, 3 and 4 weeks were on par with 229.83 days, 226.83 days and 219.00 days. Plants kept as control had a shorter duration (183.17 days).

4.2.1.5 Inflorescence length

In plants kept under rain-shelter, inflorescence length was maximum (34.70 cm) in plants exposed for 4 weeks. It was closely followed and on par in plants kept for 3 weeks (33.53 cm) and 2 weeks (33.20 cm). In control, inflorescence length was minimum (28.57 cm) but still on par with treatment plants. In fan and pad, inflorescence length was superior compared to rain-shelter. Inflorescence length was

on par in plants exposed for 2 weeks (48.53 cm), 3 weeks (48.27 cm) and 4 weeks (45.33 cm). It was minimum (39.63 cm) for control, which was on par with treatments.

4.2.1.6 Flower count

In rain-shelter, flower count was on par among treatments. Plants kept under fan and pad had a significantly superior flower count. Maximum flower count (16.33) was observed in plants exposed for 2 weeks which was on par with that of plants kept for 3 weeks (15.33) and 4 weeks (13.33). Flower count was minimum in control (9.67).

4.2.1.7 Flower size

No significant difference was noted among treatments with respect to flower size.

4.2.2 Cold room (Air conditioned) conditions

Data generated from the studies on exposure of plants to low temperature of 23°C (± 2^0C) by keeping in AC room are presented in Table 5, Figures 5,6,7,8 and Plate 7.

4.2.2.1 Time for emergence of inflorescence

Inflorescence emergence was found to be markedly influenced by cold treatment (Fig. 5). Emergence of flower inflorescence was earliest and on par in plants exposed to low temperature in the cold room for two and three weeks (14.41 and 14.43 days, respectively) compared to that of control (51.17 days).

4.2.2.2 Time for emergence of first flower bud

Emergence of the first bud was earliest (31.43 days) in control plants. The value was on par in plants kept for 2 and 3 weeks (34.30 days and 34.57 days, respectively).

Plate 7. Flower induction in phalaenopsis var. Roxenne by low temperature treatment (AC room)

Plants exposed to low temperature in an AC room

Performance of plants after low temperature treatment in AC room

Table. 5. Effect of flower induction studies in phalaenopsis plants exposed to low temperature(cold room condition

Sl.No	Duration of exposure to cold temperature	Emergence of inflorescence (days)	Emergence of first flower bud (days)	Opening of first flower bud (days)	Flowering duration (days)	Inflorescence length (cm)	Inflorescence (no)	Florets/ inflorescence (no)	Flower size Length x Breadth(cm^2)
1	Control	51.17[b]	31.43[a]	43.57[a]	45.70[b]	26.27[b]	1.00[a]	3.57[b]	10.25[a] x 8.70[a]
2	2 Weeks	14.41[b]	34.30[a]	58.01[b]	92.43[a]	34.99[a]	1.00[a]	6.71[a]	10.37[a] x 8.61[a]
3	3 Weeks	14.43[b]	34.57[a]	62.86[b]	91.86[a]	33.20[a]	1.00[a]	6.14[b]	10.18[a] x 8.57[a]

Treatments with even alphabets (a,b) as superscripts form one homogenous group

Fig. 5 Time for emergence of spike in phalenopsis var. □Roxenne kept in AC room

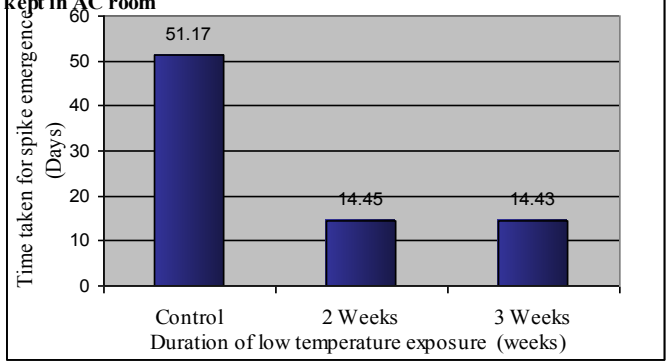


Fig. 6 Flowering duration in phalaenopsis var. Roxenne as influenced by low temperature exposure (AC room)

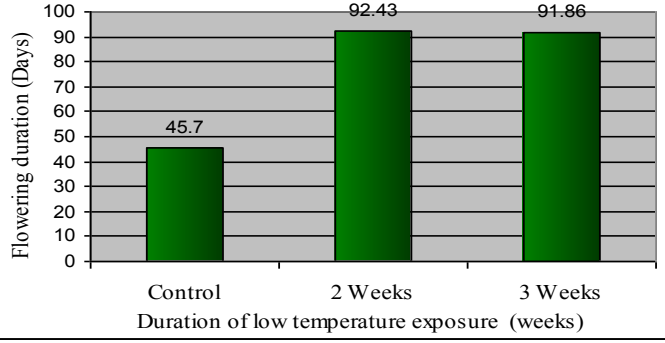


Fig 7 Spike length in phalaenopsis var. Roxenne as influenced by low temperature exposure (AC room)

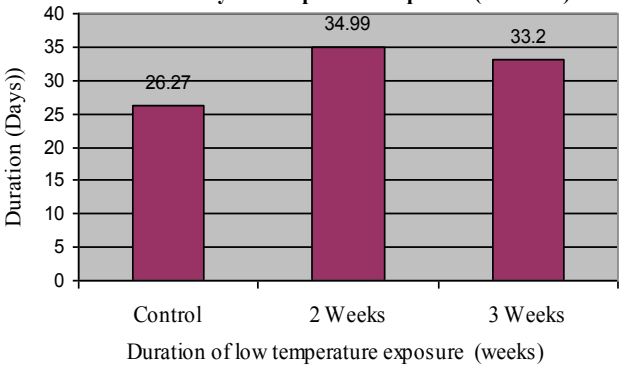
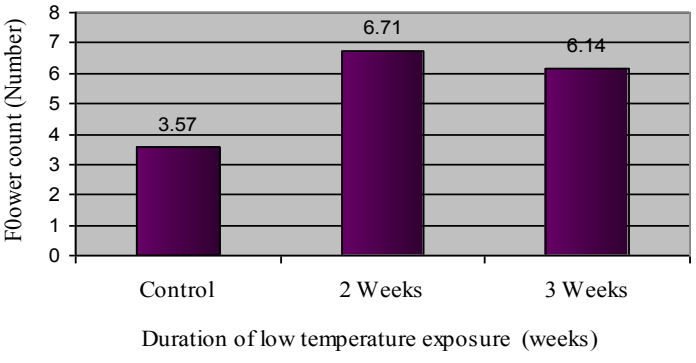


Fig. 8 Flower count in phalaenopsis var. Roxenne as influenced by low temperature exposure (AC room)



4.2.2.3 Time for opening of first flower bud

Opening of the first flower bud occurred at the earliest (43.57 days) in control plants. The plants exposed for 3 weeks of cold room temperature opened buds later (62.86 days) and was on par in plants kept for 2 weeks (58.01 days).

4.2.2.4 Flowering duration

Flowering duration was considerably higher in treatment plants than in control (Fig.6). Flowering duration was maximum in case of plants kept in cold room for 2 weeks (92.43 days) closely followed and on par in plants exposed for 3 weeks (91.86 days). Control recorded the minimum period of 45.70 days.

4.2.2.5 Length of inflorescence

Appreciable difference in inflorescence length (Fig.7) was recorded in plants exposed to cold treatment compared to that of control which recorded a minimum inflorescence length (26.27 cm). Inflorescence length of plants that received cold treatment for 2 weeks was higher (34.94 cm) but on par with the plants kept in the cold room for 3 weeks (33.20 days).

4.2.2.6 Number of inflorescence

No difference was noted among treatments with respect to inflorescence count.

4.2.2.7 Flower count

Significant difference in the number of flowers produced per inflorescence was observed in plants exposed to cold treatment compared to that of control (Fig.8). Flower count in plants exposed for 2 weeks was maximum (6.71) closely followed and on par with plants kept for 3 weeks (6.14). Flower count was minimum (3.57) in control.

4.2.2.8 Flower size

Cold treatment had no significant influence on size of flowers.

4.3 Nutrient-Growth regulator combination

Data generated from experiment involving application of nutrient and growth regulator combinations for 12 months are presented. It was carried out for pot plant (Lin Jessica) and cut flower types (Taisuco Confidence). Details are furnished in Tables 6, 7 and 8.

4.3.1 Vegetative characters

Data with respect to increase in vegetative growth is presented in Table 6

4.3.1.1. Leaf length, breadth and area

In pot plants type, leaf length, breadth and total leaf area was maximum (7.30cm, 3.00 cm and 22.17 cm^2) in plants treated with 0.1% of 20: 10: 10 of NPK. It was minimum in 0.1 % of 10:10:10 which had a length, breadth and area of 5.17 cm, 1.97 cm and 10.70 cm^2. Among treatments for cut flower varieties, 0.1% 20:10:10 NPK was the best which recorded leaf length, breadth and leaf area of 4.33cm, 2.61cm, 11.91 cm^2 respectively.

4.3.1.2. Interval of leaf production

In pot plant variety, interval of leaf production was minimum (187.10 days) in plants treated with 0.1% of 20: 10:10 and maximum (231.00 days) in plants treated with 0.1% of 10: 20:10 NPK and GA_3 1ppm. In cut flower type, interval of leaf production was minimum (228.03 days) in plants with 20:10:10 NPK and maximu in treatment of 10:20:10 in combination with Kinetin 100 ppm (312.03 days).

Table. 6. Vegetative characters of cut flower and pot plant type of phalaenopsis in nutrient growth regulator combination Studies

	Treatments (Nutrient, growth regulator combination)	POT PLANT				CUT FLOWER				
		Increase in leaf length (cm)	Increase in leaf breadth (cm)	Increase in leaf area (cm^2)	Leaf Production interval (days)	Increase in leaf length (cm)	Increase in leaf breadth (cm)	Increase in leaf area (cm^2)	Leaf production interval (days)	Leaf production interval (days)
1	10:10:10	5.17	1.97	10.70^{e}	194.00^{a}	3.11	2.30	7.24^{d}	271.33^{c}	271.33^{c}
2	20:10:10	7.30	3.00	22.17^{a}	187.10^{a}	4.33	2.61	11.91^{a}	228.03^{a}	228.03^{a}
3	10:20:10	7.05	2.24	16.23^{c}	214.07^{b}	3.04	2.19	6.88^{de}	294.22^{c}	294.22^{c}
4	10:10:10+GA3 1ppm	7.13	2.44	17.60^{b}	219.23^{bc}	3.27	2.38	8.21^{c}	248.13^{b}	248.13^{b}
5	10:10:10+GA3 2ppm	6.96	2.91	20.50^{a}	224.61^{c}	3.09	2.41	8.19^{c}	266.01^{bc}	266.01^{bc}
6	20:10:10+GA3 1ppm	7.08	2.96	19.85^{ab}	199.03^{a}	4.02	2.37	10.04^{b}	281.75^{c}	281.75^{c}
7	20:10:10+GA3 2ppm	6.03	2.69	16.57^{c}	206.12^{a}	4.71	2.33	10.81^{a}	291.68^{c}	291.68^{c}
8	10:20:10+GA3 1ppm	6.74	2.53	18.65^{ab}	231.33^{d}	3.92	2.41	9.94^{b}	252.10^{b}	252.10^{b}
9	10:20:10+GA3 2ppm	6.16	2.26	14.08^{d}	191.07^{a}	4.40	2.27	10.06^{b}	234.12^{a}	234.12^{a}
10	10:10:10+Kinetin 100 ppm	6.08	2.24	13.11^{d}	208.02^{b}	3.46	2.41	8.48^{bc}	238.21^{a}	238.21^{a}
11	10:10:10+ Kinetin 200 ppm	5.83	2.17	12.92^{de}	222.22^{d}	3.61	2.43	8.94^{bc}	244.33^{ab}	244.33^{ab}
12	20:10:10+Kinetin 100 ppm	7.01	3.54	20.71^{a}	190.51^{a}	4.48	2.42	11.03^{a}	292.45^{c}	292.45^{c}
13	20:10:10+ Kinetin 200 ppm	6.07	2.71	17.76^{c}	204.08^{b}	3.91	2.65	10.4^{ab}	233.00^{a}	233.00^{a}
14	10:20:10+Kinetin 100 ppm	6.11	2.50	17.43^{c}	196.11^{a}	3.73	2.58	9.83^{b}	312.03^{d}	312.03^{d}
15	10:20:10+ Kinetin 200 ppm	5.34	2.32	12.73^{de}	209.12^{b}	3.70	2.60	9.64^{b}	268.00^{bc}	268.00^{bc}

* Pot plants variety: Lin Jessica

* Cut flower variety: Taisuco Confidence

Treatments with even alphabets (a, b) as superscripts form one homogenous group

4.3.2 Flowering characters

Data regarding flowering and floral characters of pot plant and cut flower are presented in Table 7 and Table 8 respectively.

4.3.2.1. Time for emergence of first flower bud

In pot plant variety, Lin Jessica, there were no appreciable differences among treatments. The buds emerged earlier (30.20 days) in plants treated with 0.1% of 10:20:10 NPK and later (34.70 days) in plants treated with 0.1% of 10:10:10 combined with Kinetin 100ppm as treatment.

In cut flower variety Taisuco Confidence, there was no significant difference among treatments. The buds emerged earlier (40.20 days) in the combination involving 0.1% of 20:10:10 NPK with GA_3 1ppm and later (44.30 days) in case of 10:20:10 NPK along with GA_3 2ppm

4.3.2.2. Time for opening of the first flower bud

In pot plant variety, all the treatments were on par implying no significant differences among treatments.

In cut flower variety, there was no significant difference among treatments. The buds opened earlier (61.90 days) in the combination involving 0.1% of 20:10:10 NPK and after 67.60 days in case of 10:10:10 NPK with GA_3 2ppm.

4.3.2.3. Flowering duration

In pot plant variety, flowering duration was maximum (76.75 days) in plants treated with 0.1% of 10:10:10 NPK and 100ppm Kinetin and minimum (66.00 days) in 0.1% of 10:20:10 and GA_3 1ppm as treatment.

