# FLOWER BUD DIFFERENTIATION IN CLOVE, *Eugenia caryophyllus* (Sprengel) Bullock & Harrison

By

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## DECLARATION

I hereby declare that this thesis entitled "Flower bud differentiation in clove, Eugenia caryophyllus (Sprengel) Bullock & Harrison" 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, associateship, fellowship, or other similar title, of any other University or Society.

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Certified that this thesis, entitled "Flower bud differentiation in clove, *Eugenia caryophyllus* (Sprengel) Bullock & Harrison" is a record of research work done independently by Smt. PAULINE LINA EDWIN under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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# INTRODUCTION

# 1. INTRODUCTION

Clove, a tropical tree of medium stature, is widely acclaimed as a spice of international reputation. It is traditionally grown in a few isolated locations in Kerala, Karnataka and Tamilnadu. Clove of commerce is the mature, unopened and sun-dried flower buds. The spice, whole or ground has a number of culinary uses. The buds as well as leaves yield essential oil on steam distillation. These oils are used in perfumery, dentistry and medicine. Clove buds, apart from their use in seasoning of various foods, are used as a stimulant and carminative. In research laboratories involving microscopy, clove oil is widely used as a clearing agent during staining, due to its low refractive index. Its principal component, eugenol is easily converted into vanillin, though the use of eugenol for this purpose has been largely discontinued. One of the modern uses of clove which was evolved during the present century, is mixing the shredded spice with tobacco for the manufacture of 'Kretek' cigarettes in Indonesia. Cloves have been used in India since ancient times for fastening the betel quid or 'pan pati' of sliced arecanut smeared with lime and wrapped in a leaf of betel pepper.

Clove trees possess a distinct periodicity in flower bearing habit. The four-year cycle has more or less the following habit of flowering. During the peak year if 100 per cent production is attained, the production in the following year will be about 25 per cent and the subsequent two years will give about 50 per cent and 75 per cent of the peak year yield (Kaat and Polanja, 1982). The yield of clove in a particular year may appear to be extraordinarily high if two driest months of the preceding dry season receive a combined rainfall of 300 mm or less. Conversely, the yield decreases considerably if the two months under reference receive 500 mm or more rainfall.

Thus, if the conditions within as well as outside the plant are congenial at the time of flower bud differentiation, majority of the buds in a plant will differentiate into flowers. Information on the role of various environmental, nutritional and hormonal factors in flower bud differentiation will provide valuable tips for successful cultivation of flowering crop plants. These basic information form the scientific basis for determining the cultural, manurial and irrigative requirements and for scheduling them in such a way that factors within as well as outside the

plant could be manipulated favourably for bringing about maximum flower bud differentiation. Such information has been put to practical application in scientific culture of other crop plants like grapes, apple, orange, mango, apricot, plum and black pepper.

Information on the ontogeny of flower development and the site and time of flower bud differentiation in crop plants will aid in scheduling farm operations like manuring, irrigation and pruning to the best of advantages. Such information collected from histological studies on flower bud differentiation has been put to practical use in a number of crop plants.

The present study had the following objectives:

i) to find out the time of flower bud differentiation in clove;

ii) to study the site of flower initiation and morphogenesis of the floral parts;

iii) to examine the relationship between weather parameters (rainfall, temperature and relative humidity) on one hand, and flower bud differentiation on the other.

# REVIEW OF LITERATURE

# 2. REVIEW OF LITERATURE

Flower initiation marks the transition from vegetative to reproductive growth in seed plants. It is a significant event in the life cycle of these plants. Flowers are metamorphosed shoots which are produced by modified shoot meristems, the flower primordia. The information regarding blossom bud differentiation is highly valuable for guiding the actual time of cultural operations upon which depend ultimately the yield and returns of a farmer.

## 2.1 Factors influencing flower bud differentiation

### 2.1.1 Climatic factors

The role of different climatic factors on flowering of important crops has been investigated by several workers. Analysis of weather data to examine their possible impact on flowering of plants was conducted by Pickering (1916) who observed that the weather of England tended to form a biennial cycle. He, therefore, considered this factor as the main reason for biennial bearing in apple. According to Gibbs and Swarbrick (1930), the variation in time of flower bud differentiation depended upon the climatic conditions. Okada and Konakahara (1981) reported that due to fluctuation in climatic

conditions, the average yield of Sastuma oranges in most years deviated by about five per cent from the estimated yield. Inhibition of flowering of Mexican and Guatemalan type avocados was observed under tropical conditions by Sedgley and Scholefield (1985). At 33°C/23°C temperature regime, the trees had fewer flowers and shorter flowering period than at 25°C/15°C.

2.1.1.1 Temperature

Apple flower buds formed more readily during warm dry weather than during cool weather (Gribanovskji, 1960). Cox's Orange Pippin apple trees maintained at 24°C in growth chambers grew more vigorously than those kept at 17°C continously; but flowering behaviour was similar at these two temperature regimes (Tromp, 1980). Lowering the temperature during the period before harvest did not influence shoot growth, but markedly reduced flowering of both spur-buds and apical shoot buds. The findings postulated by Gianfagna and Mehlenbacher (1985) suggested that late flowering in apple resulted from high heat and high minimum temperature requirements for bud growth. Spurs containing flower buds were collected after completion of the chilling requirement and forced at 10, 15 and 20°C. Bud development occurred on all the samples at 20°C.

Several studies have been conducted on grapes on these lines. The number of flowers initiated in the bud was closely related to the temperature during the three-week period in which the node subtending the buds changed in position from the apex to ten nodes below (Buttrose, 1969). Fruitfulness was related to the maximum temperature recorded over a period of four hours per day (or night) rather than the temperature summation. At a temperature of 13°C, there was little initiation; but initiation increased to a maximum at 30 to 35°C. Durquety et al. (1982) suggested a model based on a Tm (sum of mean daily temperatures) of 24°C for the formation of floral primordia and 14 to 15°C for the abortion of floral primordia.

Effect of temperature on flower bud differentiation of various species of citrus has been investigated thoroughly. High mean temperature was found to induce differentiation of flower buds in the different species of citrus (Abbot, 1935; Randhawa and Disna, 1947; Singh and Dhuria 1960; Bajpai and Maurya 1963). Mishra and Yamadagni (1963) found that a warm season favoured blossom bud differentiation in grapefruit and as thetemperature rose, the number of differentiated buds increased. On the other hand, Moss (1969) reported that more inflorescences were produced at low temperatures in sweet lime.

The influence of air and soil temperatures on the flowering of several citrus species was studied by Hall et al. (1977). The bud break was increased by warm soils at 25°C than by cool soils at 15°C. In contrast, the initiation of flowers on the new shoots was more in cooler days (20°C at day and 15°C at night) than in warmer days (30°C at day and 15°C at night).

Singh (1958) reported that warm season was conducive for flower bud differentiation in mango. Ravishankar et al. (1979) found that a low temperature regime of 19°C/13°C stimulated the formation of axillary flowers of mango, while a high temperature regime of 31°C/25°C had an inhibitory effect on flowering. Zhu and Sheen (1987) found a negative relationship between the number of axillary buds and temperature. Axillary flower induction was greatest on trees subjected to a low temperature treatment of 19°C/13°C for two or more weeks.

Shukla and Bajpai (1974) showed that floral initiation in three Indian cultivars of litchi occurred about three to four weeks after the fall of daily minimum temperatures below a level of 10°C. Sedgley et al. (1985) reported that in avocados grown in controlled environmental conditions, floral initiation and early development appeared normal; but further

development was generally halted at a stage just prior to the development of stamens in flowers. It was, therefore, presumed that the differentiation of sex organs in avocado may be sensitive to high temperature regimes.

Smeets (1932) found that in strawberry, flower initiation was comparatively delayed in the chilled plants than in the fresh plants.

Magambo and Othieno (1983) reported that early flowering in tea in the exposed containers was due to the interaction between high day time soil temperatures and a marked drop in temperatures at night.

Nalini (1993) observed a rise in mean temperature at the peak period of flower bud differentiation in black pepper. Subsequently, Rajan (1995) reported that the maximum and minimum temperatures in the preceding summer and subsequent monsoon showers, played important roles in triggering the flower bud differentiation activity. Vasanthakumar (1996) found that a period of high temperature triggered the flowering mechanism in cardamom, which was in turn carried forward during the rainy period.

2.1.1.2 Rainfall and humidity

In apple, Wiggam (1919) observed that blossom showers were needed for obtaining a good crop. Collison and Harlan (1927) and Degman et al. (1933) pointed out the influence of rainfall on flower bud formation in apple.

In citrus, a moisture stress condition was created to induce flowering. During the period of stress, initiation of flower primordia occurred and flowering followed on resumption of irrigation (Monselise and Halevy, 1964).

Perold (1927) observed that in grapes, warm and dry conditions in the preceding season favoured flower bud initiation. Balasubramanyan (1971) found that a temporary water stress promoted flower bud differentiation in grapes.

Neither high humidity and rain at the time of bloom nor late rains, influenced the fruit bud differentiation during the following year in mango (Singh, 1960). Chacko and Randhawa (1971) observed that heavy rains during the period of flower bud initiation stimulated vegetative growth at the expense of fruit production. Ravishankar et al. (1979) observed that drop in the night temperature and humidity enhanced flower bud initiation.

In coffee, a gregarious flowering plant, detailed studies have been conducted on the ecophysiological aspects governing flowering (Alvim, 1960). Based on his studies in the coastal humid regions of Peru, where internal water potential of the plant is not controlled by transpiration but by the available soil moisture, he opined that flowering primarily depended on rainfall distribution followed by a period of stress. He found that under conditions favouring high transpiration rate, the moisture stress could not be controlled either by irrigation or by keeping the plant in nutrient solution. Rees (1964) observed flowering in coffee after the receipt of the first showers in plants kept watered in dry season. Alvim (1973) further observed that the water potential of coffee plants were controlled by humidity; but rainfall increased the relative humidity and influenced the water potential, thus influencing flowering indirectly.

Alvim et al. (1972) observed that a moisture stress inhibited flowering in cocoa. Two types of flowering were observed in cocoa, the 'normal' one in March to July and a 'crazy' flowering or lean flowering, at the end of the dry period.

The influence of rainfall on the productivity of cashew was evident from the investigations of Veeraraghavan and

Vasavan (1979) who stated that a well distributed rainfall during October and November months was required for the optimum flowering and fruit set in cashew.

In pepper, Nalini (1993) observed flower bud initiation to be triggered by the receipt of pre-monsoon showers, after a long spell of dry weather. Subsequently, Rajan (1995) also confirmed the beneficial effect of rainfall on the flower bud differentiation process in pepper.

2.1.1.3 Light

There is enough evidence for suggesting a quantitative role of light on flower bud initiation. Many reports indicated that shading of apple trees reduced flower bud initiation (Kraybill, 1923; Auchter et al. 1926; Jackson and Sweet, 1972) and there was evidence that the same effect occurred in apricot (Jackson, 1969) and also in peach (Kraybill, 1923).