Table7 Effect of nutrient - growth regulator combination treatment on flowering and floral characters in pot plant type* of phalaenopsis

Treatments (Nutrient , growth regulator combinations)	Time for emergence of first bud (days)	Time for opening of first bud (days)	Spike count (no)	Length of spike (cm)	Florets/ spike (no)	Flowering duration (days)	Flower size length (cm) x breadth (cm)
10:10:10- 0.1%	31.90	58.90	1.00	21.65	3.50	69.25	8.02 x 6.65
20:10:10- 0.1 %	33.40	57.20	1.00	20.75	4.25	70.50	7.63 x 6.81
10:20:10- 0.1%	30.20	59.50	1.00	21.18	3.50	68.00	7.92 x 6.71
10:10:10– 0.1 %+GA3 1ppm	33.20	58.60	1.00	23.90	4.75	68.75	8.14 x 6.70
10:10:10- 0.1%+GA3 2ppm	31.40	57.70	1.00	24.20	4.50	73.25	8.12 x 6.78
20:10:10- 0.1%+GA3 1ppm	32.60	58.80	1.00	24.95	4.00	70.25	7.73 x 6.85
20:10:10- 0.1%+GA3 2ppm	31.70	56.90	1.00	20.80	3.50	69.50	7.72 x 6.45
10:20:10- 0.1%+GA3 1ppm	32.80	57.80	1.00	27.43	4.75	66.00	7.91 x 6.72
10:20:10- 0.1%+GA3 2ppm	31.90	58.40	1.00	22.70	3.75	75.50	7.70 x 6.65
10:10:10- 0.1 %+Kinetin 100 ppm	34.70	60.30	1.25	21.80	3.25	76.75	8.00 x 6.95
10:10:10-0.1%+ Kinetin 200ppm	31.80	58.60	1.00	22.10	4.25	74.25	7.92 x 6.45
20:10:10- 0.1%+Kinetin 100ppm	33.10	60.00	1.00	22.08	3.00	74.50	7.70 x 6.45
20:10:10- 0.1%+ Kinetin 200ppm	32.40	58.30	1.00	23.55	3.75	74.00	8.00 x 6.65
10:20:10- 0.1%+Kinetin 100ppm	32.00	57.20	1.00	26.40	4.25	74.50	7.92 x 6.80
10:20:10- 0.1%+ Kinetin 200ppm	30.90	58.30	1.25	22.85	3.00	70.00	8.00 x 6.83
C.D (.05)	1.92	2.07	0.11	2.43	0.54	4.12	0.3

* variety Lin Jessica

Table.8 Effect of nutrition - growth regulator combination treatment on flowering and floral charcters in cut flower* type of phalaenopsis

Treatments (Nutrient , growth regulator combinations)	Time for emergence of first bud (days)	Time for opening of first bud (days)	Flowering duration (days)	Spike/ year (no)	Length of spike (cm)	Florets/ spike (no)	Flower size length (cm) x breadth (cm)
10:10:10- 0.1%	43.10	66.20	41.40	0.87	25.03	3.00	9.81 x 8.60
20:10:10- 0.1 %	40.20	61.90	39.20	0.80	22.80	2.00	10.51 x 9.60
10:20:10- 0.1%	44.30	64.30	42.00	1.00	23.66	3.00	9.90 x 9.20
10:10:10– 0.1 %+GA3 1ppm	41.90	62.80	37.00	1.00	34.63	2.00	9.85 x 9.12
10:10:10- 0.1%+GA3 2ppm	43.70	67.60	45.00	1.00	25.75	3.00	9.42 x 8.51
20:10:10- 0.1%+GA3 1ppm	41.90	61.90	39.40	0.85	31.30	2.00	10.05 x 9.32
20:10:10- 0.1%+GA3 2ppm	43.20	62.30	46.00	0.70	25.88	3.00	10.2 0x 9.32
10:20:10- 0.1%+GA3 1ppm	42.70	63.10	40.00	1.00	36.40	2.00	10.25 x 9.30
0:20:10- 0.1%+GA3 2ppm	44.30	65.20	52.30	1.00	33.55	3.25	10.01 x 9.60
10:10:10- 0.1 %+Kinetin 100 ppm	42.10	63.80	43.10	1.00	30.75	3.00	9.35 x 8.51
10:10:10-0.1%+ Kinetin 200ppm	40.90	62.90	39.00	1.00	29.75	2.00	10.20 x 98.8
20:10:10- 0.1%+Kinetin 100ppm	39.90	63.40	41.30	1.00	29.93	2.30	9.55x 8.50
20:10:10- 0.1%+ Kinetin 200ppm	41.80	67.40	40.60	1.00	30.00	2.70	9.70 x 9.11
10:20:10- 0.1%+Kinetin 100ppm	40.70	63.80	48.10	1.00	31.37	3.00	9.22 x 8.51
10:20:10- 0.1%+ Kinetin 200ppm	41.90	64.80	38.90	1.00	30.20	2.00	9.30 x 8.81
C.D (.05)	2.04	3.12	2.10	0.16	1.32	0.27	0.22

Variety Taisuco Kochdian.

In cut flower variety, flowering duration was maximum (52.30 days) in 0.1 % 10: 20: 10 in combination with GA_3 2ppm and minimum (37.00 days) in the combination involving 0.1% of 10:10:10 NPK with GA_3 1ppm.

4.3.2.4. Length of inflorescence

In pot plants, length of inflorescence was highest (27.43 cm) in plants treated with 0.1% of 10:20:10 along with GA_3 1ppm and lowest in plants treated with 0.1% of 20:10:10 NPK. In cut flower type, inflorescence length was highest (36.40 cm) in plants treated with 0.1 % of 10:20:10 in combination with GA_3 1ppm and lowest (22.80) in plants treated with 0.1% 20:10:10 NPK alone.

4.3.2.5. Number of florets per inflorescence

In pot plants, flower count was maximum (4.75) in plants treated with 0.1% of 10:20:10 and GA_3 1ppm and minimum (3.00) in plants treated with .1% of 20:10:10 NPK and Kinetin 100ppm. In cut flower type, flower count was highest (3.25) in plants treated with 10:20:10 along with GA_3 2ppm.There was no significant difference among treatments.

4.3.2.6. Flower size

There were no significant differences among treatments with regard to flower size.

4.4 Inflorescence pruning

Data pertaining to pruning of spent inflorescence of *Phalaenopsis* var. Roxanne at above 1st, 2nd and 3rd node is presented along with control plants (wherein inflorescences were retained) is presented in Table 9, Figure 9 and Plate 5. Inflorescence pruning was done in pot plant variety Carlotta which showed no response during the period of study.

Plate 8. Inflorescence pruning studies in phalaenopsis var. Roxanne

Levels of pruning

4.4.1 Time for emergence of inflorescence

Significant differences were observed among treatments with regard to the number of days taken for the emergence of inflorescence (Fig. 9). The number of days taken was minimum (23.80 days) in plants where inflorescences were pruned at the first node. It was followed by the third node (36.40 days) and distantly followed when pruned at second node (66.60). Treatments were significantly superior compared to non pruned plants (control) wherein new inflorescences emerged after 174.40 days

4.4.2 Time for emergence of first flower bud

No marked difference followed among treatments with respect to the number of days taken for the emergence of the first bud on the newly emerged inflorescences.

4.4.3 Time for opening of first flower bud

No marked difference followed among treatments with respect to the number of days taken for the emergence of the first bud on the newly emerged inflorescences.

4.4.4. Flowering duration

Flowering period was longer in plants pruned at the first node (38.80 days). Other treatments were on par with values 33.80 days, 32.40 days and 31.40 days for inflorescences pruned at second node, control and third node, respectively.

4.4.5 Inflorescence length

Among the treatments, no significant difference was observed with regard to Inflorescence length.

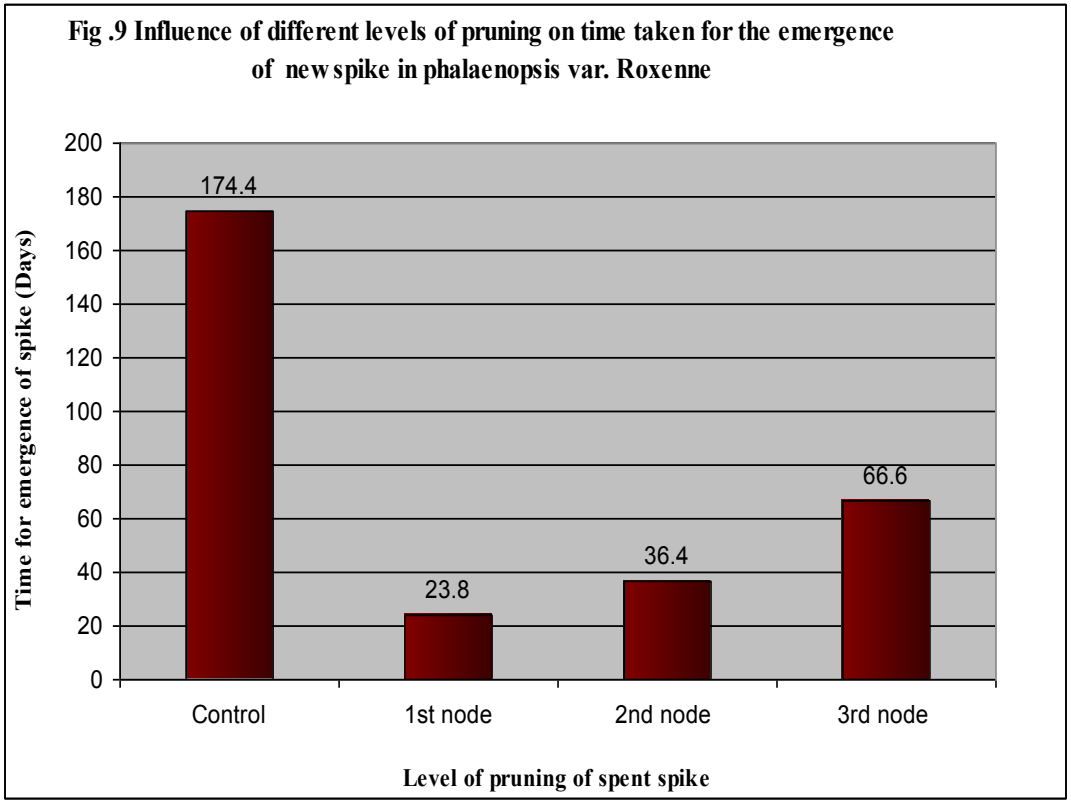
Table. 9. Influence of levels of spike pruning on flowering and floral characters in phalaenopsis var. Roxanne

Sl.No	Level of pruning	Emergence of inflorescence (days)	Emergence of first flower bud (days)	Opening of first flower bud(days)	Flowering duration (days)	Length of Inflorescence (cm)	Inflorescence (no)	Florets/spike (no)	Flower size Length (cm)x Breadth (cm)
1	Control (no pruning)	174.40 (12.61^{b})*	31.60^{a}	62.40^{a}	32.40^{ab}	33.22^{a}	1.00^{a}	4.00^{a}	10.56^{a}x8.82^{a}
2	1st node	23.80 (4.88a)*	31.00^{a}	61.40^{a}	38.80^{a}	29.44^{ab}	1.00^{a}	3.60^{a}	10.36^{a}x8.52^{a}
3	2nd node	66.60 (7.40a)*	31.03^{a}	62.40^{a}	33.80^{b}	29.30^{b}	0.60^{b}	3.20^{a}	10.40^{b}x8.70^{a}
4	3rd node	36.40 (5.95a)*	31.20^{a}	64.00^{a}	31.40^{b}	31.06^{ab}	0.80^{ab}	3.80^{a}	10.10^{ab}x8.50^{a}

* square root transformation values

Treatments with even alphabets (a,b) as superscripts form one homogenous group

Fig .9 Influence of different levels of pruning on time taken for the emergence of new spike in phalaenopsis var. Roxenne



4.4.6 Flower count

Flower count showed no significant differences among treatments.

4.4.7 Flower size

Flower size remained without significant difference among treatments.

4.5 Growing media studies

Data collected from plants of phalaenopsis var. Magic Kiss grown in different media, namely, sphagnum moss, coconut husk chips and coconut husk bits are presented in Table10, Table11, Figures 10, 11, 12, 13 and Plates 9 and 10.

4.5.1 Vegetative **characters**

4.5.1.1 Plant height

The data pertaining to the effect of different growing media on increase in plant height are presented in Table 10. The plants were significantly taller (1.64 cm) when sphagnum moss alone was used. Plants grown in husk and chips were on par with an increase of 0.21 cm and 0.40 cm respectively.

4.5.1.2 Leaf length, leaf breadth and leaf area

Significant differences were observed with regard to leaf characters among treatments (Fig.10). Leaf length, leaf breadth and leaf area were maximum with 16.72 cm, 9.87cm and 87.78 cm^2, respectively, in plants w here sphagnum moss was used. It was minimum in plants grown with coconut husk bits, recording 8.94 cm, 5.32 cm and 42.00 cm^2 which were on par with plants grown in coconut chips as growing medium.

4.5.1.2 Interval of leaf production

Plate 9. Studies on the effect growing media in phalaenopsis var. Magic Kiss (along with tile bits and charcoal)

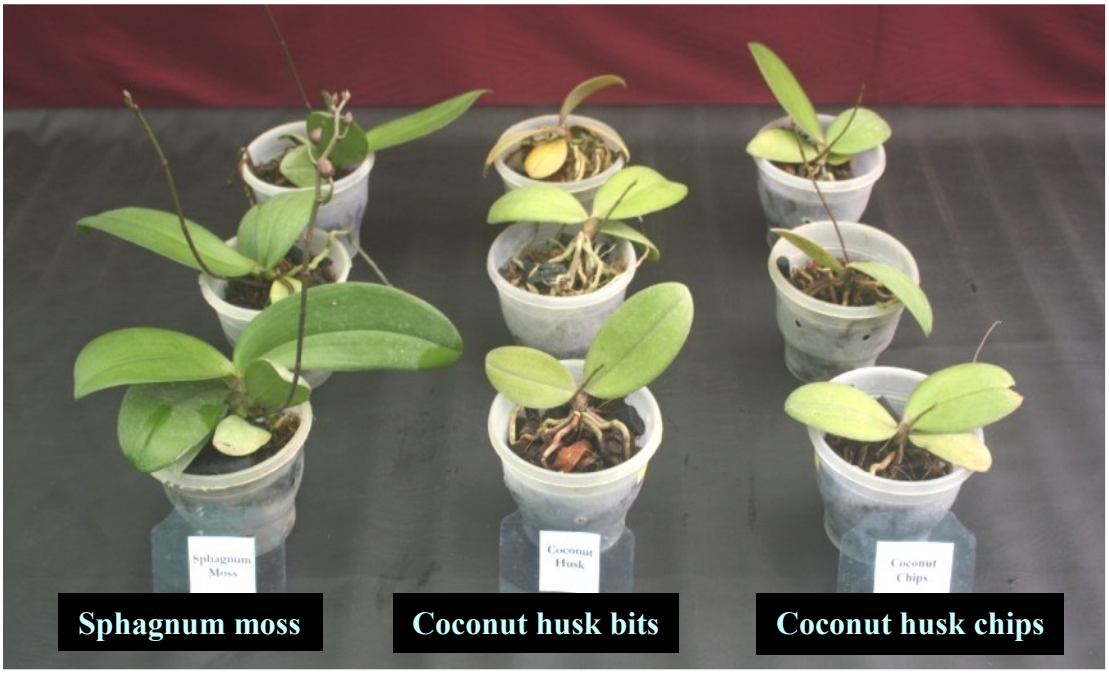


Table. 10. Influence of growing media on vegetative characters of phalaenopsis var. 'Magic Kiss' for a period of one year

	Growing Media*	Increase in plant height (cm)	Increase in leaf length (cm)	Increase in leaf breadth (cm)	Increase in leaf area (cm^2)	Interval of leaf production (days)
1	Sphagnum moss	1.64^a	16.72^a	7.02^a	87.78^a	91.40^a
2	Coconut husk chips	0.40^b	9.87^b	5.63^b	39.30^b	221.90^b
3	Coconut husk bits	0.21^b	8.94^b	5.32^b	42.00^b	260.50^b

* In addition to charcoal and tile bits

Treatments with even alphabets (a,b) as superscripts form one homogenous group

Fig. 10 Influence of growing media on increase in leaf area(cm^2) in phalaenopsis var. Magic Kiss