There was more initiation of flower buds in Vitis sp. under high light conditions (Baldwin, 1964). More precisely, May (1965) had shown that eventhough shading of Vitis leaves did not reduce flower initiation in the buds situated in the axils of shaded leaves, shading of the individual buds reduced the number and size of inflorescence primordia. Koblet (1985)

reported that shading during fruit bud differentiation caused reduction in the number of inflorescences and delayed bud break in the following year.

2.1.2. Nutritional factors

Fisher (1905) was the first to establish the relationship of carbohydrate and nitrogen as one of the most important factors responsible for fruit bud formation. Subsequently, Kraus and Kraybill (1918) reported that the concentration of sugars should be greater than that of nitrogenous compounds for successful flowering in tomato. They studied the seasonal changes in carbohydrate reserves and nitrogen content of various crop plants.

In apple, as early as in 1929 Murneek reported that flowering was characterised by a marked increase in all active forms of carbohydrates and nitrogen in the organs of bearing apple trees. Total sugar and soluble nitrogen contents were very high at full bloom. Total nitrogen also attained the maximum level at flowering time. Hooker (1930) observed higher amounts of total nitrogen in the spurs of bearing trees as compared with those of non-bearing trees and proposed that the ratio of starch to nitrogen was a better indication

of the tendency of apple trees to form flower buds than the ratio of carbohydrate to nitrogen. Faby and Nauman (1986) found that when N reserves were high, flower buds of apple opened early and the percentage fruit set was also improved.

In some preliminary studies concerned with the physiology of flowering in mango, emphasis was laid on the importance of a high C/N ratio as a causative factor for flowering (Naik and Shah, 1937; Sen, 1946 and Mallik, 1953). Later researchers also attempted to establish a correlation between high carbohydrate accumulation and flowering.

Singh (1960) found a high level of total nitrogen in the defoliated shoots of Dashehari variety of mango and a low level, in the fruited shoots. Chacko (1963) found high level of nitrogen prior to fruit bud differentiation, which was observed in the case of carbohydrates also. Suryanarayana (1990) observed that though there is a general increase in the C/N ratio of the leaves from April to November, its peak level did not synchronise with the time of flower bud differentiation in the six varieties studied. Ravishankar and Rao (1992) found that with regard to carbohydrate/nitrogen ratio, when the soluble carbohydrate fraction showed a marked increase during the period of fruit bud differentiation, the ratio in

respect of insoluble carbohydrate fraction registered a decline. The C/N ratio in respect of total carbohydrates also declined during the period of fruit bud differentiation. Experiments conducted on grapes showed that axillary buds which developed after pruning differentiated into flower buds within 40 to 90 days, when the C/N ratio was optimum (Thomas and Bernard 1937; Shantha, 1965; Rao and Suryanarayana, 1978). Chadha and Cheema (1971) observed that in Perlette cultivar of grapes starch accumulation favoured flower bud initiation. Bajua and Bindra (1982) reported that reducing sugars were high in 'Perlette' before full bloom; but in 'Thompson seedless' they were high during berry development.

Harding et al. (1962) in sweet oranges and Aiyappa et al. (1965) in mandarin oranges observed that the leaf nitrogen content was high in the non-fruiting branches than in the fruiting branches. Jones et al. (1977) correlated the alternate bearing behaviour of Valencia orange with the carbohydrate reserves of the trees and found that these reserves in the trees were reduced by an 'on' crop. In an 'off' crop following this, the carbohydrate levels were not found to be consistent.

Gopal et al. (1975) found that in coffee, uniform flower bud enlargement and opening into normal flowers were positively correlated with the starch index (reserve carbohydrates) on the wood.

Bai and Ramadasan (1976) reported that in coconut, the changes in carbohydrate fractions in the leaves followed a definite pattern year after year, under rainfed conditions. In young palms, they found a sharp increase in the soluble fractions beyond July which, in turn, was found related to the commencement of flowering. Ramadasan and Mathew (1977) found that the commencement of first flowering in coconut is preceded by a high C/N ratio. Thereafter, Bai and Ramadasan (1982) observed that peak female flower production occurred during the summer months when the starch content in stems and leaves were the maximum.

Nalini (1983) observed that in black pepper, the total soluble carbohydrates, nitrogen and C/N ratio of two types of lateral branches and new shoots varied considerably during different growth cycles. However, the C/N ratio exhibited two peaks, the first synchronising with the differentiation of flower buds and the second, with the step-up of flower bud differentiation activity. Rajan (1985) did not observe

any significant correlation between the carbohydrate content and C/N ratio with flower bud differentiation in black pepper. However, an accumulation of carbohydrates and a build up of C/N ratio were recorded prior to peak differentiation.

Vasanthakumar (1996) observed a high status of carbohydrates at the panicle initiation and flower bud development stages of cardamom. This helped the accumulation of carbohydrates, thus favouring the development of flower buds in cardamom.

## 2.2 Histological basis of flower bud differentiation

In its gross details, the morphology of flower initiation exhibit certain specific features characteristic of a species of plant. In some plants, the shoot apex having functioned as vegetative meristem for sometime, directly transforms into flower primordium. In others, flower primordia are initiated laterally, while the terminal meristem of the shoot remains in the vegetative phase or ceases active function. In yet another group, which produce inflorescence primordium, individual flower primordia are formed at a later period on the inflorescence primordium in a spatial sequence (Lang, 1965). Eventhough certain details vary from

species to species, the principal features which distinguish a flower primordium from a vegetative primordium remain more or less the same in many seed plants.

In many crop plants, the vegetative apex is characterised by a conical shape, as observed in mango (Singh, 1960), grapes (Chadha and Cheema, 1971), strawberry (Pathak and Singh, 1977) and jasmine (Subramonian and Shanmugavelu, 1980). Shukla and Bajpai (1974) found that prior to differentiation, the apical bud of a vegetative shoot is dome-shaped in litchi, with a uniform curve and surrounded by leaves. Nalini (1983) observed three distinct stages in the development of vegetative buds in black pepper. In the initial stage, the vegetative primordium was conical, undifferentiated and surrounded by leaf sheaths, which elongated as growth progressed. Rajan (1985) also found that the vegetative bud was conical in black pepper surrounded by leaf primordia.

Investigations on the physiology of flowering in cardamom by Vasanthakumar (1986) showed that the apical meristem appeared broadly conical during the initial stages, with prominently nucleated cells.

Transition stage

Broadening and flattening of the apical meristem with

two lateral protuberances, one on either side of it, was established as the phenomenon of blossom bud differentiation in citrus (Abbot, 1935; Randhawa and Disna 1947; Singh and Dhuria, 1960 and Mishra and Yamadagni, 1968).

Fruit bud initiation and differentiation in grapes cv. Anab-e-Shahi was studied by Rajaram et al. (1964). They found that the apical primordium of the bud, consisting of the apical meristem, elongated and inturn produced several lateral outgrowths viz., the primordia of leaves, bud scales, inflorescences and tendrils. Chadha and Cheema (1971) observed that the leaf primordium of grapes was pointed whereas the cluster primordium appeared broad in shape.

In mango, Singh (1958) observed high meristematic activity marked by the origin of bread conical protuberances in the axils of scales, as the sign of fruit bud differentiation.

Mishra and Bajpai (1973) observed flattening and broadening of the crown during fruit bud differentiation in jamun. After this, a rapid elevation on both the sides of growing point was observed. In litchi, Shukla and Bajpai (1974) observed that the bud flattens and broadens with a rapid elevation on both the sides of the growing point. In strawberry,

the initiation of fruit bud differentiation was found to be accompanied by flattening as well as broadening at the top and by the appearence of an irregular outline of the growing point (Pathak and Singh, 1977).

Buban and Faust (1982) narrated the histology of flower bud differentiation in apple, employing the tunica-corpus theory. When the vegetative apex received a stimulus for differentiation into a flower bud, the mitotic activity became rapid in the entire apex, thereby changing the histological structure of the apex.

In black pepper, two undifferentiated conical primordia appeared which was seen surrounded by leaf sheaths (Nalini, 1983). This was suggested as the first sign of flower bud initiation. Later, Rajan (1985) observed a change in shape from conical to hemispherical as the transition from vegetative to floral phase in black pepper which was accompanied by high meristematic activity in the primordial apex.

Histological studies on the flower bud differentiation of cardamom by Vasanthakumar (1986) showed that the panicle (inflorescence) meristem was a flattened zone. Later, this assumed an arc-like structure. The raceme initials that

appeared on the panicle primordia were larger than those of the leaf primordia that appeared on the shoot meristems.

Development of floral primordia

During the transition, the shoot meristem was reduced in size and it induced development of sepals, petals, stamens and carpels, in place of leaves. The pattern and timing of flower initiation varied from species to species. Thus, the flowers developed in terminal or axillary positions and they were either single or grouped into inflorescence.

The characteristic mode of growth followed by the inflorescence primordium in grapes, as observed by Thomas and Bernard (1937), showed a tendency to originate numerous growing apices. The primordium was a complex branched system whereas the branches elongated slowly but continued to divide rapidly. Almost the same mode of growth was observed in grapes, cv. Perlette, by Chadha and Cheema (1971).

The whole process of development of flower primordia in strawberry, cv. Pusa Early Dwarf was divided into four stages by Sharma and Singh (1980). First, there was broadening and flattening of the undifferentiated primordia. Soon after this, the growing point began to elongate with simulta-

neous lengthening of the stalk. New growing points were observed at the bases. Appearance of sepals and petals in the primary flowers was the next stage, followed by the development of rudimentary stamens and pistils on the receptacle.

The fruit bud differentiation in mango could be divided into four stages (Sen, 1943; Gunjate et al. 1977 and Ravishankar et al., 1979). After the high meristematic activity which marked the beginning of differentiation, the buds became plump and conically protruded. The main axis elongated, while the primary and secondary branches showed lobing indicating the formation of a flower cluster. In the fourth stage, the scales loosened indicating the "bud break" stage. Further elongation of the axis and loosening of the scales rendered the bud to enter the "bud burst" stage, which marked the most advanced stage of fruit bud differentiation. The floral parts developed in the order of sepals, petals, stamens and carpels.

Histological studies on the flowering behaviour of coffee, Alvim (1973) observed flattening of the apical growing point with subsequent development into two flower primordia. They developed into two lateral dome-shaped growing

points which produced additional lateral flower buds resulting in an opposite decussate inflorescence.

Mishra and Bajpai (1973) reported that in jamun the calyx primordia initiated at an early stage which further elongated and differentiated into calyx lobes. At this stage the centre appeared as a semicircular mass of tissue. Later on, the primordia of corolla lobes differentiated in the axils of calyx. After a time lag, the primordia of stamens appeared from the base of corolla lobes. The carpels differentiated at the end from the central mass of tissues. Thus, the floral parts developed in acropetal succession.