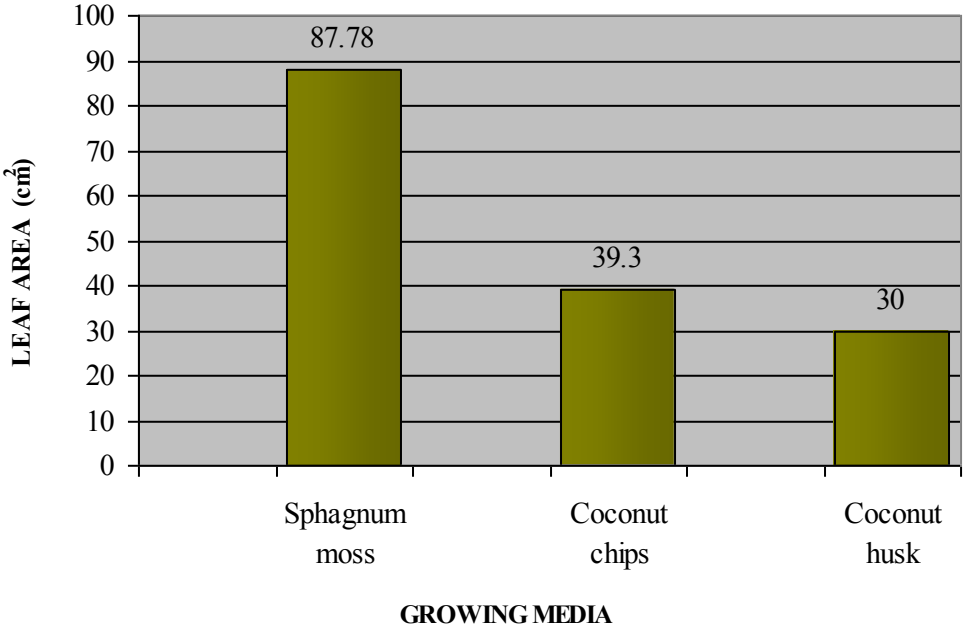
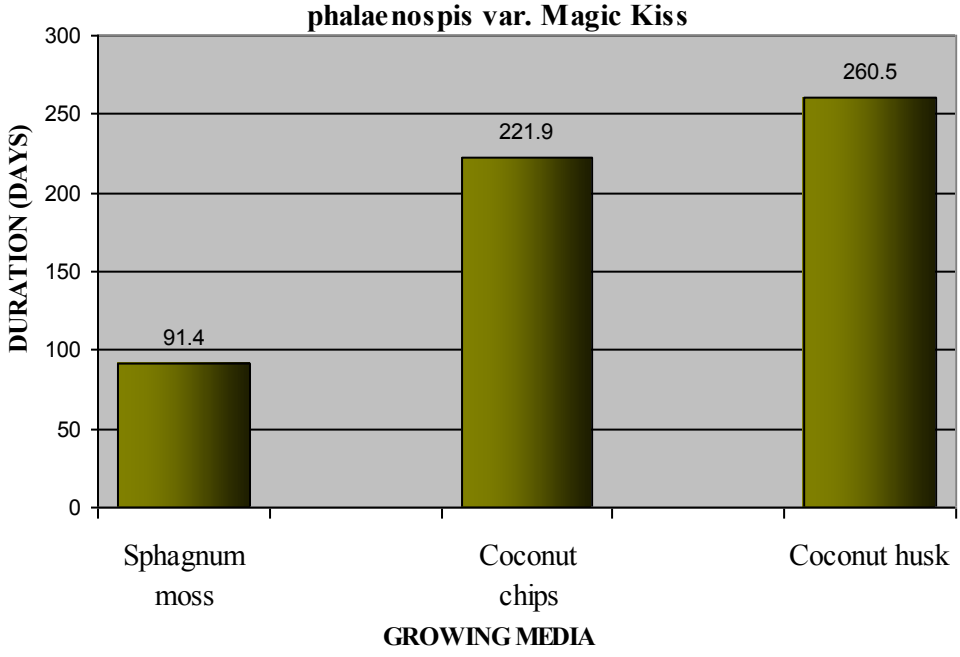


Fig. 11 Influence of growing media on leaf production interval in phalaenospis var. Magic Kiss



Remarkable differences were noted with regard to the interval of production of leaves in plants where sphagnum moss was used as the growing medium, compared to the other two (Fig.11). New leaves were produced at an interval of 91.40 days in treatment with sphagnum moss, which was significantly superior to coconut chips (221.90 days) and coconut husk (260.50 days).

4.5.2 Flowering and floral characters

4.5.2.1 Time taken for emergence of inflorescence

Time taken from imposing the various treatments was taken into consideration. Inflorescence emergence occurred at the earliest (124.90 days) in the treatment plants where moss was used as the medium. Time taken for emergence of inflorescences was on par when coconut husk chips and coconut husk bits were used with values 153.30 days and 155.90 days, respectively.

4.5.2.2 Time taken for emergence of first flower bud

The duration for emergence of the first flower bud was on par among treatments.

4.5.2.3 Time taken for the opening of first flower bud

Opening of the first flower bud occurred at the earliest (57.52 days) in treatment where moss was used. It was closely followed and on par in plants grown with coconut husk chips (58.20) and coconut husk bits (59.85) as media.

4.5.2.4 Number of inflorescence

Significant differences were observed among treatments in the number of inflorescences produced. Inflorescence count of 1.80 was recorded in plants grown in moss. This was significantly superior compared to coconut chip bits and coconut husk bits which recorded 0.90 and 0.80, respectively and were on par.

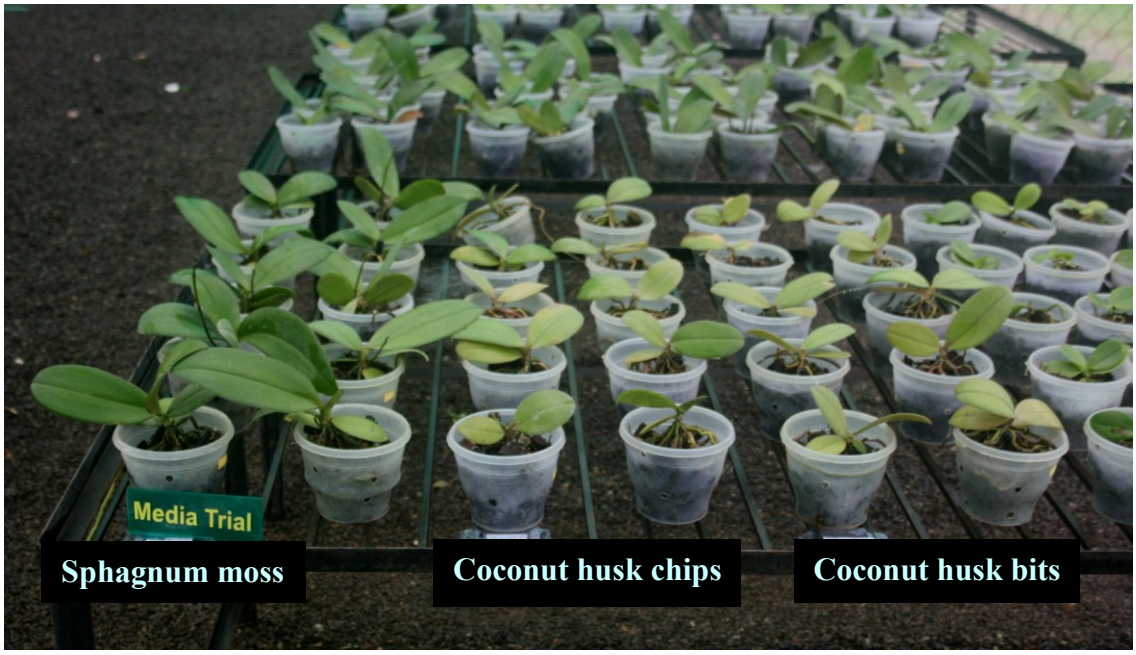


Plate 10. Studies on the effect of growing media in phalaenopsis var. Magic Kiss (field picture)

Table 11. Influence of growing media on flowering and floral charcters of phalenopsis var. Magic Kiss

Sl No	Growing Media	Time for emergence of spike (days)	Time for emergence of flower bud (days)	Time for opening of 1st flower bud	Flowering duration (days)	Length of inflorescence (cm)	Length of rachis (cm)	Spike count (no)	Flower count (no)	Flower Size length (cm)xbreadth (cm)
1	Sphagnum moss	124.90[a]	39.20[a]	57.52[a]	112.70[a]	28.17[a]	6.07[a]	1.80[a]	7.30[a]	5.94[a] x5.94[a]
2	Coconut husk chips	153.30[b]	38.80[a]	58.20[a]	73.40[b]	14.80[b]	2.26[b]	0.90[b]	3.30[b]	5.83[ab] x5.86[ab]
3	Coconut husk bits	155.90[b]	39.15[a]	59.85[a]	70.10[b]	14.03[b]	1.77[b]	0.80[b]	2.10[b]	5.68[b] x5.68[b]

*In addition to tile and charcoal bits

Treatments with even alphabets (a,b) as superscripts form one homogenous group

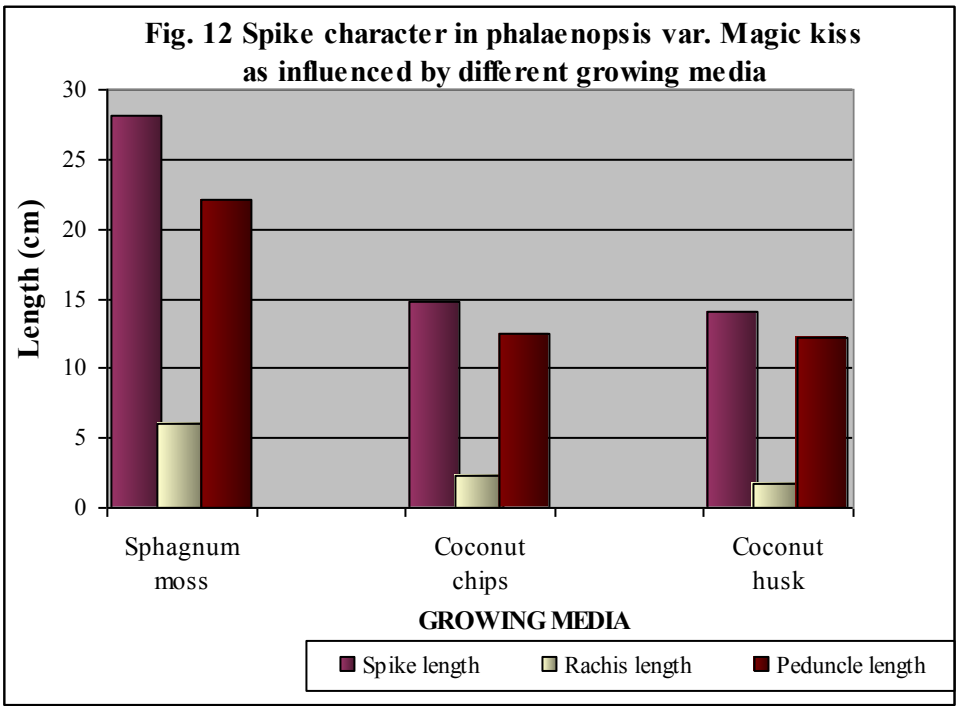


Fig. 12 Spike character in phalaenopsis var. Magic kiss as influenced by different growing media
Length (cm)
30
25
20
15
10
5
0
Sphagnum moss
Coconut chips
Coconut husk
GROWING MEDIA
Spike length
Rachis length
Peduncle length

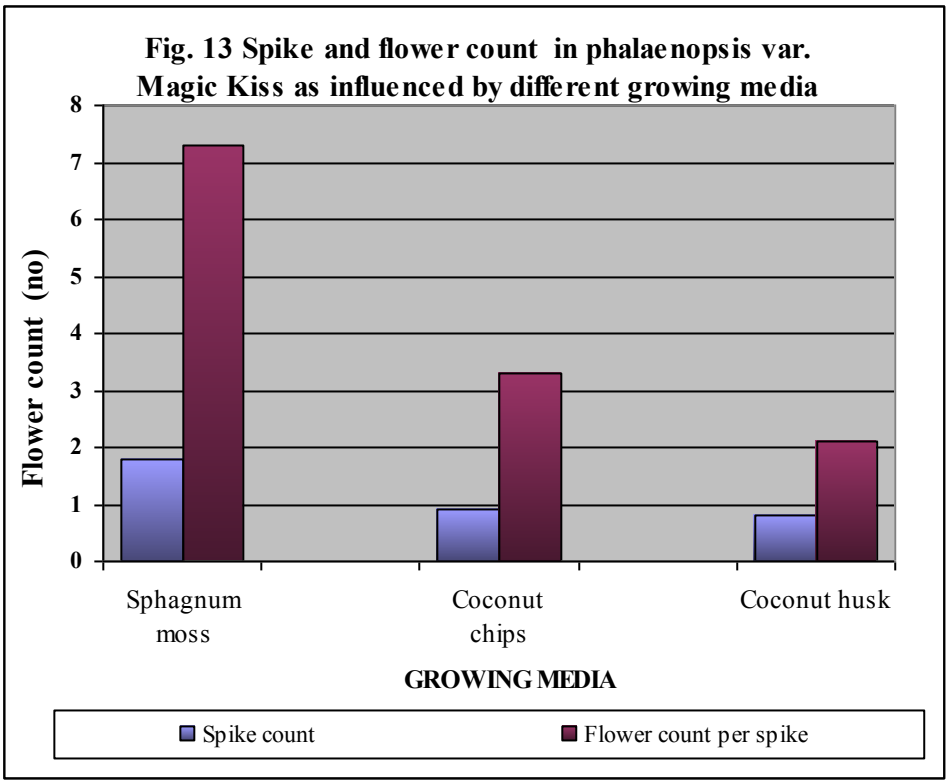


Fig. 13 Spike and flower count in phalaenopsis var. Magic Kiss as influenced by different growing media
Flower count (no)
8
7
6
5
4
3
2
1
0
Sphagnum moss
Coconut chips
Coconut husk
GROWING MEDIA
Spike count
Flower count per spike

4.5.2.5 Flowering duration

Significant differences were observed with regard to flowering duration among treatments. It was maximum (112.70 days) in plants where moss was used and the values were on par when coconut chips and coconut husk were used (73.40 and 70.10 days, respectively).

4.5.2.6 Inflorescence length

Inflorescence length recorded highest value (28.17 cm) in plants where moss was used as media. It was significantly superior to coconut husk chips and coconut husk bits as media wherein the length of inflorescence was 14.80 cm and 14.03 cm, respectively.

4.5.2.7 Rachis length

Rachis length was the highest (6.07 cm) in plants where moss was used as medium. It was significantly superior to the other two treatments with values 1.77 cm for coconut husk bits and 2.26 cm for coconut husk chips, which were on par.

4.5.2.8 Peduncle length

Peduncle length was the highest (22.10 cm) in plants where moss was used and it was significantly superior to the other treatments which were on par with values 12.54 cm and 12.26 cm in coconut husk chips and coconut husk bits as medium.

4.5.2.9 Number of flowers per inflorescence

Number of flowers per inflorescence was the maximum (7.30) in moss. The values were significantly superior to coconut husk chips and coconut husk bits (3.30 and 2.10, respectively) which were on par.

4.5.2.10 Flower size

Difference in flower size among treatments was not significant though flower size was better in moss grown plants.

4.5.3 Chlorophyll content

Data pertaining to chlorophyll content of leaf and root samples of phalaenopsis var. Magic Kiss grown in different growing media like sphagnum moss, coconut husk chips and husk bits are presented in Table 12.

4.5.3.1 Chlorophyll a.

4.5.3.2

In the leaf samples, chlorophyll a content was highest (0.19mg/g) in the leaves of plants grown with sphagnum moss as media. The values were on par in coconut husk chips and coconut husk bits as media with a value of 0.07mg/g. In the root samples, those grown in sphagnum moss as media had a value of 0.04 mg/g aga 62 0.01mg/g in coconut husk chips and husk bits as medium.