Experiments on the blossom biology of black pepper, Nalini (1983) identified five distinct stages in the development of flower buds. Two undifferentiated conical primordia surrounded by leaf sheath were observed in the first stage which indicated the beginning of flower bud differentiation. One of these primordia broadened and flattened during the latter half of the first stage. The second stage was characterised by the appearance of a dome-shaped structure at the apex of the broadened primordium which indicated the spike initiation stage. In the third stage, a structure resembling the spike was observed which denoted the floral initiation. Differentiation of floral parts were observed in the next

stage. Stamen and pistil primordia were recognised towards the end of the fourth stage. Appearance of stamens and ovaries in the fifth stage marked the end of flower bud differentiation process.

Subsequently, Rajan (1995) observed that during the transition stage in black pepper, the vegetative apex appeared flat which later transformed into a convex hemispherical structure with the configuration of a mantle core. During the next stage, spike initiation was noticed. The meristem then developed into a dome-shaped structure and the spike primordium initiated. The next stage was characterised by the initiation of primordial bracts and flowers on the sides of the cylindrical primordium in acropetal succession. He observed that the floral ontogeny of the female/hermophrodite flowers and that of the male/hermophrodite flowers were quite distinct in black pepper. In the former type, the ovary wall developed from the peripheral area and inside this structure, the differentiation of integuments took place. In the male/hermophrodite flowers, the flower primordial development led to two to four short stamens.

Vasanthakumar (1996) reported that in cardamom, the raceme initials emerged in spiral whorls at the panicle apex.

They were seen encircled by floral bracts. The floral initials developed further.

## 2.3 Time of flower bud differentiation

The time of flower bud differentiation varies depending on the cultivar, location and environmental factors. Flower bud differentiation in sweet orange took place during January in the northern hemisphere (Abbot, 1935; Ayalon and Monselise 1964). Under Delhi conditions, the blossom bud differentiation in sweet lime (Citrus limettoides) was also observed in January, as reported by Singh and Dhuria (1960).

Bergh (1985) reported that in apple, cv. Starking, the differentiation of reproductive structures initiated during the first week of January whereas Tanura et al. (1987) observed that flower bud initiation occurred between the middle and end of June.

Flower bud differentiation in grapes has been reported to take place during the period preceding the fruiting season (Bernard, 1932; Rajaram et al., 1964 and Chadha and Cheema, 1971). Krishnamurthy et al. (1962) observed that fruit bud initiation in grapes, cv. Anab-e-Shahi was found to be about sixty days from the date of pruning. Under Australian

conditions, the first evidence of the differentiation of floral parts was observed in the spring for Sultana grape vines (Scholefield, 1975).

In mango, wide variations have been reported in the time of flower bud differentiation from year to year, place to place and cultivar to cultivar (Singh, 1953). Under the coastal climate of Konkan, Gunjate et al. (1977) observed maximum fruit bud differentiation in Alphonso variety of mango during September-October period. Ravishankar et al. (1979) reported that in Dharwad which had a savannah climate, the initiation and differentiation of 'Alphonso' reached a peak in mid-November. They observed that in Totapuri, a regular-bearing mango cultivar, the time of fruit bud initiation and differentiation was about a fortnight earlier when compared to the cultivar Alphonso.

Nalini (1983) reported that in black pepper, flower bud differentiation occurred only in the new shoots that arose from the laterals. A spurt of flower bud differentiation activity was observed immediately after the receipt of the pre-monsoon showers and maximum flower bud differentiation occurred during June-July. Rajan (1985) observed that

the total time taken for differentiation of the flower buds in black pepper was about 25 days.

## 2.4 Microtechnique

Different methods of killing and fixing, dehydration and infiltration have been suggested for processing plant materials for microtome sectioning. Bernard (1932) and Snyder (1933) used FAA (Formalin Aceto Alcohol) for killing grape buds. Johansen (1940) also described FAA as the most widely used fixative in which plant specimens could be kept indefinitely without appreciable damage. The same was used by Randhawa and Disna (1947) and later by Mishra and Yamadagni (1968) in citrus. Further evidences for the effectiveness of FAA as an ideal fixative was clear from the works of Mishra and Bajpai (1973) in jamun, Gunjate et al. (1977) in mango, Pathak and Singh (1977) and Sharma and Singh (1980) in strawberry and Subramonian and Shanmugavelu (1981) in jasmine.

Among the different methods of dehydration studied by various authors, graded series of tertiary butyl alcohol (TBA)-isopropyl alcohol-water mixture was found to be promising. The odour of TBA was also found quite agreeable for its

wide spread use in experiments involving microtechnique (Sass, 1951). Ethyl alcohol, which was used widely earlier, was found to cause shrinking and hardening action on the tissue (Johansen, 1940). Sass (1951) suggested that isopropyl alcohol, which can be purchased without restrictions, could be used in exactly the same manner as rather scanty ethyl alcohol for the purposes of dehydration, along with TBA.

Johansen (1940) and Sass (1951) reported that Saffranin and Saffranin combinations were the most important and suitable stains for morphological and cytological studies. They found that Saffranin stained the lignified, cutinized, suberized and chitinised structures as well as the chromosomes, nucleoli and centrosomes.

# MATERIALS AND METHODS

# 3. MATERIALS AND METHODS

Investigations on flower bud differentiation in clove were carried out at the College of Agriculture, Vellayani from August 1996 to December 1997. Bearing clove trees (seven years old) at the Instructional Farm, attached to the College were utilised for the study. These clove trees received uniform cultural practises as per the package of practices recommendations of the Kerala Agricultural University (1996).

## 3.1 Factors influencing flower bud differentiation

### 3.1.1 Carbohydrate and nitrogen reserves

For studying the carbohydrate and nitrogen reserves in relation to flower bud differentiation, leaf samples were collected from five clove trees selected for this purpose. From each tree, four samples were collected for chemical analysis at fortnightly intervals from 15th April 1997 to 30th December 1997. The leaf samples were labelled, dried in an oven at 60°C for 48 hours and powdered, using a grinder to a fineness of 14 mesh. The total soluble carbohydrates and nitrogen content in the powdered plant material were estimated and correlated with flower bud differentiation.

Total soluble carbohydrates in the samples were determined colorimetrically as per the method suggested by Deiraz (1961). Nitrogen in the samples were estimated by the micro-kjeldhal method.

### 3.1.2 Climatic factors

The data on the meteorological parameters were collected daily from the meteorological observatory in the campus. From the daily mean data tabulated, fortnightly averages were computed for mean temperature, relative humidity, rainfall and sunshine hours. These parameters were correlated statistically with the data on flower bud differentiation.

### 3.1.3 Correlation of different factors vs. flower bud differentiation

The influence of climatic and nutritional factors on flower bud differentiation were assessed by correlating the data obtained during each fortnight with that of the data on flower bud differentiation (lag zero). In addition, the climatic factors during the fortnight immediately preceding (lag 1) were correlated with the data on flower bud differentiation during a particular fortnight. Similarly, the climatic factors during two fortnights before the period of flower bud differentiation during a particular

fortnight were also correlated (lag 2). Likewise, analyses were conducted upto lag 16.

## 3.2 Histological studies

### 3.2.1 Selection of material

For studying the site and stages of flower bud differentiation, five trees were selected. Seventy five branches were tagged separately on each tree. From the tagged branches, four shoot-tips per plant were collected randomly at fortnightly intervals, commencing from the first fortnight of April, 1987 to the second fortnight of December, 1987. The specimens (shoot tips) were collected during the morning hours and they were then separated, labelled and processed as per the standard microtechnique procedures (Johansen, 1940).

### 3.2.2 Killing and fixing of plant specimens

FAA (Formalin-Aceto-Alcohol), a widely used fixative (Sass, 1940), was used for killing and fixing the plant specimens. The plant specimens were kept immersed in glass specimen tubes (1.5 cm x 7.0 cm). The tubes were then labelled, closed with tight fitting corks and stored under room temperature.

Formalin-Aceto-Alcohol (FAA)

| | | |
|---|---|---|
| Ethyl alcohol (95%) | - | 50 ml |
| Glacial acetic acid | - | 5 ml |

Formaldehyde (40%) - 10 ml

Distilled water - 35 ml

### 3.2.3 Dehydration

The specimens were stored for one week in FAA and the series of dehydration started subsequently. Two methods of dehydration were tried. The killed specimens were passed through a graded series of ethyl alcohol-xylene solution as indicated below:

Specimens from FAA

↓

Decanted and flooded with Solution I (12 hrs.)

↓

Decanted and flooded with Solution II (12 hrs.)

↓

Decanted and flooded with Solution III (12 hrs.)

↓

Decanted and flooded with Solution IV (12 hrs.)

| Grade Number | Absolute ethyl alcohol (ml) | Xylene (ml) |
|---|---|---|
| Solution I | 75 | 25 |
| Solution II | 50 | 50 |
| Solution III | 25 | 75 |
| Solution IV | 0 | 100 |

A second set of specimens were passed through a graded

series of isopropyl alcohol-tertiary butyl alcohol solution as indicated below:

Specimens from FAA

↓

Decanted and flooded with 50% isopropyl alcohol (3 hrs.)

↓

Decanted and flooded with solution I (3 hrs.)

↓

Decanted and flooded with solution II (12 hrs.)

↓

Decanted and flooded with solution III (3 hrs.)

↓

Decanted and flooded with solution IV (3 hrs.)

↓

Decanted and flooded with solution V (12 hrs.)

↓

Rinsed with three changes of tertiary butyl alcohol (TBA)

↓

Flooded with TBA in the last change (12 hrs.)

| Grade Number | Isopropyl alcohol (ml) | Tertiary butyl alcohol (ml) | Water (ml) |
|---|---|---|---|
| I | 50 | 10 | 40 |
| II | 50 | 20 | 30 |
| III | 50 | 35 | 15 |
| IV | 50 | 50 | — |
| V | 25 | 75 | — |

Based on the two methods of dehydration tested, the TBA (tertiary butyl alcohol) method was found suitable for

the specimens of clove, in terms of firmness of tissues, absence of shrinkage of the protoplasm and absence of distortion of cell walls and contents. Consequently, the TBA series was adopted for further dehydration of all the specimens.

### 3.2.4 Paraffin infiltration

Immediately after the last stage of dehydration series, infiltration was started. Chips of paraffin wax containing ceresin (m.p.58-60°C) were added to the specimens in tertiary butyl alcohol. The addition of paraffin was continued till a layer of undissolved wax remained on top of the solution. The specimen tubes were then corked and placed in an oven at a temperature of 55°C. The specimen tubes were then taken out, one-half of the solution decanted, an equal quantity of paraffin added and the tubes were replaced into the oven quickly. The melted paraffin in the specimen tubes were partially replaced as described above at intervals of four hours. The melted paraffin was then poured out and replaced completely with pure paraffin. After keeping for four hours at 60°C in the oven, another complete replacement of paraffin was done to remove all the traces of the solvent.

### 3.2.5 Embedding

Paraffin wax with ceresin (m.p.53-60°C) was used for embedding the specimens. Embedding was done in boats made of 7.0 cm x 4.5 cm pieces of stiff paper with slightly glazed surface. The paraffin wax containing the specimens were then poured into the boats. The specimens were arranged in a proper order with a heated needle before the solidification of wax. A distance of 1 cm was given between the neighbouring specimens. The identity of different specimens were noted. After hardening of the wax, the paper boat was stripped out and the paraffin blocks containing the specimens were separated.

### 3.2.6 Microtomy

The paraffin blocks were cut into pieces such that one piece contained one specimen. The edges of the pieces were trimmed to make the surfaces flat and uniform.

For microtome sectioning, the paraffin blocks were fastened to metal mounting blocks. Hot paraffin was added to the top of each mounting block to form mounds and was allowed to solidify. The bottom of each embedded piece was then heated, pressed firmly on the top of the mounting blocks and held in contact till the wax cooled down. A fillet of paraffin

was also made around the embedded specimen block to provide a firm basement. Excess paraffin was then removed and the sides of the block, levelled. Sections were cut from the paraffin blocks at a thickness of 10-12 $\mu$, using a rotary microtome.

### 3.2.7 Affixing paraffin sections to the slides

The sections were obtained in the form of ribbon. The ribbon was cut into suitable size of about 5 cm and floated in luke-warm water. The slide was then brought beneath the section(s) so that the section(s) stuck on to the slide. The excess water was drained off. The slides were then immersed in coplin jars filled with xylene and the sections were de-waxed.

### 3.2.8 Staining

Sections affixed to the slides were stained by immersing in staining jars containing various preparations of stains. Saffranin and Saffranin-Fast Green combination were tried.

Staining chart for Saffranin

Pre-staining

Xylene (5 min.)

↓

Absolute alcohol (5 min.)

95% alcohol (5 min.)

↓

70% alcohol (5 min.)

↓

50% alcohol (5 min.)

↓

30% alcohol (5 min.)

↓

Distilled water (2 min.)

↓

Aqueous Saffranin (2 hrs.)

↓

Distilled water (5 min.)

↓

30% alcohol (2 min.)

↓

50% alcohol (2 min.)

↓

70% alcohol (2 min.)

↓

95% alcohol (2 min.)

↓

Absolute alcohol, first change (2 min.)

↓

Absolute alcohol, second change (2 min.)

↓

Clove oil (2 min.)

↓

Xylene, first change (2 min.)

↓

Xylene, second change (2 min.)

↓

Balsam and coverglass

**Staining chart for Saffranin-Fast Green**

Pre-staining operations and time intervals given for various reagents were the same as adopted for Saffranin staining.

Aqueous Saffranin (12 hrs.)

↓

Three changes in distilled water (2 min. each)

↓

30% alcohol (2 min.)

↓

50% alcohol (2 min.)

↓

70% alcohol (2 min.)

↓

95% alcohol (2 min.)

↓

Fast Green in 95% alcohol (30 sec.)

↓

Absolute alcohol, first change (2 min.)

↓

Absolute alcohol, second change (2 min.)

↓

Clove oil (2 min.)

↓

Xylene, first change (2 min.)

↓

Xylene, second change (2 min.)

↓

Canada balsam and coverglass

### 3.2.9 Microscopic examination

The slides were examined through a binocular mono-objective microscope (Olympus KIC B1) with 10X/5X objectives and 10X eyepiece. Critical examinations were done at higher magnification using a binocular research microscope (Nikon Optiphot) available at the Central Instruments Laboratory, National Agricultural Research Project, Vellayani.

### 3.2.10 Photomicrography

Photomicrographs of the selected sections were taken using a photomicrographic system (Nikon Optiphot with FX-35A). Black and white negative film of 120 ASA (ORWO) and colour negative film of 100 ASA (Kodak) were used for taking the photomicrographs.

# RESULTS

# 4. RESULTS

The results of the investigations conducted on flower bud differentiation in clove are presented here. The study consisted of two parts, one on the factors influencing flower bud differentiation and the other on the histological aspects. The observations were recorded over a nine-month period, from April 1987 to December 1987 (Fig. 1a). The climatological factors during sixteen fortnights prior to the period of study were also recorded (Fig. 1b) and examined to find out their influence on flower bud differentiation.

## 4.1 Factors influencing flower bud differentiation

### 4.1.1 Temperature

#### 4.1.1.1 Maximum temperature

During the period of study, the maximum temperature (Table 1) ranged between 29.77°C (2nd fortnight of August 1987) to 34.19°C (2nd fortnight of April, 1987). The maximum temperature recorded during April and May 1987 were slightly higher than that registered during the other periods of the year. The period from June to December, 1987 revealed a more or less steady pattern with regard to the daily maximum temperature.

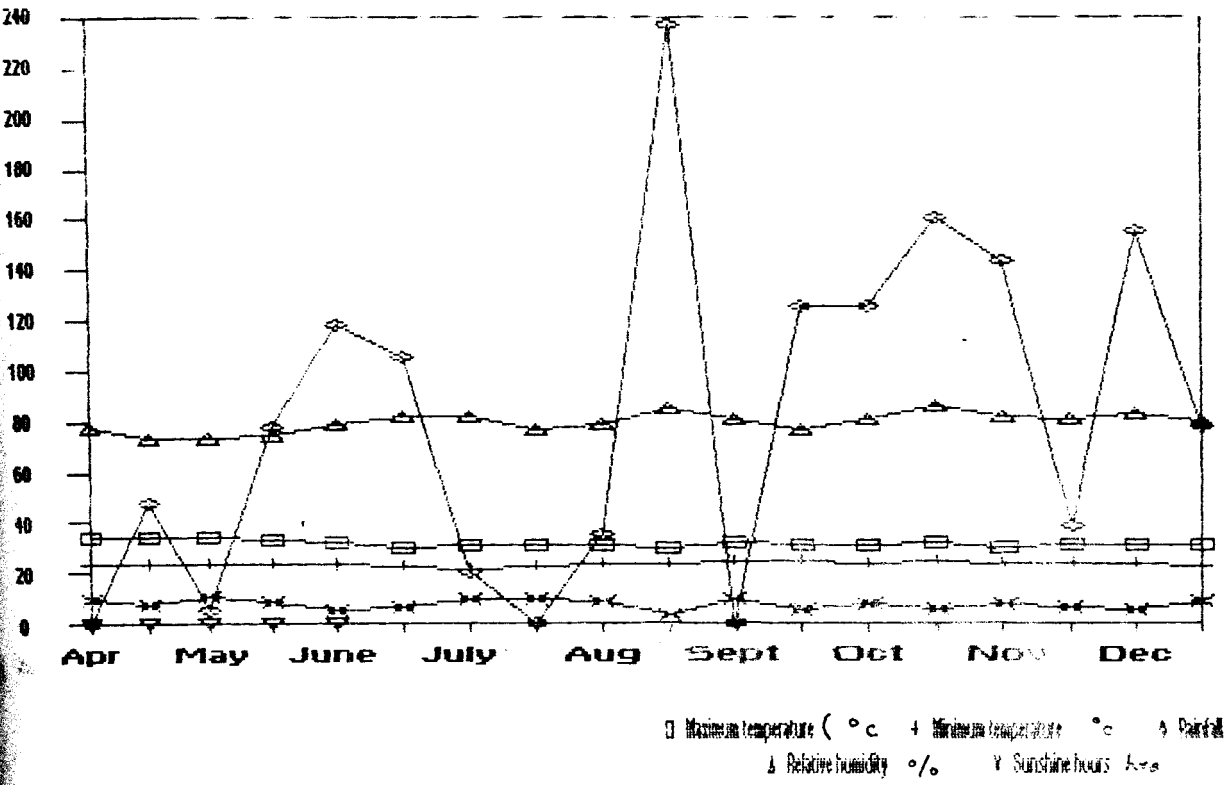


Fig. 1.a. Meteorological data of Agricultural college farm ,Vellayani from April - December, 1987

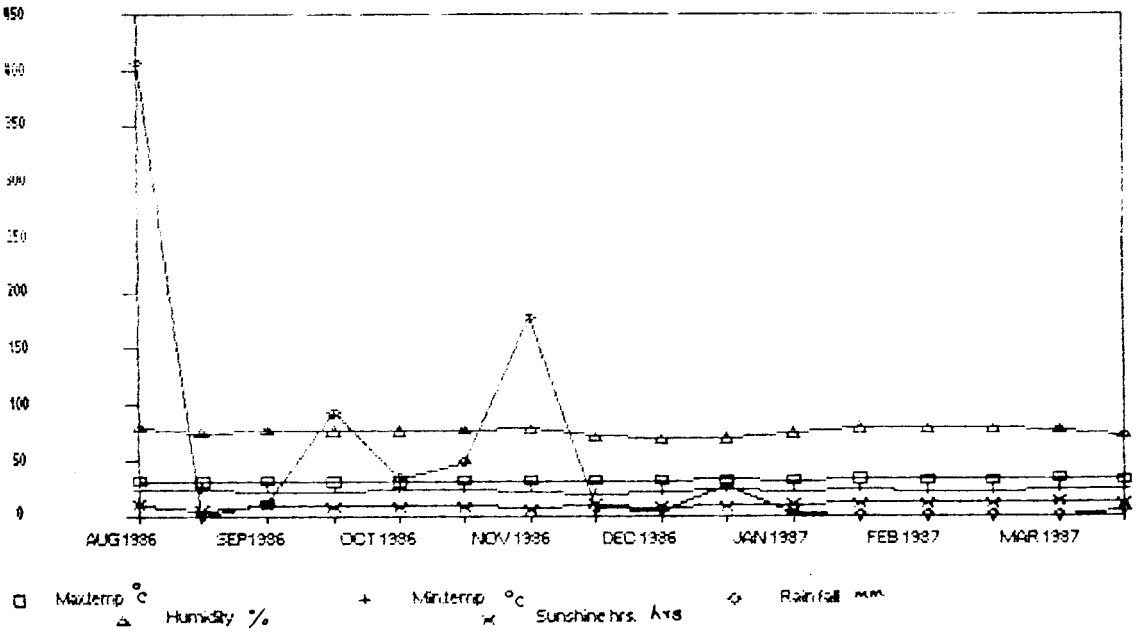


Fig. 1.b Meteorological data of Agricultural college farm ,Vellayani during the lag period (Aug 1986-Mar 1987)