4.5.3.3 Chlorophyll b.

Among leaf samples, chlorophyll b was on par (0.10mg/g) in samples where sphagnum moss and coconut husk chips were used as medium. It was closely followed in leaf sample where coconut husk bits were used with value 0.07(mg/g).In root samples, chlorophyll b content was higher (0.02mg/g) in plants grown in moss and it was 0.01 mg/g in roots of plants grown in coconut husk.

4.5.3.4 Total chlorophyll

In leaf samples, total chlorophyll content was highest (0.29mg/g) in plants where moss was used as medium. It was 0.15mg/g in samples with coconut husk bits as media and 0.17mg/g where coconuthusk chips were used.

Table. 12. Chlorophyll content in leaves and roots of phalaenopsis var. Magic Kiss grown in different media

Plant part	Growing media	Chl a (mg/g)	Chl b (mg/g)	Total Chl (mg/g)	Chl a/b
LEAF	Sphagnum moss	0.19^{a}	0.10^{a}	0.29^{a}	1.88^{a}
	Coconut husk chips	0.07^{b}	0.10^{a}	0.17^{b}	0.68^{b}
	Coconut husk bits	0.07^{b}	0.07^{a}	0.15^{b}	1.02^{b}
ROOT	Sphagnum moss	0.04^{a}	0.02^{b}	0.06^{b}	2.18^{a}
	Coconut husk chips	0.01^{b}	0.01^{a}	0.03^{b}	0.93^{b}
	Coconut husk bits	0.01^{b}	0.01^{a}	0.03^{b}	0.93^{b}

Treatments with even alphabets (a,b) as superscripts form one homogenous group

Table 13. Tissue analysis (leaves and roots) of phalaenopsis var. Magic Kiss grown in different growing media

		Nutrients									
Plant part/ media	Growing media	N	P	K	Ca	Mg	S	B	Zn	Cu	Mn	Fe
		%	%	%	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
LEAF	Sphagnum moss	1.428^{a}	0.200^{a}	2.37^{ab}	2052^{b}	1272.6^{a}	3583^{a}	128^{b}	287^{ab}	673^{b}	234^{a}	15886^{a}
	Coconut husk bits	0.840^{b}	0.158^{b}	2.896^{a}	3760^{a}	1011.2^{b}	2383^{b}	148^{a}	308^{a}	793^{a}	214^{b}	15670^{a}
ROOT	Sphagnum moss	0.924^{a}	0.138^{ab}	0.384^{b}	2170^{a}	1320.0^{a}	4050^{a}	163^{a}	347^{a}	1384^{ab}	19^{b}	2170^{b}
	Coconut husk chips	0.868^{a}	0.148^{a}	0.918^{ab}	1398^{b}	1157.4^{b}	3558^{b}	157^{a}	305^{b}	256^{c}	36^{a}	4225^{a}
	Coconut husk bits	0.546^{b}	0.160^{a}	1.28^{a}	1286^{b}	1137^{b}	3383^{b}	121^{b}	233^{c}	1979^{a}	35^{b}	4175^{a}
MEDIA	Sphagnum moss	1.316^{a}	0.822^{a}	0.344^{b}	1054^{a}	2294^{a}	854^{ab}	68^{b}	58^{b}	98^{b}	182^{a}	25220^{a}
	Coconut husk bits	0.322^{b}	0.151^{b}	1.708^{a}	362^{b}	2230^{ab}	923^{a}	196^{a}	88^{a}	213^{a}	42^{b}	4380^{b}

Treatments with even alphabets (a,b) as superscripts form one homogenous group

Fig. 14 Influence of growing media on Nitrogen content in phalaenopsis var. Magic Kiss

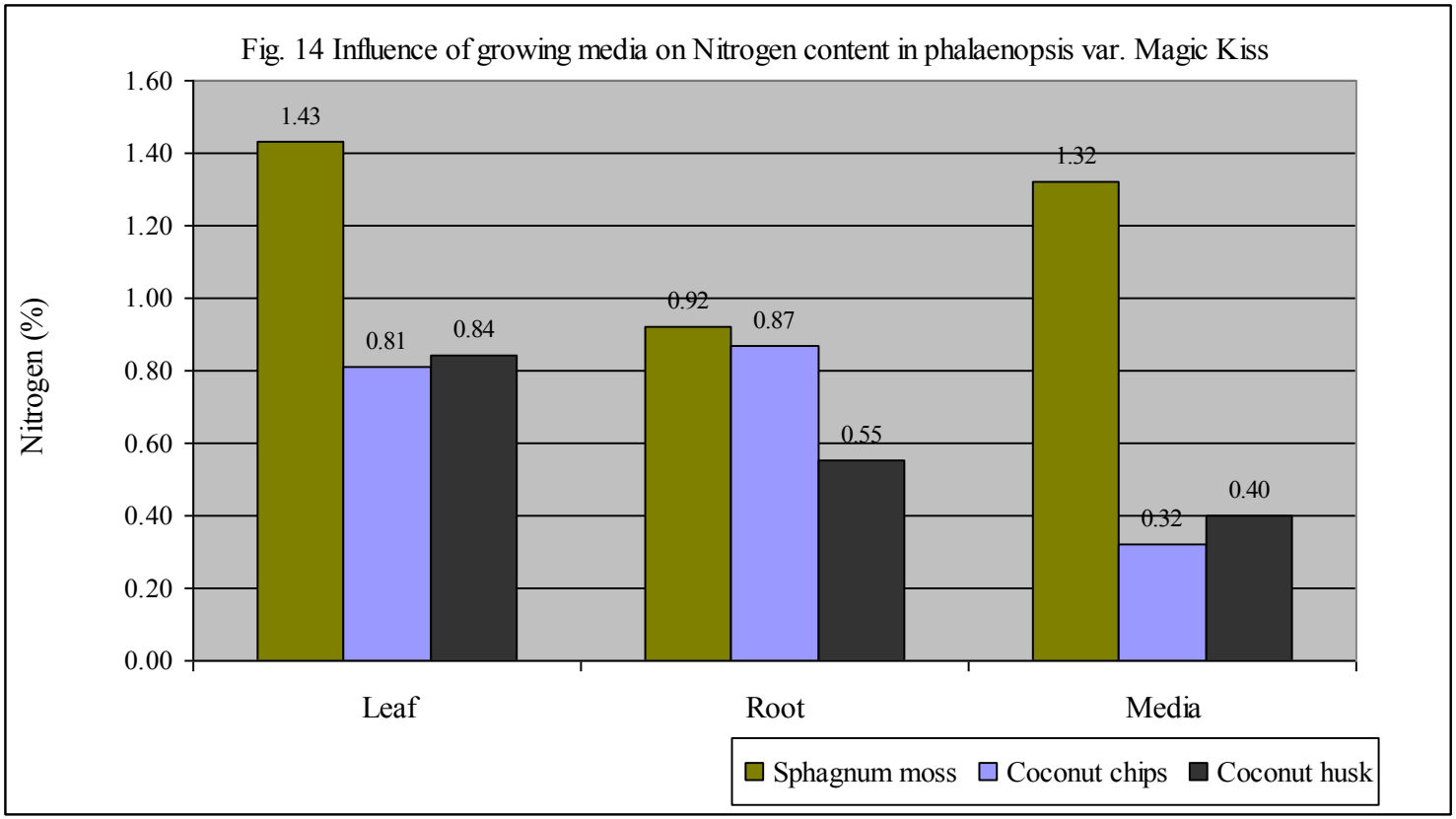


Plate 11. Roots of *Phalaenopsis* ‘Magic Kiss’ grown in different media

Sphagnum moss

Coconut husk chips

Coconut husk bits

In root sample, total chlorophyll content was highest 0.06 mg/g in plants grown in moss against 0.02 mg/g in coconut husk bits and coconut chips as media.

4.5.3.5 Chlorophyll a/b

In leaf samples as well as root samples, chlorophyll a/b was higher in plants grown in moss with values 1.88 and 2.18 respectively against 0.68 and 0.93 in leaf and root sample from coconut husk chips as media.

4.5.4 Plant tissue analysis

Leaves, roots and media were analysed for macronutrients, secondary and micronutrients. Details are furnished in Table13.

Nitrogen content of leaves (1.43 %) and roots (0.92 %) was high in plants grown in moss compared to coconut husk recording values 0.84 per cent (leaves) and 0.87 per cent (roots). With regard to analysis of media, N content was very high i.e. 1.32 per cent in moss against 0.32 per cent in coconut husk. Phosphorus content was higher in leaves of plants grown in moss (0.20 %) and in roots of plants grown in coconut husk (0.16 %). Moss was richer in P (0.82 %) compared to coconut husk (0.15 %). Potassium content was higher in leaves (2.90 %) and roots (1.12 %) of plants grown in coconut husk. In growing media, K content was higher in coconut husk (1.70 %) compared to moss (0.34 %).

Among secondary and micronutrients, moss grown leaf sample was rich in magnesium (1273 ppm), sulphur (3583 ppm) and manganese (234 ppm). Roots of plants grown in moss was rich in calcium (2170 ppm), magnesium (1320 ppm) and zinc (347 ppm). Between the media, moss recorded high content of calcium (1054 ppm), copper (98 ppm) and iron (25220 ppm).

Table 14 Details of bacteria isolated from moss & Phalenopsis (grown with and without moss)

No.	Name of isolate	Source	Cell shape	P-solubilisation (zone in cm)	IAA production (µg/ml)	HCN Production	Strains isolated by 16sDNA sequencing
1.	M1-MM1	Moss -surface	G+ve rods	_	44	_	*Bacillus aryabhattai*
2.	MD1	Moss -surface	G+ve chain	_	40	_	*Bacillus thuringiensis*
3.	MY	Orchid in presence of moss-Surface of leaf & root ; endophytic in leaf	G-ve rods	2.2	72	+	*Klebsiella Pneumoniae*
4.	MW	Orchid leaf surface (grown with moss)	G+ve rods	1.7	30	_	*Not identified*
5.	LWMY	Orchid leaf surface (grown in coconut husk)	G-ve	1.7	47	_	*Enterobacter sp*
6.	LWMW	Orchid leaf surface (grown in coconut husk)	G-ve	1.5	64	_	*Enterobacter ludwigii*
7.	Moss-red (imprinting)	Moss- surface	G+ve	-	NA	-	*Bacillus muralis*
8.	Moss- (imprinting)	Surface of moss	G+ve	NA	NA	NA	*Bacillus cereus*

4.5.5 Bacterial isolates

Details of bacteria isolated are presented in Table 14. From the moss surface G +ve bacterial isolates of *Bacillus thuringensis* and *B. aryabhattai* were identified. *Klebsiella pneumoniae* (G –ve)*, B. muralis and B. cereus* were the endophytes isolated from *Phalaenopsis* leaves and roots grown in moss. From leaves and roots of phalaenopsis samples grown without moss G-ve bacteria *Enterobacter sp* were isolated.

P solubilisation zone was 2.2 cm in MY isolate from plants grown in moss. Strain isolated was *Klebsiella pneumoniae*. The isolate also tested positive for HCN production and 70 mg/ml of IAA production. The isolate was associated both in leaves and roots as endophytes and also on the surface of leaves in the plants grown in moss. Population was more in leaf (203x 10^3cfu/ g) than in roots (11x10^3cfu).

4.5.6 Anatomy of root section

The root anatomy of *Phalaenopsis* var. Magic Kiss grown in different media was studied. The cross section of aerial roots of plants in moss as media revealed well developed rhizodermis followed by velamen tissues and many layered parenchymatous cortex which were green in colour (Plate 12). The cross section of roots from coconut husk bits and coconut husk chips did not reveal a well developed green coloured paranchymatous cortex.

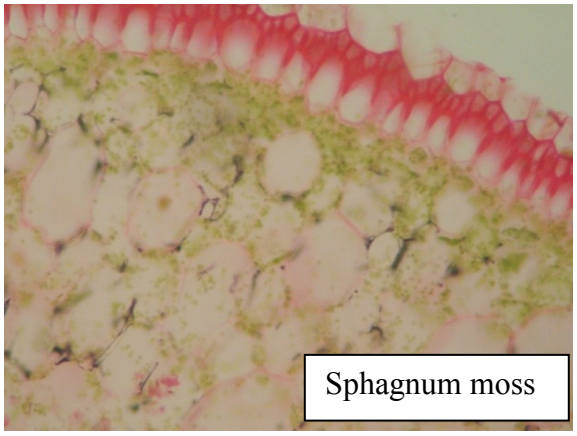
4.6 Post harvest studies

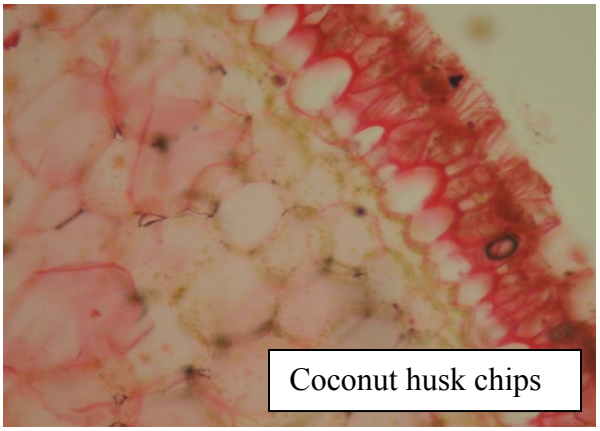
Post harvest studies were conducted in five varieties viz., Roxanne, Taisuco Confidence, Magic Kiss, Goldie and Mimi and the details are presented in Table 14.

4.6.1 Time taken for wilting of the first floret (days)

Wilting of the first floret occurred after a longer period in Mimi (7.00 days). It

Plate 12. Cross section of phalaenopsis roots grown in different media combinations (along with tile bits and charcoal)





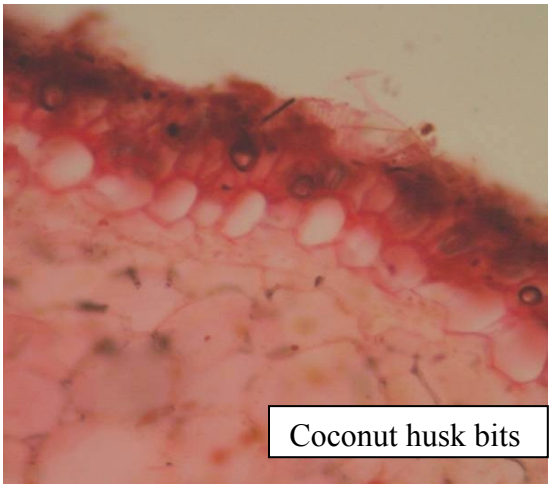


Table.15 Post harvest longevity of different varieties of phalaenopsis

Variety	Time taken for first flower to wilt	Spike longevity (Days)
Roxanne	2.00	3.40
Taisuco Confidence	2.20	3.82
Magic Kiss	2.23	14.40
Goldie	5.75	12.62
Mimi	7.00	16.70

Treatments with even alphabets (a,b) as superscripts form one homogenous group

Table. 16 Stomatal density in different varieties of Phalaenopsis

Variety	Adaxial	Abaxial	Spike (pere mm2)
Roxanne	22-35	5	4.0-6.0
Magic Kiss	15-22	5	4.0-5.0
Medium Pinki	30-42	5	3.0-4.0
Chin Shang Stripe	16-28	5	4.0-5.0
Kathleen Ai	17-21	5	5.0-7.0

occurred at the earliest in Roxanne (2.00 days) which was on par with Taisuco Confidence (2.20 days).

4.6.2 Inflorescence longevity (days)

Inflorescence longevity was maximum for Mimi (16.70 days) which was on par with Goldie (12.62 days). Longevity of inflorescence was minimum in Roxanne (3.40 days) and Taisuco Confidence (3.82 days).