Table 1. Weather data and nutrient status of leaves during the period of study

| Period | | | Maximum temperature °C | Minimum temperature °C | Rainfall (mm) | Relative humidity (%) | Sunshine hours | Carbohydrate (%) | Nitrogen (%) | C/N ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| Month and Year | | Fortnight | | | | | | | | |
| April | 1987 | 1 | 33.76 | 23.50 | 0.00 | 78.16 | 9.67 | 9.11 | 1.50 | 6.07 |
| | | 2 | 34.19 | 23.03 | 48.00 | 73.89 | 9.06 | 8.23 | 1.60 | 5.15 |
| May | 1987 | 1 | 33.83 | 23.60 | 5.80 | 73.30 | 10.68 | 9.34 | 1.55 | 6.04 |
| | | 2 | 32.64 | 23.67 | 77.20 | 74.31 | 8.74 | 8.18 | 1.55 | 5.26 |
| June | 1987 | 1 | 32.15 | 22.95 | 117.70 | 79.36 | 5.68 | 9.23 | 1.22 | 7.59 |
| | | 2 | 30.21 | 22.29 | 105.40 | 81.56 | 6.48 | 9.04 | 1.36 | 6.67 |
| July | 1987 | 1 | 30.91 | 23.19 | 20.00 | 81.66 | 9.58 | 8.70 | 1.74 | 4.99 |
| | | 2 | 31.18 | 22.60 | 0.40 | 77.06 | 9.28 | 7.64 | 1.77 | 4.32 |
| August | 1987 | 1 | 30.99 | 23.99 | 35.20 | 78.20 | 8.82 | 7.94 | 1.01 | 7.86 |
| | | 2 | 29.77 | 23.10 | 237.40 | 84.63 | 3.73 | 7.98 | 1.54 | 5.20 |
| September | 1987 | 1 | 31.57 | 24.44 | 0.00 | 81.33 | 9.94 | 7.99 | 1.45 | 5.52 |
| | | 2 | 30.99 | 24.09 | 125.70 | 76.90 | 5.10 | 7.29 | 1.59 | 4.59 |
| October | 1987 | 1 | 30.84 | 23.70 | 126.00 | 81.20 | 7.20 | 7.68 | 1.22 | 6.32 |
| | | 2 | 32.26 | 24.21 | 160.00 | 86.45 | 5.02 | 8.02 | 1.82 | 4.39 |
| November | 1987 | 1 | 30.26 | 23.63 | 143.80 | 81.72 | 7.36 | 8.33 | 1.63 | 5.10 |
| | | 2 | 30.66 | 23.50 | 38.50 | 81.00 | 6.76 | 8.24 | 1.44 | 5.72 |
| December | 1987 | 1 | 30.69 | 23.81 | 155.50 | 82.50 | 5.61 | 7.34 | 1.80 | 4.07 |
| | | 2 | 31.03 | 22.66 | 77.70 | 80.29 | 8.94 | 8.17 | 1.66 | 5.04 |

The lag period (2nd fortnight of March 1987 to first fortnight of August, 1986) gave a trend almost similar to that of the period of study. Here, the highest value was observed (Table 3) during the first fortnight of March, 1987 (33.81°C) and the lowest, during the second fortnight of August, 1986 (30.21°C).

4.1.1.2 Minimum temperature

Regarding minimum temperature, the lowest reading of 22.29°C was observed during the second fortnight of June, 1987. The peak reading of 24.44°C was recorded during the first fortnight of September, 1987 (Table 1). With the onset of the South-West monsoon during the first fortnight of June, 1987 a comparatively low value (22.95°C) was recorded. During the second fortnight of December, 1987 also the minimum temperature recorded (Table 1) was rather low (22.66°C). The other months registered a more or less uniform trend for the mean minimum temperature.

During the lag period, wide fluctuations of minimum temperature ranging from 19.28°C during the 2nd fortnight of November, 1986 to 23.68°C during the 2nd fortnight of March, 1987 were seen (Table 3).

Table 3. Weather data during the lag period of experiments (March 1987 to August 1986)

| Period | | | Maximum temperature °C | Minimum temperature °C | Rainfall (mm) | Relative humidity (%) | Sunshine hours |
|---|---|---|---|---|---|---|---|
| Month and | Year | Fortnight | | | | | |
| March | 1987 | 2 | 32.09 | 23.68 | 4.90 | 72.50 | 10.50 |
| | | 1 | 33.91 | 22.91 | 0.00 | 77.00 | 10.52 |
| February | 1987 | 2 | 31.73 | 20.84 | 0.00 | 77.60 | 10.22 |
| | | 1 | 32.12 | 20.77 | 0.00 | 77.43 | 10.25 |
| January | 1987 | 2 | 32.05 | 21.95 | 0.00 | 78.40 | 10.46 |
| | | 1 | 31.03 | 21.60 | 0.60 | 73.86 | 8.37 |
| December | 1986 | 2 | 31.65 | 22.70 | 24.80 | 69.21 | 7.97 |
| | | 1 | 31.23 | 20.17 | 4.80 | 68.46 | 7.19 |
| November | 1986 | 2 | 30.71 | 19.28 | 6.00 | 70.75 | 9.49 |
| | | 1 | 30.53 | 21.47 | 177.40 | 78.00 | 5.28 |
| October | 1986 | 2 | 30.66 | 22.39 | 47.90 | 77.20 | 7.53 |
| | | 1 | 30.37 | 22.87 | 32.40 | 76.40 | 7.89 |
| September | 1986 | 2 | 30.30 | 21.20 | 92.60 | 76.25 | 7.78 |
| | | 1 | 31.40 | 21.39 | 9.80 | 77.16 | 8.32 |
| August | 1986 | 2 | 30.21 | 22.86 | 0.00 | 74.63 | 3.42 |
| | | 1 | 30.99 | 22.43 | 406.50 | 79.50 | 9.42 |

### 4.1.2 Rainfall

Rainfall showed wide variation, recording nil during the first fortnight of April, 1997 and reaching a peak of 237.40 mm during the second fortnight of August, 1997 (Table 1). The South-West monsoon showers started during the first fortnight of June and rainfall was rather scanty during July which otherwise is considered as a heavy rainy month of the locality. Total rainfall recorded was 1474.30mm which is a low figure compared with the total rainfall of Kerala, registered during the past several years.

Rainfall during the lag period was generally scanty with a dry period intervening between the second fortnight of January, 1997 and the first fortnight of March, 1997 (Table 3). During the lag period, maximum rainfall was received during the first fortnight of August, 1996 (406.50mm). Total rainfall recorded during the lag phase was 907.50mm for a period of eight months.

### 4.1.3 Relative humidity

The relative humidity recorded during the period of study, did not show wide variations. The lowest value of 73.30 per cent was recorded during the first fortnight of

May, 1987 and the highest (96.45 per cent), during the second fortnight of October, 1987. The second fortnight of August, 1987 which registered the maximum rainfall during the study period also registered comparatively high relative humidity (84.63 per cent). The months of November and December 1987 were relatively humid, registering values 81.72, 81.00, 92.50 and 80.29 per cent respectively.

During the lag period, relative humidity ranged between 69.46 per cent during the first fortnight of December, 1986 and 79.50 per cent during the first fortnight of August, 1986. Here again, the maximum for relative humidity and rainfall coincided during the first fortnight of August.

4.1.4 Sunshine hours

During the study period, the duration of sunshine hours (Table 1) was maximum during the first fortnight of May, 1987 (10.68) which declined to 5.69 during the first fortnight of June when the South-West monsoon started. The duration of sunshine hours was minimum (3.73) during the second fortnight of August, when rainfall obtained was the maximum.

During the lag phase period from January to March

1987 (Table 3) higher values for sunshine hours were registered. Maximum sunshine (10.52 hrs.) was obtained during the first fortnight of March, 1987 during which period the maximum temperature recorded was also the highest. Sunshine hours was minimum (3.42) during the second fortnight of August, 1986.

### 4.1.5 Nutritional factors (carbohydrates, nitrogen and C/N ratio)

Data on fortnightly variation of total soluble carbohydrates, nitrogen and C/N ratio in clove leaves during the period of study are presented in Table 1.

During the period of observation, the total soluble carbohydrates did not show wide variation. Carbohydrate content ranged between 7.29 per cent (during the second fortnight of September, 1987) to 9.34 per cent (during the first fortnight of May, 1987). The carbohydrate content recorded during the months of April to June were relatively high (8.13 to 9.34 per cent). Carbohydrate levels in the leaves were comparatively low during the months of August (7.94 and 7.93 per cent) and September, 1987 (7.29 and 7.99 per cent).

Maximum nitrogen content in the leaves was recorded during the second fortnight of October, 1937 (1.92 per cent) and the minimum, during the first fortnight of August (1.01 per cent). Nitrogen content was rather high during the two fortnights of July (1.74 and 1.77 per cent, respectively).

The carbohydrate - nitrogen ratio ranged between 4.07 during the first fortnight of December, 1937 to 7.96 during the first fortnight of August (Table 1). A well-defined monthly variation for C/N ratio was not seen from the data recorded for the study period.

4.1.6 Correlation between environmental and nutritional factors Vs flower bud differentiation

The weather parameters and the nutritional factors recorded during the period of study were correlated with flower bud differentiation. In addition, correlation was worked out between the weather parameters recorded during the different fortnights preceding the period of flower bud differentiation and the percentage of flower bud differentiation observed during a particular fortnight of the study period. The fortnight just preceding the fortnight of the study period was considered as 'lag 1' and the second fort-

night preceding the fortnight of the study period was considered as 'lag 2'. Likewise, analysis was conducted upto 'lag 16' (15 fortnights preceding the fortnight of the study period). The correlation coefficients are presented in Table 4.

During the lag zero or the fortnight corresponding to the observation, neither the climatic factors nor the nutritional factors showed significant correlation with flower bud differentiation.

Maximum temperature during the 6th fortnight prior to differentiation (lag 6) and the 7th fortnight prior to differentiation (lag 7) showed significant positive correlation with flower bud differentiation ($r = 0.6424^{**}$ and $0.8116^{**}$ respectively). A similar trend was found in the fortnights eight to ten also (lag 8, $r = 0.8420^{**}$; lag 9, $r = 0.7059^{**}$ and lag 10, $r = 0.5038^{*}$). The highest correlation between the maximum temperature and flower bud differentiation was noted during the eighth fortnight prior to flower bud differentiation (lag 8, $r = 0.8420^{**}$).