4.7 Physiological studies

Physiological studies were conducted in five phalaenopsis varieties *viz*., Magic Kiss, Medium Pink, Chin Shang Stripe, Kathleen Ai and Roxanne.

4.7.1 Stomatal density on leaves and inflorescence

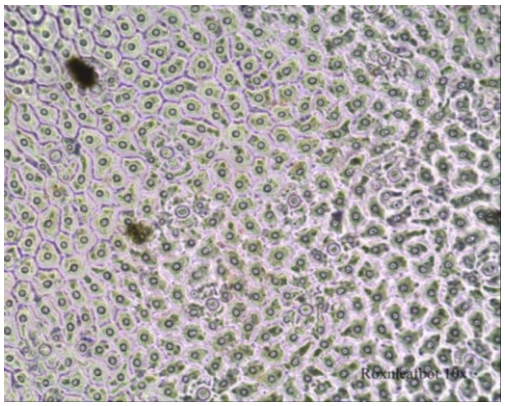
Stomatal density varied among varieties and is given in Table 15. The stomatal density was more in leaves than on stalk of inflorescence. In leaves, the adaxial region maintained a stomatal density of 5 per mm^2 in all the varieties. On abaxial surface of the leaf the stomatal density was maximum in Medium Pink (30- 42 per mm^2) followed by Roxanne (22 -35 per mm^2) and Chin Shang Stripe (16-28 per mm^2). The lowest number was observed in Kathleen Ai (17-21 per mm^2) followed by Magic Kiss (15-22 per mm^2). In inflorescence, the maximum number of stomata was observed in Kathleen Ai (5-7 per mm^2) followed by Roxanne (4-6 per mm^2).

4.7.2 Stomatal conductance

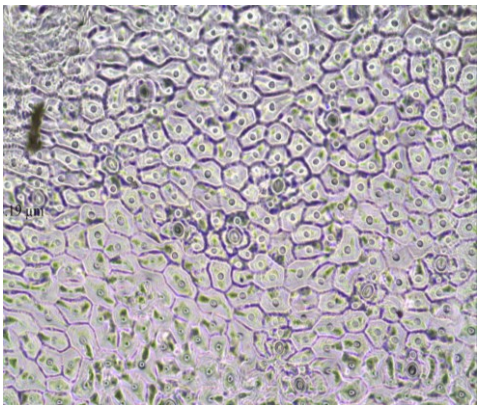
Stomatal conductance values are recorded in Table17 and depicted in Fig. 15. It varied among varieties. Stomatal conductance was maximum at 4 am and was neglibible between 10 AM and 4 PM for all the varieties. Chin Shang Stripe recorded the maximum values throughout the period of study reaching a peak of 2.84.

Plate 13. Stomatal distribution in different varieties of *Phalaenopsis*

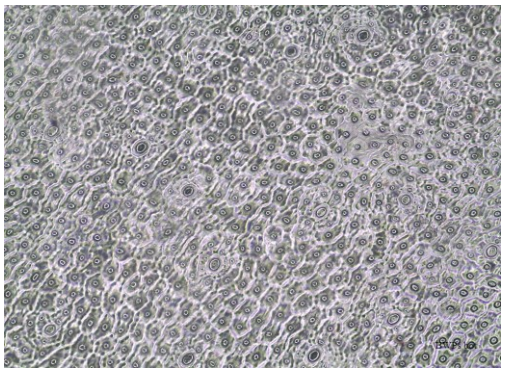
a. Stomatal distribution on abaxial surface of leaves



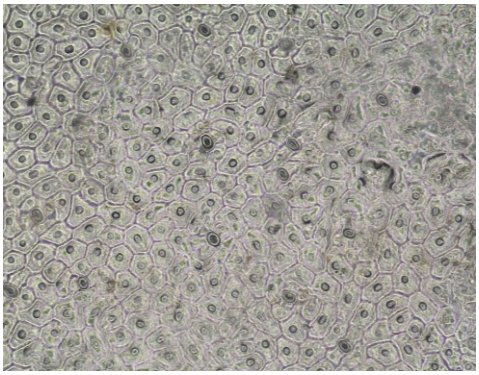
Roxenne



Kathleen Ai

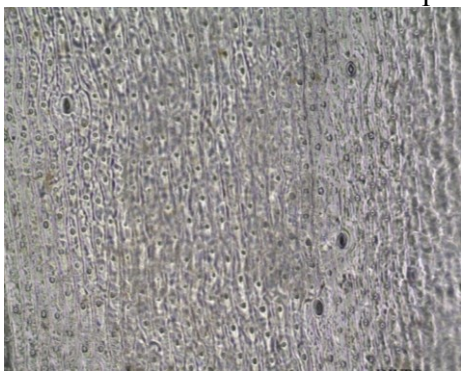


Chin Shang Stripe

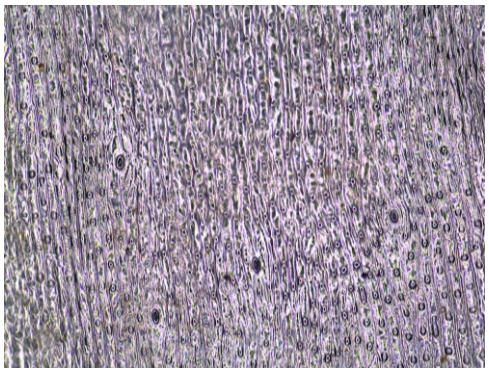


Medium Pink

a. Stomatal distribution on spikes



Chin Shang Stripe



Roxenne

Table 17 Stomatal conductance (mol/m^2/s) recorded in a 24 hour cycle in different varieties of phalaenopsis

Sl. No.	Variety	8pm	10pm	12am	2am	4am	6am	8am	10am	12pm	2pm	4pm	6pm
1	Magic Kiss	0.20	0.83	0.72	1.36	2.08	0.95	0.14	0.03	0.00	0.00	0.01	0.01
2	Medium Pink	0.28	0.91	1.19	0.85	2.07	0.51	0.02	0.04	0.00	0.00	0.01	0.02
3	Kathleen Ali	0.18	0.89	1.11	1.28	2.07	0.46	0.03	0.04	0.01	0.01	0.01	0.03
4	Roxanne	0.16	0.83	1.13	1.26	2.03	0.46	0.03	0.02	0.00	0.01	0.01	0.05
5	Chin Shang Stripe	0.38	1.24	1.62	1.77	2.84	0.89	0.12	0.04	0.01	0.01	0.01	0.06

Table 18 Net CO_2 uptake (m mol CO_2/m^2/s) recorded in a 24 hour cycle in different varieties of phalaenopsis

Sl. No	Variety	8pm	10pm	12pm	2am	4am	6am	8am	10am	12pm	2pm	4pm	6pm
1	Magic Kiss	7.00	12.23	15.05	26.03	27.36	13.89	2.11	0.07	0.00	0.00	0.07	2.44
2	Medium Pink	8.02	13.19	16.50	24.62	26.72	8.31	2.17	0.00	0.00	0.00	0.00	2.52
3	Kathleen Ali	6.63	12.71	15.57	24.12	26.28	8.92	1.91	0.00	0.00	0.00	0.00	3.32
4	Roxanne	6.24	11.52	15.87	24.46	26.10	10.42	2.21	0.00	0.00	0.00	0.00	2.92
5	Chin Shang Stripe	9.44	13.69	17.31	26.27	27.88	11.26	2.41	0.01	0.00	0.00	0.57	4.32

Fig. 15 Stomatal conductance (rhythm) in a 24 hour cycle in different varieties of phalaenopsis

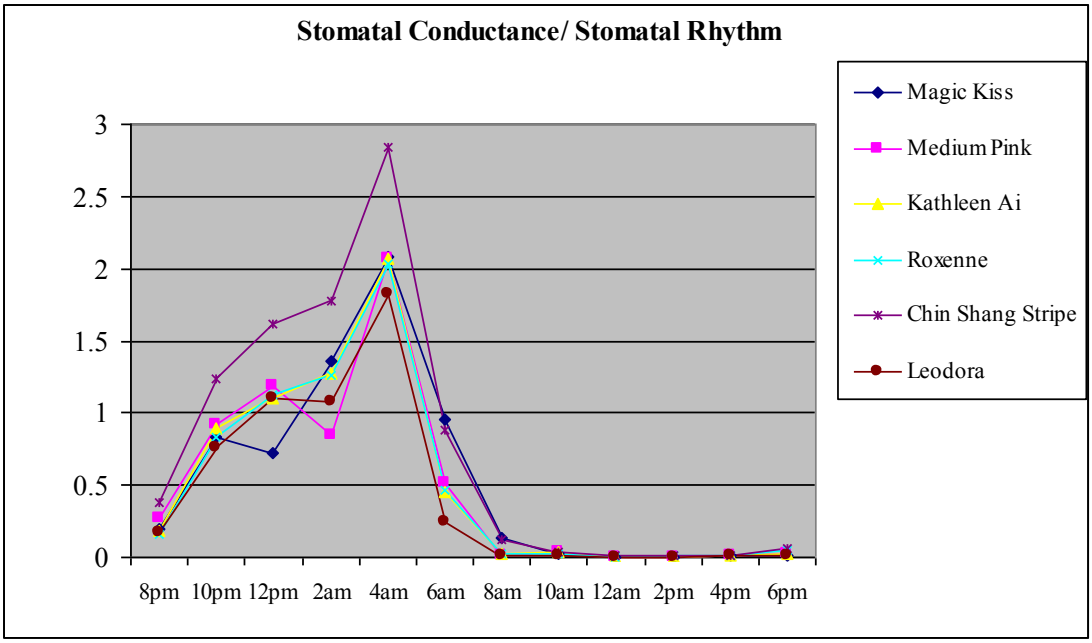
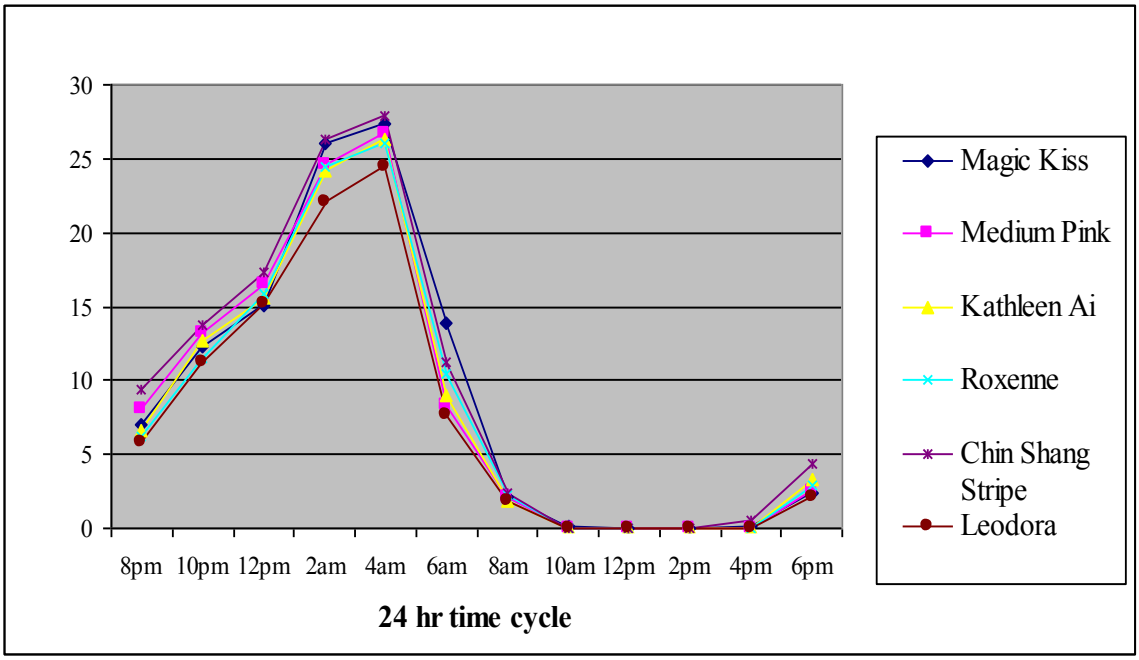


Fig.16 Net CO2 uptake recorded in a 24 hours cycle in different varieties of phalaenopsis



mol/m^2/s at 4am. Minimum value was recorded in Roxanne 2,03 mol/m^2/s at 4am.

4.7.3 Stomatal rhythm

Stomatal rhythm was proportional to stomatal conductance. The stomata opened wide at 4 am in all the trial varieties. It closed post dawn or was negligible during mid day

4.7.4 Net CO_2 assimilation

It is presented in Table 18 and Fig. 16. Net CO_2 absorbed in all the varieties was maximum at 4 am and reached 0.00 between 10am and 2pm. Net $C0_2$ uptake was maximum for variety Chin Shang Stripe (27.88 μ mol CO_2/m^2/s) and minimum for Roxanne (26.10 μ mol CO_2/m^2/s) at 4am

4.7.5. Chlorophyll content

The chlorophyll a, chlorophyll b, total chlorophyll content and a/b ratio varied between different systems of growing and also between varieties. It is presented in Table 19.

The total chlorophyll content was maximum in rain-shelter than fan and pad for all the varieties under study. Variety Roxanne recorded maximum value for total chlorophyll under rain-shelter (1.491 mg/g) and Fan and Pad (1.079 mg/g). The lowest value was recorded in Chin Shang Stripe (0.107 mg/g) under Fan and Pad and by Medium Pink (0.160 mg/g) under rain-shelter.Kathleen Ai showed higher content of chlorophyll a content under fan and pad greenhouse(0.116 and 0.183 mg/g, respectively).

Chlorophyll a/b ratio was more in fan and pad greenhouse (1.568 to 4.513)

Table 19 Chlorophyll content in different phalaenopsis varieties in different growing systems.

Sl. No.	Variety	Chlorophyll a		Chlorophyll b		Total Chlorophyll		Chlorophyll a/b	
		RS	FP	RS	FP	RS	FP	RS	FP
1	Magic Kiss	0.105	0.101	0.098	0.043	0.207	0.145	1.075	2.349[c]
2	Medium Pink	0.092	0.116	0.066	0.074	0.160	0.192	1.390	1.568[d]
3	Chin Shang Stripe	0.109	0.082	0.073	0.022	0.196	0.107	1.493	3.727[b]
4	Kathleen Ai	0.166	0.183	0.086	0.057	0.261	0.248	1.930	3.210[b]
5	Roxenne	1.090	0.880	0.398	0.195	1.491	1.079	2.738	4.513[a]

* RS - Rain-shelter

*FP - Fan and Pad.

Treatments with even alphabets (a, b) as superscripts from one homogenous group

Table 20 Titrable acidity as malic acid equivalent (mg/g) in different varieties Of phalaenopsis

Variety	Top ventilated rain-shelter		Fan and pad greenhouse	
	Timeof sample collection and conversion			
	6am	6pm	6am	6pm
Magic Kiss	7.59	1.34	5.58	1.34
Medium Pink	6.36	1.34	6.03	1.34
Kathleen Ai	7.31	1.34	5.68	0.67
Chin Shang Stripe	5.14	1.34	4.91	1.34
Roxenne	4.02	1.34	3.35	0.67

Treatments with

Table 21 Diurnal variation in pH in different varieties of Phalaenopsis.

Variety	Top ventilated rain-shelter		Fan and pad greenhouse	
	Timeof sample collection and difference in acidity			
	6am	6pm	6am	6pm
Magic Kiss	4.73	6.28	4.90	5.99
Medium Pink	5.15	6.60	4.88	7.85
Kathleen Ai	5.20	6.78	4.99	6.80
Chin Shang Stripe	5.17	6.90	4.87	7.51
Roxenne	5.36	6.71	5.18	6.95

Treatments with even alphabets (a, b) as superscripts from one homogenous group

when compared to rain-shelter greenhouse (1.075 to 2.738). The lowest value in rain-shelter was recorded in Magic Kiss (1.075) followed by Medium Pink (1. and Chin Shang Stripe (1.498). The highest value of 2.738 was recorded ın Roxanne. In fan and pad system, the lowest value of 1.568 was recorded in variety Medium Pink and the highest value for a/b ratio was recorded in Roxanne (4.513)

4.7.6 Titrable acidity as malic acid equivalent

Among plants grown in rain-shelter, malic acid concentration was higher (7.59 mg/g at 6am against 1.34mg/g at 6pm) in Magic Kiss and lower in Roxanne (4.02 mg/g at 6am against 1.34mg/g at 6pm). In fan and pad, malic acid concentration was higher in Kathleen Ai (5.68 mg/g at 6am against 1.34 mg/g at 6pm) and lower in Roxanne (3.35 mg/g at 6am against 0.67 mg/g at 6pm). The diurnal fluctuation difference in malic acid content was more in rain-shelter in comparison with fan and pad (Table 20).

4.7.7. Diurnal variation in pH in different varieties of phalaenopsis

In rain-shelter, diurnal variation in acidity was maximum in Chin Shang Stripe (6.90 at 6pm against 5.17 at 6am) and minimum in Roxanne (6.71 at 6pm against 5.36 at 6 am). In fan and pad, diurnal variation in acidity was maximum in Medium Pink (7.85 at 6pm against 4.88 at 6am) and minimum in Magic Kiss (5.99 at 6pm against 4.90 at 6 am). The details are presented in Table 21.

Discussion

5. DISCUSSION

The results of the trials conducted on the regulation of flowering in *Phalaenopsis* orchids through performance evaluation, low temperature treatments, nutrient growth regulator combinations and use of different growing media are discussed briefly in this chapter.

Genus *Phalaenopsis* is classified under short-stemmed monopodial orchids. They are epiphytic in nature and in their native habitat grow in the shades of the canopy of jungle trees. They are slow growing; a fully mature plant attains a maximum of 15 cm. Plants remain in juvenile stage for over eighteen months after hardening. Leaves are fleshy, near elliptical with different shades of green depending on the variety; combined with purplish pigmentation underneath in certain varieties. Plants have a crassulacean acid metabolism pathway with stomata opening during night. Roots are fleshy, chlorophyllous and greyish-green in colour. The inflorescence arises from third or fourth node and is long, attractive and an arching raceme. Flowers are zygomorphic and occur in a wide variety of colours. The tepals have a li75 shade and labellum is of a darker colour. The column like structure 'gynostemium' is positioned above the labellum. Gynostemium is a special feature of orchids, a fusion of androecium and gynoecium, separated by a wall like structure called rostellum. It separates the male and female organs restricting self pollination. *Phalenopsis* occupies the number one position in the international trade in pot plants market and is ranked one among the top ten in the cut flower segment. In orchid trade, phalaenopsis plants are grouped into grandiflora types and multiflora types. Grandiflora types have larger flowers and are used as cut flower along with their usage as pot plant. The multiflora types are smaller in size and flower profusely and thus are exclusively used as pot plants. Major countries growing phalaenopsis are Taiwan, Thailand, Malaysia, Singapore, United States and Netherlands. In India, orchid cultivation is still in infancy being just a couple of decades old. Though the humid tropics of Kerala is the hub of orchid cultivation, there are problems faced by the growers here. The plants produce inflorescence when the temperature is low, which is usually during

the onset of monsoon or in some varieties during winter. The experiments were designed so as to make the plants flower during the off season.

5.1 Performance evaluation

Phalaenopsis plants are basically subtropical plants which can be grown in the tropical and temperate regions with appropriate modifications in the growing environment. Plants attain good vegetative growth at temperature above 26°C and a lower temperature of 15-26°C favours flowering.

The trial was carried out for a period of 12 months under two different growing systems, *viz*., top ventilated rain-shelter greenhouse and fan and pad greenhouse. Day temperature was higher in rain-shelter greenhouse compared to the fan and pad greenhouse by 5-7ºC. In the present study, two varieties each under the pot plant (multiflora) type namely, Magic Kiss and Medium Pink and cut flower types (grandiflora), namely, Chin Shang Stripe and Kathleen Ai were used.

Good vegetative growth indicates better accumulation of photosynthates in plants. Faster growth is essential especially during juvenile stage for better flowering later on. Between the growing systems, vegetative characters were better in plants grown under rain-shelter greenhouse. Plant height, leaf length, leaf breadth and leaf area were the highest in plants grown here. This could be because of the higher temperature and ventilation prevailing inside the structure. This was in agreement with Fitch (2004) who reported that higher temperatures force faster vegetative growth when accompanied by bright light, humidity and air movement. Among the varieties, Magic Kiss performed significantly better in rain-shelter and the growth was poor in fan and pad system. Besides the advantage of rain-shelter, varietal difference is also indicated in this case. It has been reported by Kano (2001) that most of the commercial hybrids originating from tropical and subtropical areas grow and leaves expand better at high temperatures of about 30°C.

Flowering was better in fan and pad where the average day temperature was usually lower by 5-7ºC compared to rain-shelter. Emergence of inflorescences occurred earlier in fan and pad for all the varieties but the number of days taken for emergence of bud and opening of flowers was lower under rain-shelter greenhouse. It is reported that lower temperature favours flower induction in phalaenopsis. Inflorescence length was better for varieties Magic Kiss and Chin Shang Stripe under rain-shelter but varieties Medium Pink and Kathleen Ai had better inflorescences in fan and pad greenhouse. Flower count was significantly superior in fan and pad greenhouse for varieties Medium pink, Chin Shang Stripe and Kathleen Ai. Blanchard and Runkle (2006) reported a parallel observation that the number of flower buds in phalaenopsis was generally greater at cooler temperature treatments than at treatments with a higher average day temperature. The number of inflorescence per plant was more in variety Chin Shang Stripe grown under fan and pad greenhouse where temperature was lower. It is in accordance with Chen (2002) who reported that the spiking percentage is a function of the accumulated cooling temperature. The flowering period was significantly higher in fan and pad for all the varieties. The flowering season extended from September to April. On the other hand, the flowering period ended by January in rain-shelter. High temperature causes increased respiration, thus reducing the longevity of flowers. Thus in summer, up to May, plants in fan and pad continued flowering unlike those in rain-shelter where the temperature crossed 35 ºC during the above period.

5.2 Induction of flowering through low temperature treatment

Production of flowers in the off season fetches premium price in the market. Commercial producers of *Phalaenopsis* and their related hybrids usually manipulate flowering by controlling temperature and applying chemicals that influence endogenous hormone levels. Thus they produce architecturally desirable and floriferous plants for predetermined market dates. The interaction of endogenous phytohormones, which are markedly influenced by various environmental conditions, is an important regulatory factor for the induction of flowering. It has been report by Wang (1995) that phalaenopsis plants develop at least two undifferentiated bud primordia at each node which later

becomes dormant. Under appropriate environmental and cultural conditions, the upper bud elongates and emerges through the epidermis of the stem and develops into an inflorescence. Wu and Chang (2011) reported that the timing of flowering is controlled through timing of spiking. Growers can modulate flowering time in each *Phalaenopsis* cultivars, based on reliably controlling the time of spiking.

Temperature is the most important environment factor, associated with light, which controls the performance of a plant, both in terms of growth as well as development. In the present study, *Phalaenopsis* plants were induced to produce inflorescence by exposing plants to a lower temperature. In their native habitats, tropical conditions persist throughout the year with temperatures ranging between 28ºC and 35ºC during the day and 20ºC to 24ºC at night. Flower induction in the trial was carried out by exposing plants to lower temperature by taking them to a higher altitude and by keeping them in cold room for a given period of time.

5.2.1 Higher altitude

Tropical zone is blessed with the unique advantage of having near sub tropical and temperate weather conditions due to altitudinal differences.

Study was conducted by exposing the mature plants to low temperature by taking plants to a higher altitude, Nelliampathy situated at an altitude of 1050 m above mean sea level. Nelliampathy, had a maximum temperature of 26.9ºC and minimum of 18.3ºC during the period of study (April 2011). Hew and Yong (1997) reported that flowering induced by low temperature had contributed significantly to the large scale production of Phalaenopsis cut flowers and potted plants in Taiwan and Japan. In the trial carried out, initially plants were grown in the plains of Vellanikkara and later taken to Nelliampathy. Three different sets of plants were exposed to low temperature (mean varied between 26.9ºC and 18.3ºC) for a period of 2 weeks, 3 weeks and 4 weeks respectively. It was compared with a set of plants kept in Vellanikkara, in rain-shelter and fan and pad system. The mean maximum temperature recorded in rain-shelter was 36.21 ºC and mean

minimum was 23.52ºC while in fan and pad it was 28.7ºC and 24.0ºC during April 2011. It was observed that the plants exposed to low temperature for 3 weeks produced inflorescence earlier. For high altitude exposure, flowering characters like duration taken for emergence of inflorescence was significantly superior to the set of plants taken as control. The emergence and opening of flower bud were earlier in the control set of plants. Flower count and flower size were on par among treatments and control. Inflorescence length and flowering duration improved with period of exposure to low temperature. Hew and Yong (1997) reported that the age of plant, timing of transfer to highlands and their eventual return to the warmer lowlands are critical for flower induction and development

5.2.2 Cold room

The statement made earlier asserts that plants flower only on exposure to appropriate environment. Though exposing plants to low temperature prevailing at higher altitude has yielded convincing results, it may not always be practical. In the subsequent trial, plants of *Phalaenopsis* var. Roxanne were exposed to low temperature by keeping them in an air-conditioned room. The temperature inside the room was set at 23 ± 2ºC. The maximum and minimum temperature varied between 24.5ºC and 20.6ºC. The plants were kept in the room for a period of 2 and 3 weeks. Artificial lighting was provided during daytime in the cold room in addition to the natural light which together ranged between 400 and 900 lux during the period of exposure. Phalaenopsis requires an average light intensity of 12,000-15,000 lux for proper growth and development. The positive result recorded here, in spite of the extremely low light intensity of 400-900 lux reaffirms that it is the temperature alone that regulates flowering. Moreover, the plants were exposed to such low temperatures only for a short period of 2 to 3 weeks and hence it did not affect the general growth. This contradicts Wang (1995) who reported that during the low-temperature period, exposure to high-intensity light was necessary to induce spikes.

The treatment plants were compared with the control set of plants, which were kept in rain-shelter greenhouse. It was noted that on exposing the plants to low temperature for just 2 weeks, new inflorescence emerged. The treatment plants were far

superior to the control plants in this character. The number of days taken for emergence of first bud was on par among treatments but the opening of flower bud occurred earlier in plants exposed to higher temperature i.e. the control set of plants. This is in agreement with the reports by Lopez and Runkle (2005) who stated that once flower buds have initiated, flower development time depends upon temperature and genotype.

Flowering duration was significantly superior in plants kept in the AC room. This is because of the higher number of flowers produced in treatment plants compared to that of control. Levy (2002) reported that in *Arabidopsis VRN* genes mediate vernalization, the process by which a long period of cold induces a mitotically stable state that leads to accelerated flowering during later developmental stages. Inflorescence length was far superior in treatment plants compared to control. The endogenous growth regulators especially gibberellins have contributed to an increase in inflorescence length. Runke (2010) reported that exogenous application of gibberellic acid (GA) can be used to increase inflorescence length in phalaenopsis. Flower count was significantly higher in plants exposed to low temperature. A good inflorescence length and a higher flower count are the desirable traits for a cut flower. Low temperature exposure can thus help growers in inducing blooms as per demand. There could be some differences with regard to the temperature and duration of exposure for different varieties.

5.3 Nutrient – growth regulator combinations

Phalaenopsis orchid being an epiphytic plant, its nutrition needs could be very different from terrestrial plants. The plants absorb nutrients and moisture from the atmosphere. The roots are exposed and they anchor to the bark of forest trees. They derive their nutrition from air, decaying bark, bird and animal droppings in addition to the dissolved nutrients in rain and dew.

In the present study, the vegetative growth was higher in both cut flower and pot plant types where 20:10:10 of NPK (0.1% at fortnightly interval) was applied compared to other treatments with or without growth regulator combinations. Plants with growth regulators applied at monthly intervals rather performed poorly in certain combinations.

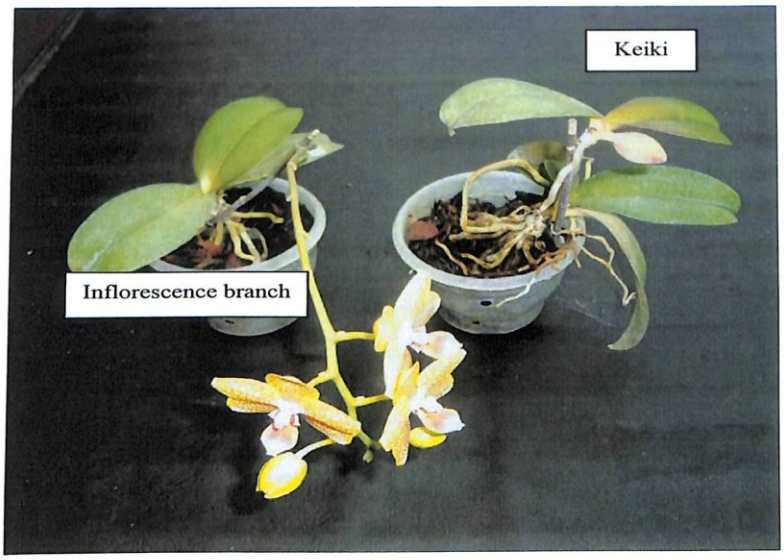
Chen (2002) had also reported against the application of hormones. The concentration of hormones is not easy to control. If the concentration is too high, the shape of flower could change unpredictably. Floral characters did not improve on application of Kinetin. Flower count rather declined with the application of 200ppm Kinetin in both cut flower and pot plant types. 10:20:10 NPK with GA_3 at 2 ppm was better in cut flower type as it had an improved inflorescence length. This is supported by findings of Runkle (2010) who stated that gibberellin is not effective at inducing flowering, it increases inflorescence length. Gibberellin need not be applied in pot plant as inflorescence length is not a desirable character for pot plant. Chen (2002) stated that the use the hormones have a small effect on the spiking and the application of hormones with the cooling stress could increase the spiking percentage and shorten the cooling period.

5.4 Inflorescence pruning

Pruning is a widely followed operation targeted at an increased productivity in a range of horticultural crops. The idea is to direct the energy towards a desired region. The physiological stress caused in the process can induce blooms as well.

To conserve the plant's energy and force it to rest in preparation for a good presentation of flowers in the following blooming season, the inflorescence is cut off at its lowest point with a sterilised tool. This prevents an enzyme produced in the nodes and tip of the inflorescence (which keeps the plant in the reproductive mode) from entering the plant, thus allowing the plant to devote all its energies to growth following a brief rest. Yaqin *et al.,* (2006) analysed endogenous hormones (including GA_3, GA_{4+7}, IAA, ABA, ZR and iPA) in different ds located in different nodes of two-year-old Kyoho grape seedlings. The results revealed that the reproductive phase appeared at about 20th node. In the present study, in grandiflora type of *Phalaenopsis* var. Roxanne, the spent inflorescences were pruned at three different levels; above 1st node, 2nd node and 3rd node which was compared with the plants where spent inflorescence was not pruned. The plants where inflorescences was pruned at the 1st node produced inflorescence within a month of pruning and was better than pruning inflorescence at 2nd and 3rd node. All the

Plate 14. Branching of inflorescence and production of keiki in phalaenopsis var. Goldie after pruning of inflorescence



treatments were significantly superior compared to control. Physiological stress caused due to inflorescence pruning could have caused the initiation of new inflorescence in the plants. Stress was severe when pruned at first node. Partitioning of assimilates occurred to the maximum extent in plants where inflorescences were pruned above the first node and the time taken for producing the new inflorescence was also the minimum. This further necessitates study of translocation of nutrients or other factors within the plant as a result. The concentration of flowering hormones could be high in the first node. Quantification of endogenous hormones at different nodes needs to be quantified in further studies.