Table 4. Correlation of weather data (simultaneous and lag periods) and nutritional factors (simultaneous period) vs. flower bud differentiation

| Factors affecting flower bud differentiation | lag zero | lag 1 | lag 2 | lag 3 | lag 4 | lag 5 | lag 6 | lag 7 | lag 8 |
|---|---|---|---|---|---|---|---|---|---|
| 1. Maximum temperature | -0.4122 | -0.3549 | -0.2327 | -0.0926 | -0.0655 | 0.3367 | 0.6424** | 0.8116** | 0.8420** |
| 2. Minimum temperature | -0.0627 | -0.4607 | -0.4706* | -0.2616 | -0.0434 | 0.1261 | 0.2752 | 0.2751 | 0.2728 |
| 3. Rainfall | -0.0133 | -0.0320 | -0.1761 | -0.1664 | 0.0115 | 0.1030 | 0.0224 | -0.1148 | -0.2975 |
| 4. Relative humidity | 0.1760 | 0.0768 | -0.0566 | -0.0944 | -0.1423 | -0.3294 | -0.5028* | -0.4281 | -0.1888 |
| 5. Sunshine hours | -0.0185 | 0.0123 | 0.1285 | 0.0704 | -0.0545 | -0.1224 | 0.0691 | 0.2551 | 0.4531 |
| 6. Carbohydrates | -0.1308 | | | | | | | | |
| 7. Nitrogen | -0.2292 | | | | | | | | |
| 8. C/N ratio | 0.3071 | | | | | | | | |

* Significant at 5% level ** Significant at 1% level

Table 4. Correlation of weather data (simultaneous and lag periods) and nutritional factors (simultaneous period) vs. flower bud differentiation

(cont.....2)

| Factors affecting flower bud differentiation | lag 9 | lag 10 | lag 11 | lag 12 | lag 13 | lag 14 | lag 15 | lag 16 |
|---|---|---|---|---|---|---|---|---|
| 1. Maximum temperature | 0.7059** | 0.5038* | 0.2772 | 0.1305 | -0.0968 | -0.2329 | -0.2902 | -0.3815 |
| 1. Minimum temperature | 0.2350 | 0.1840 | -0.0860 | -0.3878 | -0.5438* | -0.4936* | -0.4016 | -0.4258 |
| 3. Rainfall | -0.3284 | -0.4873* | -0.5655* | -0.5476* | -0.4723* | -0.2516 | 0.0246 | -0.1294 |
| 4. Relative humidity | 0.0237 | 0.0452 | -0.0365 | 0.0103 | -0.0057 | -0.1578 | -0.3678 | -0.6484** |
| 5. Sunshine hours | 0.4689* | 0.6076** | 0.6252** | 0.5518* | 0.3761 | 0.0855 | -0.0372 | -0.2197 |
| 6. Carbohydrates | | | | | | | | |
| 7. Nitrogen | | | | | | | | |
| 8. C/N ratio | | | | | | | | |

* Significant at 5% level ** Significant at 1% level

Significant negative correlation was obtained for minimum temperature during the second fortnight prior to differentiation (lag 2, r = -0.4706*). The minimum temperature during the thirteenth fortnight prior to differentiation (lag 13) and fourteenth fortnight prior to differentiation (lag 14) were also negatively correlated with flower bud differentiation (r = -0.5433* and r = -0.4936*).

Rainfall showed significant negative correlation with the percentage of flower buds differentiated during the tenth fortnight (lag 10) prior to differentiation (r = -0.4973*). During the eleventh fortnight prior to flower bud differentiation (lag 11) also rainfall showed significant negative correlation (r = -0.5655*). The trend was similar for lag 12 (r = -0.5476*) and lag 13 (r= -0.4723*). Of these, maximum negative correlation between rainfall and flower bud differentiation was in the eleventh fortnight prior to flower bud differentiation.

Percentage of flower bud differentiation was negatively correlated with relative humidity recorded during the sixteenth fortnight (lag 16) prior to flower bud differentiation (r = -0.6494**). Significant negative correlation was also seen during the sixth fortnight prior to differentiation (r= -0.5028*).

Sunshine hours during the ninth fortnight prior to differentiation (lag 9) showed significant positive correlation with the percentage of flower bud differentiation ($r = 0.4639^{*}$). Significant positive correlation between sunshine hours and flower bud differentiation was seen during the preceding three fortnights also (lag 10, $r = 0.6076^{**}$; lag 11, $r = 0.6252^{**}$ and lag 12, $r = 0.5513^{*}$). Sunshine hours during the eleventh fortnight had maximum correlation with flower bud differentiation.

The carbohydrate, nitrogen and C/N ratio were correlated with the data on flower bud differentiation during the simultaneous fortnight of study period only (simultaneous or lag zero analysis). Neither carbohydrate content nor nitrogen content showed any significant correlation with the data on flower bud differentiation. C/N ratio also did not reveal any significant correlation with flower bud differentiation (Table 4).

## 4.2 Histological studies on flowering

### 4.2.1 Flower bud differentiation

The pattern of flower bud differentiation was studied for a nine-month period from April, 1987 to December, 1987.

Flower bud differentiation, as presented in Table 2, markedly increased from the second fortnight of May and attained a peak (90 per cent) during the second fortnight of August. Thereafter, flower bud differentiation steadily declined. No flower buds were seen differentiated during the months of April, November and December 1987.

### 4.2.2 Floral ontogeny

Apical meristems of clove plants collected at fortnightly intervals were processed as per the standard microtechnique procedures and serial median longitudinal sections were taken to observe the internal morphology of developing organs.

The vegetative shoot apex appears dome-shaped, surrounded by leaf primordia (Plate 1). The deeply stained region depicts a zone of high meristematic activity.

The apex is seen hidden midway between two leaf primordia which are larger in dimension and are projected above the primordial site.

In the next stage, the apex is seen slightly raised above the primordial site (Plate 2). Vascular strands appear

Table 2. Flower bud differentiation in clove at fortnightly intervals from April to December, 1987

| Period | | Fortnight | Differentiation of flower buds (%) |
|---|---|---|---|
| Month and Year | | | |
| April | 1987 | 1 | 0 |
| | | 2 | 0 |
| May | 1987 | 1 | 5 |
| | | 2 | 15 |
| June | 1987 | 1 | 30 |
| | | 2 | 45 |
| July | 1987 | 1 | 50 |
| | | 2 | 60 |
| August | 1987 | 1 | 85 |
| | | 2 | 90 |
| September | 1987 | 1 | 75 |
| | | 2 | 25 |
| October | 1987 | 1 | 10 |
| | | 2 | 5 |
| November | 1987 | 1 | 0 |
| | | 2 | 0 |
| December | 1987 | 1 | 0 |
| | | 2 | 0 |

Plate 1 Vegetative primordium of clove (L.S. x 25)

1. Dome-shaped apex
2. Leaf primordium


PLATE - 1

Plate 2 Vegetative primordium of clove showing commencement of vascularization (L.S. x 4)

1. Shoot apex
2. Vascular strands
3. Leaf primordium


PLATE – 2

at this stage. Leaf primordia are seen emerging, subtending the apex at lateral positions.

The dome-shaped apex has elongated above the primordial site and the shape has become nearly elliptical (Plates 3 and 4). On either side of the dome, two bud initials can be seen. Vascular development is more prominent at this stage.

Secretory spaces are seen developed during the above stages (Plates 2 and 3). In clove, the number of such secretory spaces are more in the bracts towards the periphery. They are fewer in number, towards the lower region. The spaces are lined with secretory cells, composing the epithelium (Plate 5). These secretory spaces are schizogenous in origin. The cells oppressing these spaces, divided and enlarged further thus making possible their expansion. The schizogenous spaces or cavities are observed to be somewhat round.

### 4.2.3 Floral morphology and differentiation of floral organs

In clove, the inflorescence is a terminal corymbose, trichotomous panicle, shortly pedunculate and branched from the base (Plate 6) shorter than the leaves and variable in

Plate 3 Vegetative primordium of clove showing elongation and prominent development of vascular zones (L.S. x 10)

1. Shoot apex
2. Bud initial
3. Vascular zones


PLATE - 3

Plate 4 Vegetative primordium of clove in Plate 3 magnified (L.S. x 25)

1. Shoot apex
2. Bud initial
3. Vascular zones
4. Secretory spaces


PLATE - 4

Plate 5 Secretory spaces (schizogenous cavities) shown in Plate 4 magnified (L.S. 100)

1. Schizogenous cavities

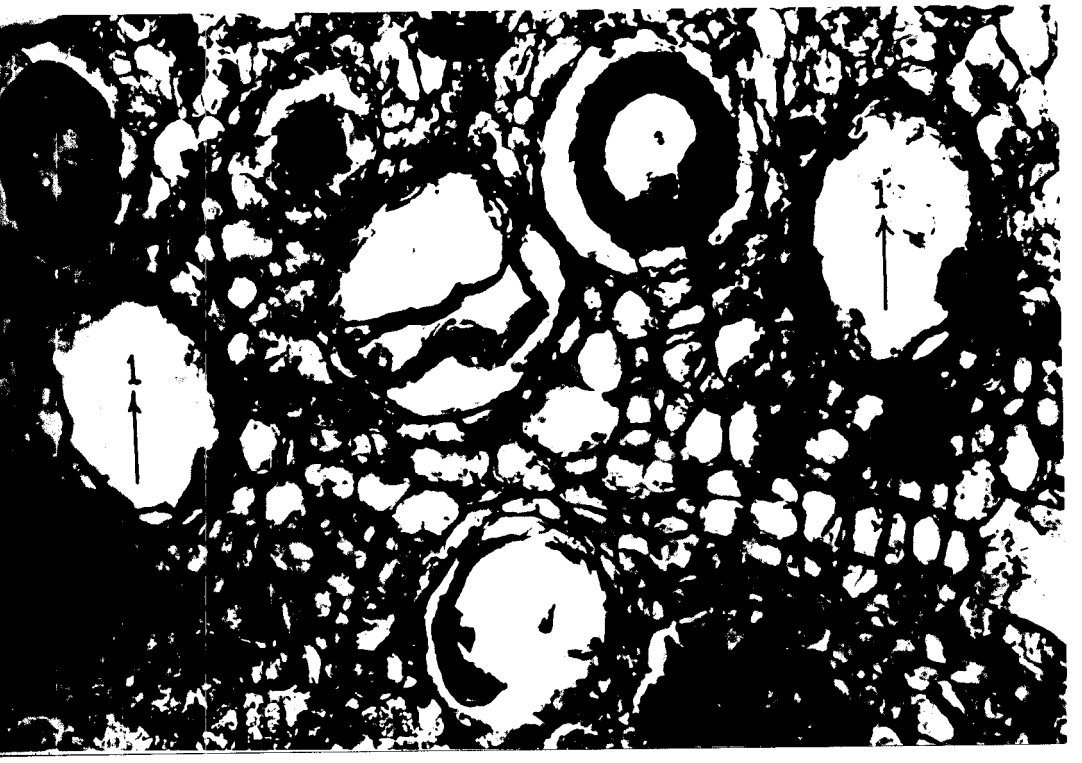


PLATE - 5

Plate 6 Inflorescence of clove showing the trichotomous branching (x 0.5)

PLATE - 6

the number of flowers ranging from three flowers on a simple three-forked peduncle to as many as fifty or more when conditions favour the triple divisions of the peduncle.

The central bud initials end in a flower. Two more subtending flower bud initials arise on the lateral sides (Plate 7). This is seen clearly demarcated where the central bud is seen much raised above the two lateral initials. The lateral initials observed in Plate 7 is magnified and depicted in Plate 8 where the peripheral cells are observed to be prominently nucleated.