The other floral characters like emergence and opening of first bud, flowering duration, inflorescence length and flower count were on par among treatment plants and control. In multiflora type (variety Carlotta), the pot plant did not produce new inflorescences unlike the grandiflora types. Additional work was done in variety Goldie; where inflorescences pruned at 3rd node and above usually produced keiki or baby plant and at times produced an inflorescence branch. The reason for keiki production could be genetic.

5.5 Growing media studies

Orchids exhibit a wide range of habitats, terrestrial and epiphytic plants occupying the major share of plant genera. In the epiphytic orchids, a variety of media are used, the main functions of such media being, providing good aeration, holding moisture and allowing good drainage. Media like tree bark, coconut husk, charcoal, brick pieces, tile bits etc are popular with epiphytic orchids. The media is also reported to be genus specific. Wang (2005) reported that, while growing phalaenopsis in containers filled with artificial medium, important considerations are aeration, capillary action, water and nutrient-holding capacities, stability and weight of the medium components, as well as cost and consistency. In the present study, three different growing media were used namely coconut chips, z for growing orchids. There have been reports on

sphagnum moss being a good media for orchids in terms of good moisture holding capacity but not in terms of nutrient supply and microbial load. Although moss from New Zealand is of higher quality, many growers use Chilean moss because it is less expensive. For the trial, high quality long threaded moss growing on trees and shrubs especially from coffee plantations (where it is considered a parasite) along the Western Ghats were used. They were superior and very well suited for phalaenopsis growing. Vegetative and floral characters were studied and also plant tissue and media were analysed for nutrients, chlorophyll content and bacterial colonies.

Good vegetative growth is an indication of the photosynthetic ability of plants. Among the treatment plants, plants grown using sphagnum moss as media was found to have a significantly better vegetative growth. Plant height, leaf length, leaf breadth and leaf area were far superior compared to those grown in coconut husk chips and coconut husk bits as media. This is in agreement with the studies of Wang (2005) who reported that pure sphagnum moss could probably be the single best material for growing young phalaenopsis in warm (tropical and subtropical) conditions. The plants grown using moss as media, had deep green foliage, the total chlorophyll content recorded was noted to be almost double that of plants where coconut chip bits and coconut husk bits were used. The health of the roots is very important for flowering as root is a major producer of cytokinins in plants. In the case of epiphytic plants, where the velamen tissue of roots favour absorption of water and nutrients through their entire length, a physiological function of extracting as much favourable elements as possible, even a slight increase in the functional capability of the roots would bring in significant changes in the performance of plants. The advantage with epiphytic roots is that they get better opportunity to communicate with the environment in compared to terrestrial roots as they are exposed to free air. Among the different media tried in the present study, the roots of plants grown using moss were healthy and deep green in colour unlike the other media where it was grayish to pink. The root chlorophyll content was thrice the amount of that in coconut husk as medium. The root cross section depicted healthy tissues in plants grown using moss along with chlorophyll content in parenchymatous cortex. Wang *et al*., (2007) opined that managing the root zone of potted orchids can be one of the most

critical aspects to growing a healthy crop. If one can grow plants with healthy roots, making them flower is a much easier task. Good flowering is attributed to a healthy root system which is a site for cytokinin production. Leaves of *Phalaenopsis* contain more chlorophyll-a and chlorophyll-b than roots. Chlorophyll a/b ratio varied among treatments, with ratio being higher for plants grown in sphagnum moss and this was in contradiction to Trelka *et al*., (2010) who reported that the .growing media did not affect chlorophyll concentration in leaves and roots

A strong base of vegetative characters resulted in better flowering characters of plants grown in moss, compared to those using coconut chip bits and coconut husk bits. The inflorescence produced was significantly longer. The endogenous growth regulators could possibly be responsible for the high growth rate in these plants. Hiller *et al*., (1979) reported that the changes in endogenous gibberellin like activity were related with stem elongation, but not with floral initiation. Flower count was significantly superior in plants grown in moss. But some of the flowers in the variety Magic Kiss was found to be malformed. This influence could be genetic. Runkle (2010) reported that in some hybrids, inflorescences can develop abnormally if cytokinins are applied at an excessive rate or after flower initiation. Excess of cytokinins or any related plant growth regulator in moss could be the reason for malformation.

Plants grown in moss as media were rich in photosynthetic elements, moss the media as such was rich in nitrogen, calcium, manganese, magnesium and iron compared to husk. Leaf samples of plants grown in moss were rich in nitrogen, sulfur and manganese. Roots were rich in nitrogen, calcium, magnesium, sulphur and zinc. Generalizing orchid plant with no reference to media, Trelka *et al*., (2010) reported that the leaves and substrate roots of orchids differ significantly with regard to the accumulation of macro and microelement; leaves accumulate more nitr[84] phosphorus, potassium, calcium and manganese, while roots accumulate more magnesium, iron and zinc. Though sphagnum moss had a very high iron content, it showed no symptoms of toxicity. Instead, plants were very healthy.

Stoessl and Arditti (1984) reported that orchids are known to produce various phenolic compounds and phytoalexins, which were shown to suppress a number of different microorganisms. Plant exudates also supply the rhizosphere with tryptophan that is the main precursor in microbial IAA biosynthesis (Kravchenko *et al*., 2004). IAA producing bacteria transform it into auxin, increasing its exogenous level. The plants grown using sphagnum moss had no incidence of disease. Plants grown in coconut based media recorded higher incidence of *Fusarium* rot. Lugtenberg *et al.*, (1991) reported that besides the provision of nurients, plant-associated bacteria are important for supporting growth, health and stress resistance of plants. Lindow and Brandl (2003) reported that the orchid leaf surface (phyllosphere) may be colonized by a range of different bacteria and fungi and that the bacterial communities on leaves are limited by nutrient availability and it is known that mainly simple sugars, which leach from the interior of plants, are the available source of carbon.

In order to further understand the superiority shown by sphagnum moss, the population and types of microflora (surface as well as endophytic) associated with the three substrates were assessed. A Gram negative rod shaped bacterium that formed highly fluidal yellow colonies on nutrient agar was found to be the most predominant in moss. This isolate fixes nitrogen, solubilizes insoluble P and produces HCN. It also produces IAA from tryptophan, to the extent of 72 µg ml^{-1} of the medium, in a period of two weeks, in pure culture. This isolate was identified as *Klebsiella pneumoniae* by 16S rDNA sequencing. The isolate was associated with phalaenopsis, both in leaves and roots as endophytes and also on the surface of leaves, when the orchids were grown on moss. Population was more in leaf (203x 10^3cfu/ g) than in roots (11x10^3cfu). The bacterium could not be detected when the plants were grown in the other two media. *In vitro* studies with cowpea seeds indicated enhancement of germination percentage and seedling vigour. Sachdev *et al*., (2009) report that six 85 producing strains of *Klebsiella* significantly increased root length and shoot height of inoculated wheat seedlings over control. *Bacillus thuringensis* and *B*. *aryabhatti* were recorded as well in plants grown in moss as media.

5.6.1 Post harvest studies

Orchid flowers are very sensitive to ethylene. Vase life studies were carried out in five varieties of *Phalaenopsis* by keeping them in plain water. Flowers lost turgor and wilting of the first floret and inflorescence longevity varied among treatments. The variety Mimi had the longest flower and inflorescence longevity because of the waxy texture of petals while Roxanne wilted earlier because of the petals being thin. In-folding of the flowers was very common in all the varieties. Porat (1994) reported that accelerated flower wilting as a result of ethylene evolution following pollination in *Cymbidium*, *Dortitaenopsis*, *Dendrobium*, and *Phalaenopsis* orchids. It is induced by a loss of water from cells of the upper layer of the petals, leading to their in-folding and causing water-soaked appearance

5.7 Physiological observations

It has been observed that phalaenopsis orchids exhibit crassulacean acid metabolism pathway of photosynthesis. Stomatal opening and CO_2 uptake is performed mainly during night time and the absorbed CO_2 is fixed as malic acid and stored in vacuoles. During day time, the stomata get closed and malic acid is released from vacuoles and decarboxylated. The study also confirmed that CAM photosynthetic pathway occurred in phalaenopsis orchids through physiological observation on stomatal density, stomatal conductance, net CO_2 assimilation and diurnal fluctuation in malic acid concentration and pH.

The study revealed that stomatal density was considerably higher in the leaves of all the varieties compared to the inflorescences. It was in confirmation with the findings of Hew *et al.,* (2005) who reported that the density of stomata in floral parts of orc 86 was considerably lower than the leaves. In the study, in all the *Phalaenopsis* varieties, the maximum number of stomata was observed on abaxial surface. The adaxial sur 86 which is exposed to light recorded a uniform number of 5/mm^2 leaf area in all the varieties. Earlier reports on leaf anatomy of phalaenopsis also revealed that stomata are

common on abaxial surface, but are usually sparse or absent on the adaxial surface. Ferry (2008) reported absence of stomata on the adaxial leaf surfaces of *Spiranthes* orchid. In the present study, high stomatal density (30-42 / mm^2) was observed in the abaxial surface of Medium Pink and lower values were observed in Kathleen Ai (17-21 / mm^2). A similar observation was reported in orchid species *Aerides lour* by Malgaonkar (2005). He observed a stomatal density of 5.33/mm^2 on adaxial side and 42.66/mm^2 on abaxial surface.

Low stomatal density and low stomatal conductance were related to high water content. In CAM plants, .the stomatal conductance of all the varieties gradually increased from 6pm to 4am and the peak was observed between 2am and 4am. Present findings showing maximum stomatal opening in these varieties occurring very early in the morning (2am to 4am) is in corroboration with the findings in *Cattleya* sp. (Goh *et al*., 1977). The gradual decrease in stomatal opening was observed from 4am to 8am and it completely closed by midday. Gradual opening of stomata was observed after 4pm. The rhythmic opening of stomata was in relation to the stomatal conductance.

Net CO_2 uptake was observed to be in relation to stomatal conductance in all the varieties where the maximum uptake was recorded between 2am and 4am. The uptake was negligible between 12 pm and 2pm. In general, CO_2 uptake gradually increased from 6pm to 4am registering a peak at 4am for all the varieties. Sudden decrease in CO_2 uptake was observed from 4am to 8am and the uptake was almost negligible up to 4pm. The CO_2 pathway was similar to that of other CAM plants reported by many researchers. Kano (2001) reported that, in phalaenopsis the optimum temperature for photosynthesis as measured by CO_2 absorption was 15-25°C and this temperature range was almost the same as the optimum temperature for floral transition.

Chlorophyll a/b ratio provides information on plant behaviour in relation to distinct light intensities and lower chlorophyll a/b ratio of orchid leaves may reflect on adaptation to shade (Boardman, 1977). The results obtained in this study also indicates similarity in findings as observed in Magic Kiss which recorded low a/b ratio under both

the systems. The higher value recorded in Roxanne may be an indication of its adaptation to higher light intensities as reported by Konow and Wang (2001) in some phalaenopsis hybrids.

The photosynthetic leaves in CAM plants accumulate malic acid at night and decarboxylation of malic acid occurs during the day. The study was carried out to confirm or to ascertain CAM pathway in *Phalaenopsis* by recording titrable acidity equivalent to malic acid at 6am and 6pm. Between the growing systems, it was observed that in rain-shelter, the malic acid concentration was higher for all the varieties, indicating high CO_2 uptake and fixation thereby supporting a better vegetative growth in rain-shelter compared to fan and pad. Diurnal variation in pH values revealed a inverse relation with malic acid concentration. Leaf samples recorded a lower pH in rain-shelter compared to fan and pad with variations among varieties. The lower pH at 6am indicates an increased accumulation of malic acid at the end of night period and higher pH at 6pm indicates decarboxylation of malic acid at the end of day or light period. Similar relationship between pH and titrable acidity equivalent to malic acid was reported by Kubota *et al.*, (1997). The diurnal fluctuation in titrable acidity as malic acid equivalent resulting from CO_2 fixation in the dark period was reported previously in orchids by Guo and Lee (2006). A consistent increase in titrable acidity overnight relates to high malic acid accumulation thereby indicating CAM in the *Phalaenopsis* varieties studied. High titrable acidity and malic acid equivalent at 6 am and low values at 6 pm reveals acidification during night and deacidification during daytime.

Summary

6. SUMMARY

Studies on 'Regulation of flowering in *Phalaenopsis* orchids' were conducted in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara from November 2010 to April 2012. The main objectives of the trial were to evaluate performance of varieties under different growing systems, improve vegetative growth, induction of inflorescence by different treatments like low temperature, inflorescence pruning and nutrition and growth regulator combinations. Study on effect of media compositions, post harvest studies and physiological studies were also taken up. The results of the study are summarised below.

Two varieties each of pot plant and cut flower varieties were evaluated under rain-shelter and fan and pad systems of greenhouse. Significant differences with regard to vegetative growth were observed between growing systems. Plants in rain-shelter performed better than fan and pad. Increase in plant height (1.09 cm), leaf length (6.46 cm), leaf breadth (1.54 cm) and leaf area (10.50 cm^2) was higher in variety Magic Kiss grown in rain-shelter greenhouse. Leaves produced at a significantly shorter interval of 97.92 days in variety Magic Kiss grown under rain-shelter. Differences were not significant in other varieties. Emergence of the first flower bud and opening of the first flower were earlier in rain shelter for all four varieties. Flowering duration was significantly higher in fan and pad for all varieties compared to rain-shelter with values 158.85 days for Magic Kiss, 222.54 days for Medium Pink, 180.05 days for Chin Shang Stripe and 149.46 days for Kathleen Ai. Length of inflorescence was maximum (33.27 cm) in rain shelter for Magic Kiss, in fan and pad for Medium Pink (38.55 cm), in rain-shelter for Chin Shang Stripe (34.04 cm), and in fan and pad for Kathleen Ai (34.85). number of flowers per inflorescence was higher in fan and pad for all varieties but was significantly higher with values 16.69 and 9.77 for Medium Pink and Kathleen Ai respectively. Inflorescence count was significantly higher (1.31) for cut flower variety Chin Shang Stripe in fan and pad. Branching was observed in all varieties when grown in

fan and pad. Flowering season for all varieties was better in fan and pad (July- August to April- May).

Mature plants of Phalaenopsis were exposed to low temperature by keeping at higher altitude for 2 weeks, 3 weeks and 4 weeks. The plants were brought back and kept separately in rain-shelter and fan and pad. It was compared with that of control plants. Inflorescences emerged in 18.00 days in plants kept in Nelliampathy for 3 weeks. In rain-shelter, emergence of the first bud was earlier (27.70 days) in control set of plants. Under Fan and pad system, emergence of bud was earlier in plants that were kept for 3 weeks (30.00 days). Days taken for opening of the first bud was minimum (51.97 days) in plants kept in rain-shelter as control. In fan and pad, days taken for opening was minimum (55.66 days) in control. In rain-shelter, flowering duration was higher (74.33 days) in plants kept for 4 weeks at higher altitude. In fan and pad, plants exposed for 2 weeks of low temperature had longer (229.83 days) flowering duration. In rain-shelter, inflorescence length was higher (34.70 cm) in plants exposed for 4 weeks and in fan and pad it was in case of 2 weeks (48.53 cm). In rain-shelter, flower count was higher (5.84) for 3 weeks of cold exposure and in plants exposed for two weeks (16.33) in case of fan and pad. In rain-shelter, flower count was higher (5.84) for four weeks of exposure and under fan and pad higher flower count (16.33) was observed in plants kept for two weeks.