The transformation from the vegetative to reproductive stage in clove is marked by the appearance of two lateral protuberances on either side of the apical dome (Plate 9). Soon after the transformation, initials of non-essential whorls of flower (sepals and petals) begin to appear (Plate 10). Here, the sepal primordia can be seen overlapping the inner petal primordia. Sepal primordia are seen more developed. Initials of stamens are visible, as small dots; but no trace of carpels could be detected.

Plate 7 Bud initials (flower primordia) of clove before transformation (L.S. x 10)

1. Apical bud
2. Lateral bud

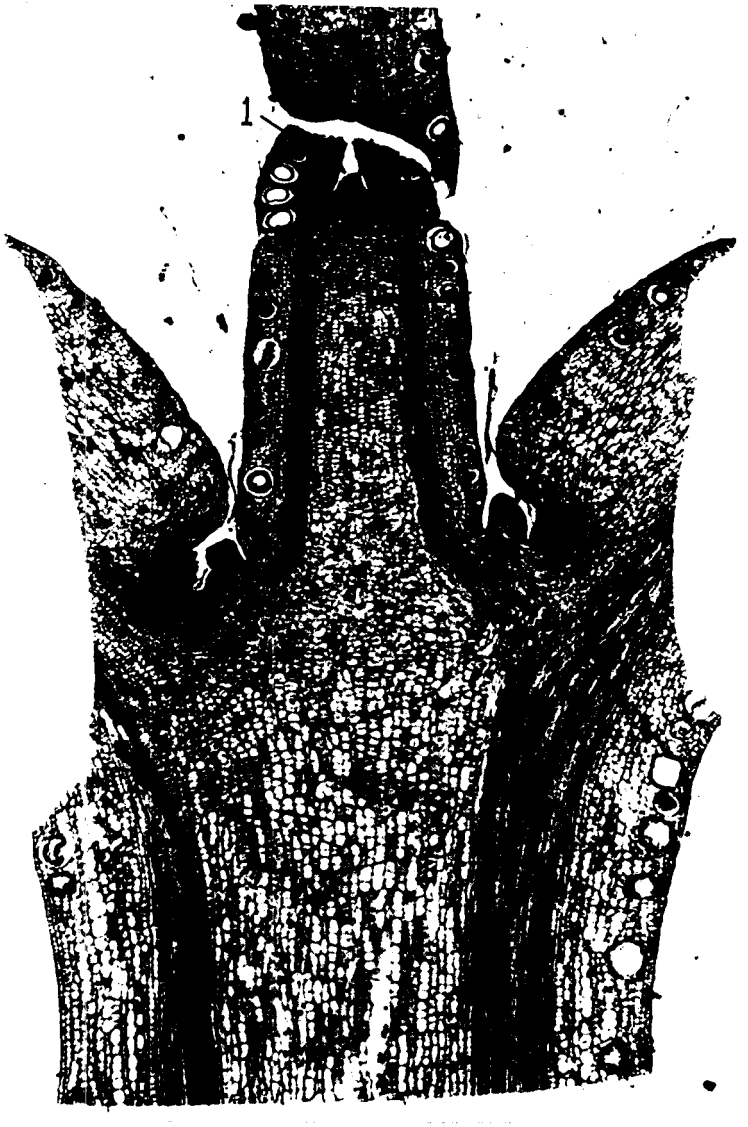


PLATE - 7

Plate 8 Lateral bud initial in Plate 7 magnified (L.S. x 50)

1. Bud initial
2. Deeply stained nuclei

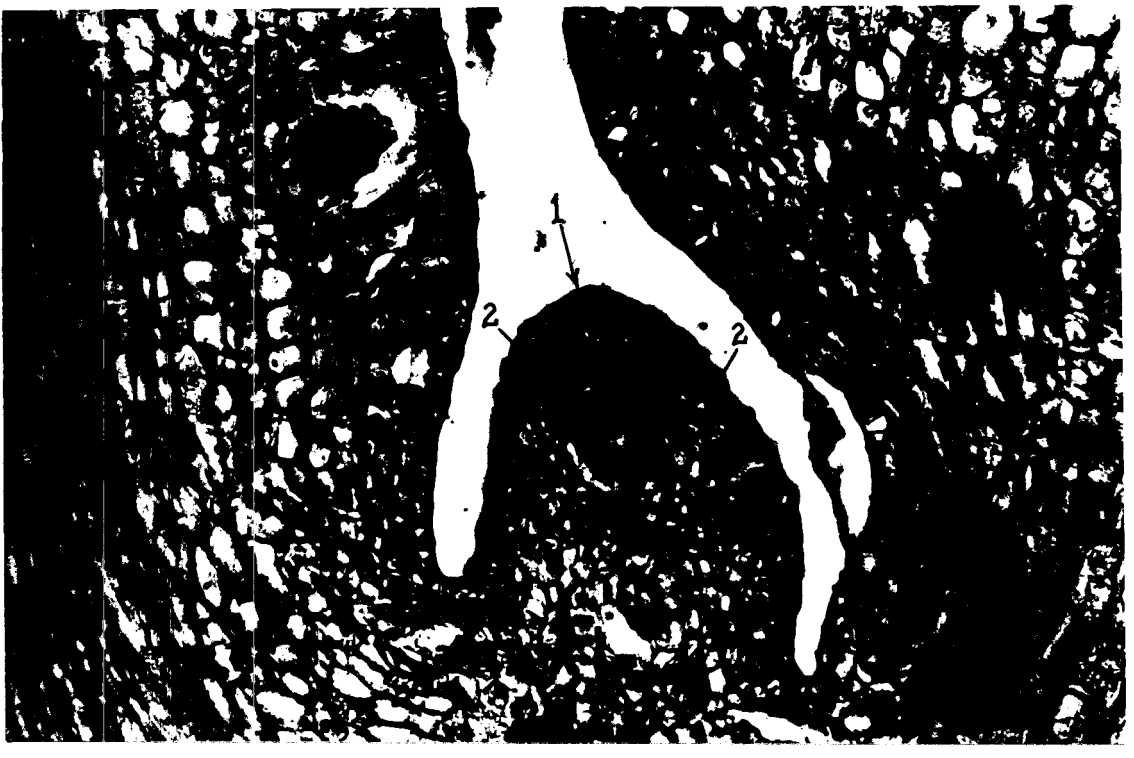


PLATE - 8

Plate 9 Transformation from vegetative to floral phase in clove (L.S. x 10)

1. Apical dome
2. Lateral protuberance


PLATE – 9

Plate 10 Initiation of non-essential floral parts in clove (L.S. x 10)

1. Sepal primordium
2. Petal primordium


PLATE - 10

4.2.3.1 Development of androecium

Clove flower is protrandrous. The stamens are numerous (Plate 11), appearing in clusters of four.

The pollen mother cells are closely packed in their early stages of development. Towards the later stage, the four nuclei of microspores inside the pollen mother cells round off forming tetrads (Plate 12). This is seen enlarged in Plate 13. Further advancement of anther development is characterised by disintegration of walls, thereby releasing the microspores (Plate 14).

4.2.3.2 Development of gynoecium

The carpels are short and stalky with a large ovary, short style and capitate stigma (Plate 15). The carpels develop below the androecium. The ovary has two cells, multiovulate, inferior and is situated (embedded) on top of the hypanthium (Plate 16).

The flower bud of clove which is green in the young stage (Plate 6) develops a pinkish tinge before and after anthesis (Plates 17 and 18). The four calyx lobes turn fleshy and attain a triangular shape which are slightly incurved (Plate 19) as the fruits advance to maturity.

Plate 11 Development of androecium in clove at an early stage (L.S. x 10)

1. Stamens

PLATE - 11

Plate 12 Development of androecium in clove at a later stage (L.S. x 10)

1. Outer stamens
2. Anther

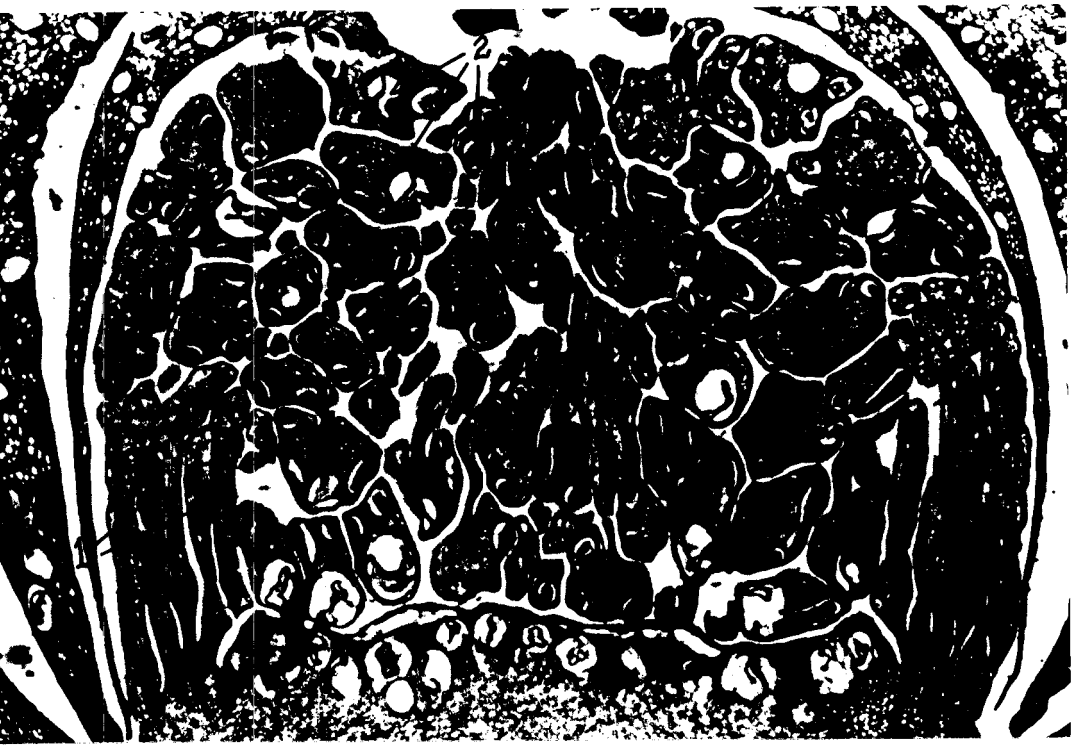

PLATE - 12

Plate 13 Anthers shown in Plate 12 magnified (L.S. x 100)