Plants were exposed to low temperature of AC room with temperature set at 23˚C (± 2 ^{0}C) for 2 weeks, 3 weeks and it compared with that of control. Emergence of inflorescence occurred in 14.41 days in plants exposed to low temperature for 2 weeks. Emergence of the first bud was earliest (31.43 days) in control plants. Opening of the first flower bud occurred earlier (43.57 days) in control plants. The plants exposed for 3 weeks of cold room temperature opened buds later (62.86 days) which was on par in plants kept for 2 weeks (58.01 days). Flowering duration was superior in case of plants kept in cold room for 2 weeks (92.43 days). Inflorescence length and flower count were higher (34.98 cm and 6.71) in plants that received cold treatment for 2 weeks.

Nutrient and growth regulator combination studies were carried out in pot plant (var. Lin Jessica) and cut flower (var. Taisuco Confidence) types. Leaf length, 90 breadth and total leaf area was maximum (7.30 cm, 3.00 cm and 22.17 cm^2 respectively) for pot plant types and 4.33 cm, 2.61 cm, 11.91 cm^2 for cut flower types in plants treated with 0.1per cent of 20: 10: 10 of NPK. Interval of leaf production was minimum (187.10 days in pot plant and 228.03 days in cut flower type) in plants treated with 0.1per cent of 20: 10:10 of foliar NPK.

In pot plant variety, Lin Jessica, buds emerged earlier (30.20 days) in plants treated with 0.1per cent of 10:20:10. Time for opening of bud showed no significant differences among treatments. Flowering duration was maximum (76.75 days) in plants treated with 0.1per cent of 10:10:10 NPK and 100ppm Kinetin. Length of inflorescence was highest (27.43 cm) in plants treated with 0.1per cent of 10:20:10 along with GA_3 1ppm. Flower count was higher (4.75) in plants treated with 0.1per cent of 10:20:10 and GA_3 1ppm

In cut flower variety Taisuco Confidence, buds emerged earlier (40.20 days) in the combination involving 0.1per cent of 20:10:10 NPK with GA_3 1ppm, the buds opened earlier (61.90 days) in the combination involving 0.1per cent of 20:10:10 NPK. Flowering duration was maximum (52.30 days) in plants sprayed with 0.1 per cent of 10: 20: 10 in combination with GA_3 2ppm. Inflorescence length was highest (36.40 cm) in plants treated with 0.1 per cent of 10:20:10 in combination with GA_3 1ppm. Flower count was highest (3.25) in plants 10:20:10 and GA_3 2ppm.

The spent (flowered) inflorescences were pruned at three different levels, above1st, 2nd, 3rd node which was compared with that of control, wherein spent inflorescences were retained. The number of days taken was minimum (23.80 days) in plants wherein inflorescences were pruned at the first node. The number of days taken for first bud emergence, first flower opening showed no significant difference. Flowering period was longer in plants pruned at the first node (38.80 days). Inflorescence length and flower count were on par among treatments.

Sphagnum moss had a significantly higher vegetative growth and floral characters compared to coconut husk chips and coconut husk bits. Plant height, leaf length, leaf breadth, and leaf area was maximum in sphagnum moss (1.64 cm, 1 91 cm, 7.02 cm and 87.77 cm^2 respectively) and interval of leaf production was minimum (91.40 days) in plants grown in moss. Emergence of inflorescence was early (124.90) in plants grown in moss as medium. Flowering duration (112.70 days), inflorescence count (1.80), inflorescence length (28.17 cm), rachis length (6.07 cm) and peduncle length (22.10 cm) was highest where sphagnum moss was used as medium.

Chlorophyll a was highest (0.19 mg/g) in the leaves and roots (0.01 mg/g) of plants grown with sphagnum moss. Chlorophyll b was on par (0.10 mg/g) in samples where sphagnum moss and coconut husk chips were used as medium. In root, chlorophyll b content was higher (0.02 mg/g). Total chlorophyll content was highest (0.29 mg/g) in leaves and roots (0.06 mg/g) in plants grown in moss. Chlorophyll a/b in plants grown in moss was higher with 1.88 (leaves) and 2.18 (roots). From the moss surface, G + ve bacterial isolates of *Bacillus thuringensis* and *B. aryabhattai* were found. *Klebsiella pneumoniae* (G –ve)*, B. muralis and B. cereus* were the endophytes isolated from *Phalaenopsis* leaves and roots grown in moss. From leaves and roots of phalaenopsis samples grown without moss G-ve bacteria *Enterobacter sp* were isolated. P solubilisation zone was 2.2 cm in MY isolate from plants grown in moss. The isolate also tested positive for HCN and IAA production (70 mg/ml).

Nitrogen content of leaves (1.43 %) and roots (0.92 %) was higher in plants grown in moss. Among media, nitrogen content was very high (1.32 %) in moss. Phosphorus content was higher in leaves of plants grown in moss (0.20 per cent) and in roots of plants grown in coconut husk (0.16 %). Moss was richer in P (0.82 %) compared to coconut husk (0.15 %). Potassium content was higher in leaves (2.90 %) and roots (1.3 %) of plants grown in coconut husk. In growing media, K content was higher in coconut husk (1.70 %) compared to moss (0.34 %). Among secondary and micronutrients, moss grown leaf sample was rich in magnesium (1273 ppm), sulphur (3583 ppm) and manganese (234 ppm). Moss grown root was rich in calcium (2170 ppm), magnesium

(1320 ppm) and zinc (347 ppm). Between the media, moss recorded high content of calcium (1054 ppm), copper (98 ppm) and iron (25220 ppm). Root cross section (plate) revealed healthy velamen in plants grown in moss. Root parenchyma tissues (cortex region) were rich in chlorophyll and green in colour.

Post harvest studies varied among varieties. Wilting of the first floret occurred later in mimi (7.00 days). Inflorescence longevity was maximum for Mimi (16.70 days).

Physiological studies were conducted in five varieties *viz*., Roxanne, Chin Shang Stripe, Kathleen Ai, Medium Pink, Magic Kiss. Stomatal conductance was highest at 4 am and in variety Chin Shang Stripe (2.84mol/m^2/s). Stomatal rhythm was in accordance with the stomatal conductance. The stomata opened wide after 12 am was higher at 4 am. Net CO_2 absorbed in all the varieties was higher at 4 am.

Total chlorophyll content was higher in fan and pad compared to rain-shelter for all varieties. Variety Roxanne recorded maximum value for total chlorophyll under rain-shelter (1.491 mg/g) and fan and pad (1.079 mg/g). Chlorophyll a and chlorophyll b was maximum for variety Roxanne grown under rain-shelter (1.090 and 0.398 mg/g) and fan and pad system (0.880 and 0.195 mg/g). Magic Kiss and Chin Shang Stripe showed higher chlorophyll a content under rain-shelter greenhouse (0.105 and 0.109 mg/g respectively). Whereas Medium Pink and Kathleen Ai showed higher content of chlorophyll a content in fan and pad greenhouse (0.116 and 0.183 mg/g respectively). Chlorophyll a/b ratio was more in fan and pad greenhouse (1.568 to 4.513) when compared to rain-shelter greenhouse (1.075 to 2.738).

Among plants grown in rain-shelter, malic acid concentration was higher (7.59 mg/g at 6am against 1.34 mg/g at 6pm) in Magic Kiss. In fan and pad, malic acid concentration was higher in Kathleen Ai (5.68 mg/g at 6am against 1.34 mg/g at 6pm). The diurnal difference in malic acid content was more in rain-shelter when compared to fan and pad. In rain-shelter, diurnal variation in acidity was maximum in Chin Shang Stripe (6.90 at 6pm against 5.17 at 6am). In fan and pad, diurnal variation in acidity was maximum in Medium Pink (7.85 at 6 pm against 4.88 at 6am).

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* Originals not seen

ABSTRACT

Studies on 'Regulation of flowering in *Phalaenopsis* orchid' were conducted in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara from November 2010 to April 2012. The main objectives were to evaluate cut flower and pot plant varieties of *Phalaenopsis* hybrids under two microclimatic conditions, to observe their flowering behaviour with respect to the weather elements and to study the effect of cultural practices on flowering and floral characters

Orchidaceae is the largest family in the plant kingdom with over 25,000 species in over 600 genera. Despite the diversity, very few genera like *Cymbidium, Phalaenopsis, Dendrobium, Oncidium* and *Cattleya* are ranked high commercially. The genus has around 80 species and more than 40,000 hybrids. It is a short stemmed epiphytic monopodial orchid with Crassulacean Acid Metabolism (CAM) pathway of photosynthesis.

Based on floral characters, phalaenopsis are broadly classified as grandiflora (cut flower) and multiflora (pot plant) types. Grandiflora types have long, arching inflorescence with large flowers, whereas, the multiflora types have short, branched inflorescence with numerous smaller sized flowers. *Phalaenopsis* is now among most valuable flowers used as cut flower and as pot plant.

As a part of the study, phalaenopsis varieties were evaluated under two different growing systems, *viz*., top ventilated rain-shelter and fan and pad system of greenhouse. Vegetative growth was better in rain-shelter whereas longer flowering period and higher flower count were obtained under fan and pad system.

In an attempt to induce flowering by exposing to lower temperature, plants were taken to Nellaimpathy, having an altitude of 1050 meters, and were kept for different periods. Keeping for three weeks was the best in terms of inflorescence induction as compared to control. Subsequently, comparable situation was provided in an AC room. Plants kept for two weeks initiated inflorescence. Nutrient and growth regulator treatments had no significant impact on growth and flowering. Pruning of spent

inflorescence induced new inflorescence and the best treatment was pruning above the first node.

Commonly used media for growing phalaenopsis are coconut husk chips and coconut husk bits, along with charcoal and tile bits. Use of sphagnum moss has been reported from other countries only as media for good moisture retention. In the present study, plants grown in sphagnum moss were significantly superior to coconut husk chips and coconut husk bits as media, both in terms of vegetative and floral attributes. It was high in chlorophyll content along with high N, Mg, S, Fe and Mn, which are responsible for photosynthesis. N fixing bacteria *Klebsiella pneumonia* and *Bacillus thuringensis* were also found in plants grown using sphagnum moss.

Post harvest studies conducted to evaluate the longevity of floret and spike in plain water indicated differences among varieties. Var. Mimi had maximum inflorescence longevity and variety Roxenne the minimum. Physiological studies showed stomatal conductance, net CO_2 assimilation reaching peak at 4am. Stomatal density was higher on the abaxial leaf surface in all varieties. Titable acidity as malic acid accumulation was higher in rain-shelter compared to fan and pad, indicating better growth.

AAppendix1. weather data of COH (2011-2012)

	Growing system	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Temperature (Maximum	RS	34.51	30.6	30.26	30.52	31.27	33.33	36.31	32.88	34.38	35.47	36.59	36.21
	FP	28.24	24.15	24.22	24.94	25.84	27.87	25.78	25.31	26.49	28.48	28.37	28.77
	Open	33	29.3	29.1	29.4	30	32	31.4	31.9	32.8	35.1	35.2	34.8
Temperature (Maximum	RS	24.68	23.99	23.82	23.92	23.36	23.39	23.04	22.4	21.76	22.7	23.68	23.52
	FP	24.82	23.63	23.33	23.19	23.81	23.27	22.13	21.37	20.74	21.05	23.3	24.05
	Open	24.9	23.6	22.9	22.9	23.1	23.5	22.9	22.6	21.3	22.1	24.2	24.8
Relative Humidity (%)	RS	71.14	79.9	79.45	78.7	75.05	68.52	52.18	58.53	57.38	53.23	56.86	64.65
	FP	81.3	95.07	94.93	95.36	92.6	89.5	85.29	82.7	81.03	75.95	84.48	89.8
	Open	76.8	88.99	87.83	87.04	84.41	77.87	68.1	62.01	57.54	54	67.87	71.6
Light Intensity (lux)	RS	6300	6825	6337	5458	6523	10898	10837	9494	10888	10968	6308	6815
	FP	4717	3789	4256	4688	5532	5354	4367	4132	5129	4690	5580	7231
	Open	68000	72064	65643	71000	78000	76005	94912	83651	92654	89567	71240	70323

*RRS Rain-shelter

*FFPFan and Pad

Appendix 2. Weather data of Nelliampathy for April 2011

Date	Relative Humidity (%)	Temperature		Rainfall (mm)
		Maximum (°C)	Minimum (°C)	
04/01/2011	79	26.00	16.00	5.00
04/02/2011	80	28.00	16.00	0.00
04/03/2011	79	26.00	16.00	0.00
04/4/2011	80	26.00	18.00	2.40
04/05/2011	78	27.00	18.00	0.00
04/06/2011	79	27.00	18.00	0.00
04/07/2011	79	27.00	17.00	0.00
04/08/2011	79	27.00	17.00	0.00
04/09/2011	80	26.00	18.00	0.00
04/10/2011	80	26.00	18.00	0.00
04/11/2011	78	27.00	22.00	0.00
04/12/2011	80	28.00	21.00	0.00
13/4/2011	79	28.00	21.00	0.00
14/4/2011	79	28.00	20.00	0.00
15/4/2011	79	28.00	18.00	0.00
16/4/2011	78	28.00	18.00	0.00
17/4/2011	78	28.00	18.00	0.00
18/4/2011	79	28.00	18.00	0.00
19/4/2011	79	28.00	19.00	0.00
20/4/2011	79	26.00	18.00	7.50
21/4/2011	78	26.00	18.00	0.00
22/4/2011	80	26.00	18.00	4.50
23/4/2011	79	26.00	18.00	12.00
24/4/2011	79	26.00	18.00	5.00
25/4/2011	78	26.00	18.00	4.80
26/4/2011	78	27.00	18.00	1.40
27/4/2011	77	27.00	19.00	0.00
28/4/2011	79	28.00	19.00	0.00
29/4/2011	79	28.00	19.00	0.00
30/4/2011	79	30.00	20.00	0.00

Appendix 3. Environment inside the cold room (AC room)

	Temperature °C		Light intensity (lux)	Relative humidity (%)	
	Maximum	Minimum		Maximum	Minim
25-Jan-11	23.90	20.40	533	69	51
26-Jan-11	22.80	20.20	689	73	52
27-Jan-11	22.40	20.40	524	68	53
28-Jan-11	26.10	21.20	441	72	55
29-Jan-11	25.00	20.60	552	69	52
30-Jan-11	23.90	20.60	540	62	51
31-Jan-11	24.00	21.00	519	68	49
1-Feb-11	26.00	20.60	682	76	49
2-Feb-11	22.40	21.20	491	69	53
3-Feb-11	25.80	20.50	352	79	53
4-Feb-11	25.80	20.40	588	68	50
5-Feb-11	22.40	20.10	461	74	52
6-Feb-11	26.30	20.10	453	68	53
7-Feb-11	26.90	20.10	657	67	53
8-Feb-11	22.40	20.40	453	73	54
9-Feb-11	26.10	21.20	636	67	56
10-Feb-11	25.00	20.60	564	77	53
11-Feb-11	23.90	20.60	1034	71	52
12-Feb-11	24.00	21.00	823	75	55
13-Feb-11	26.00	20.60	787	68	52
14-Feb-11	22.40	21.20	786	65	51
Average	24.45	20.62	598.3	70.4	52.3