1. Tetrads

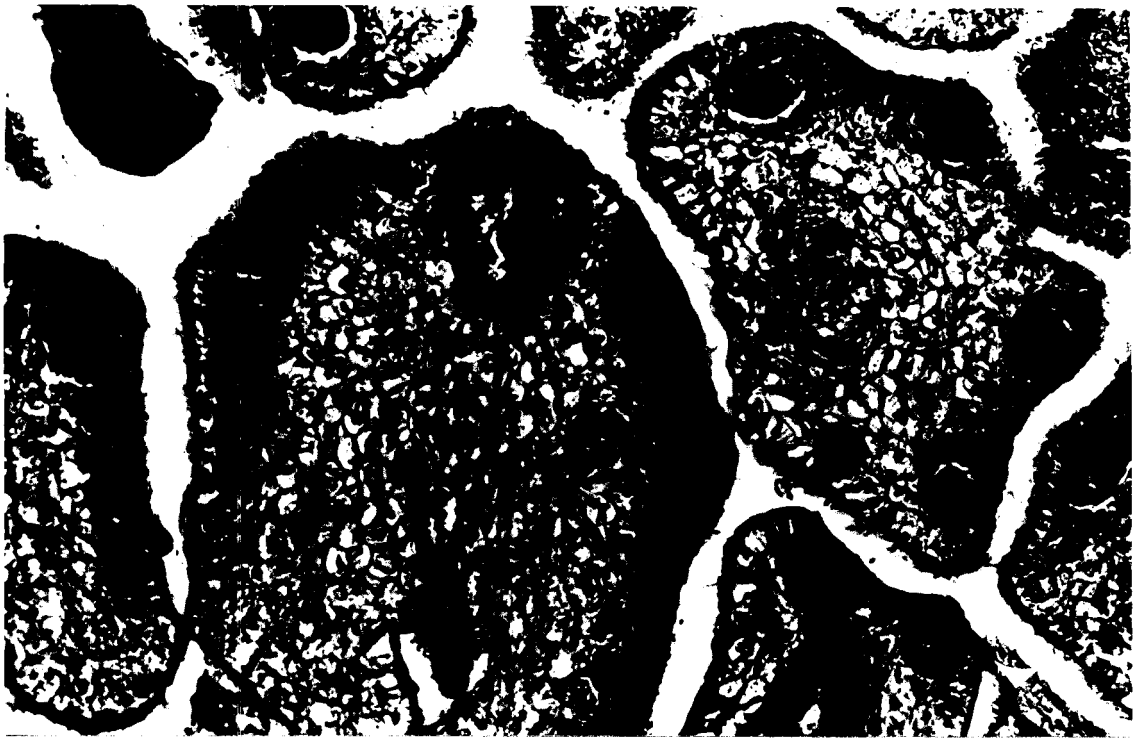

PLATE – 13

Plate 14 Disintegration of anthers, releasing microspores (L.S. x 10)

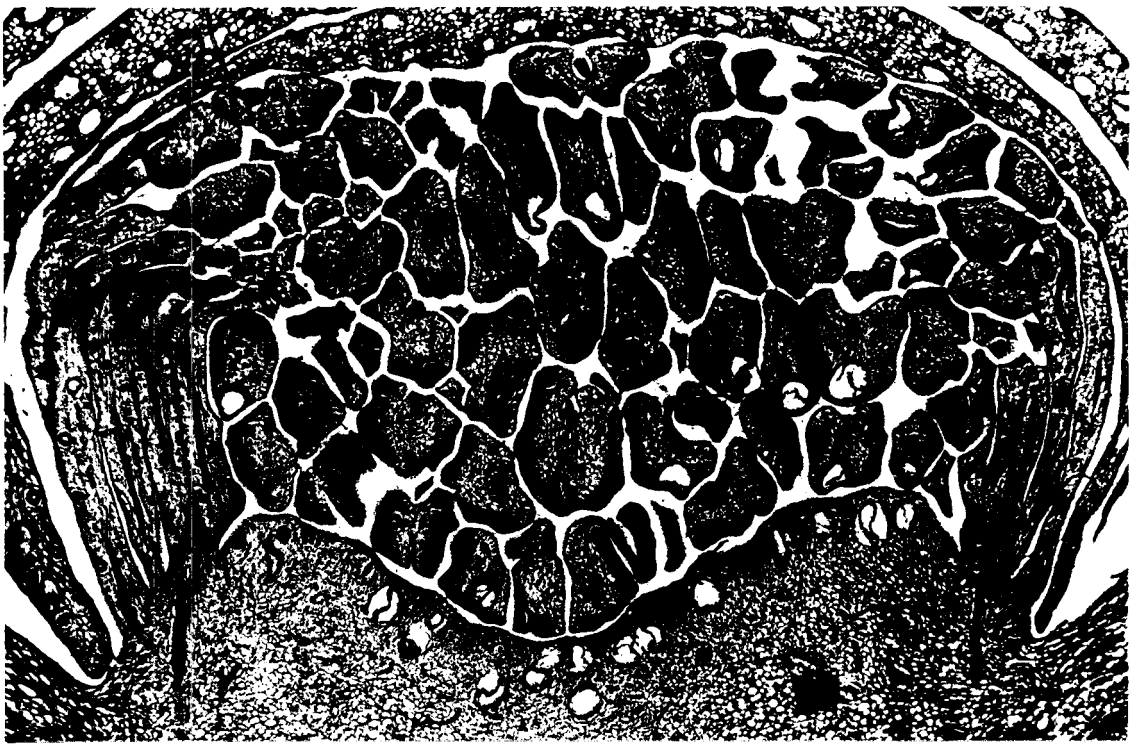

PLATE - 14

Plate 15 Development of gynoecium in clove (L.S. x 10)

1. Hypanthium
2. Locule
3. Style
4. Stigma

PLATE - 15

Plate 16 Ovary of clove flower bud in Plate 15 magnified (L.S. x 100)

1. Ovule

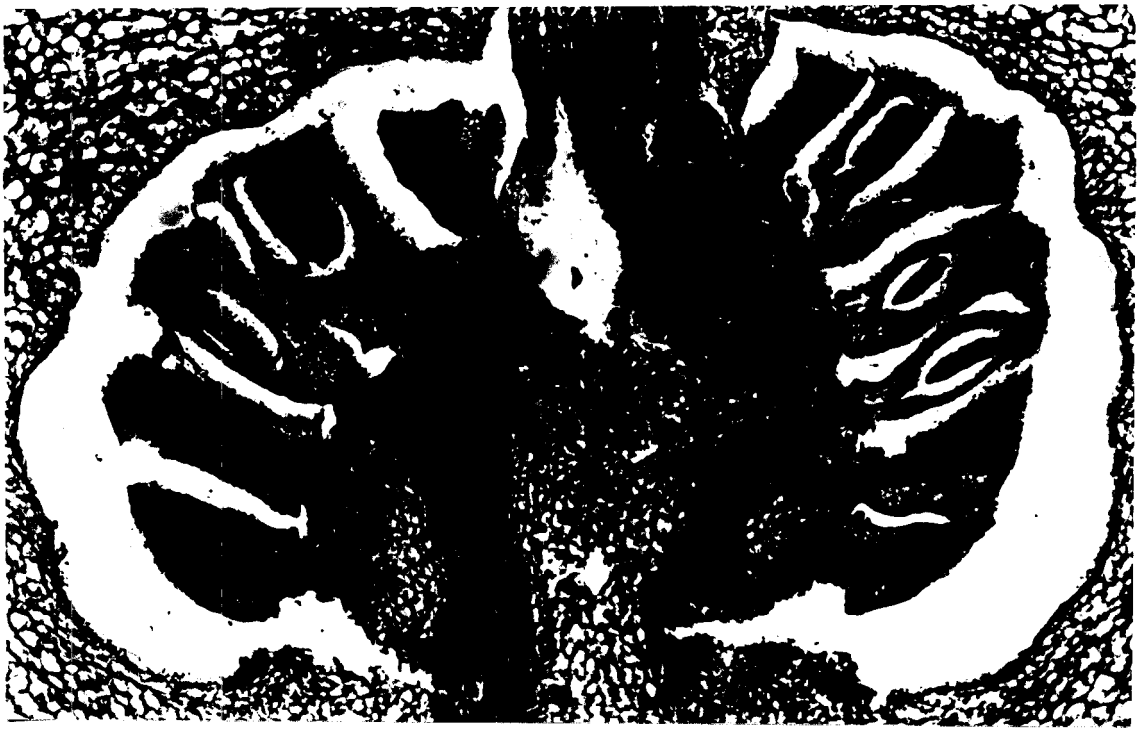

PLATE - 16

Plate 17 Flower bud of clove before anthesis (x 2.6)


PLATE - 17

Plate 13 Flower bud of clove after anthesis (x 2.6)


PLATE - 18

Plate 19 Fruit of clove at a maturing stage (x 2.5)

1. Calyx lobes

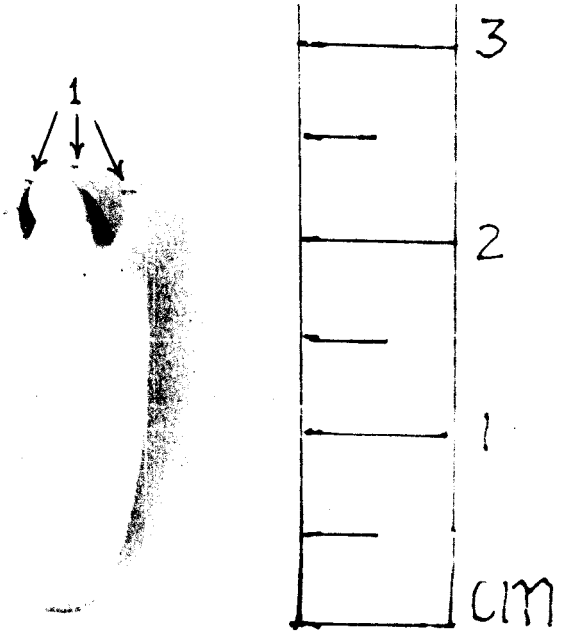


PLATE - 19

# DISCUSSION

# 5. DISCUSSION

For obtaining steady and optimum yield in any perennial crop, the various cultural practices will have to be timed in relation to the cycle of flower bud differentiation. It is known that flower buds may be differentiated a few days to several months prior to the bud burst. With precise information on the site and time of flower bud differentiation, the application of fertilizer can be timed to help the build up of carbohydrate and nitrogen reserves as well as favourable C/N ratio. By withholding irrigation at the appropriate time, the physiological maturity of fruiting branches/shoots can be brought about. The nature and quantity of vegetative portion to be removed by way of pruning can be decided only with a clear understanding of the site and time of flower bud differentiation, and on the basis of an estimate of the expected crop. In short, precise information on the various aspects of flower bud differentiation will aid in the scientific management of any crop including perennials.

## 5.1 Factors influencing flower bud differentiation

### 5.1.1 Temperature

5.1.1.1 Maximum temperature

During the period of observation, the maximum temperature varied from 30.26°C during the second fortnight of November, 1987 to 34.19°C during the second fortnight of April, 1987. The maximum temperature remained high during the summer months of March to May, till the onset of the South-West monsoon in June.

The peak flower bud differentiation was recorded in August. The maximum temperature registered during six to ten fortnights prior to differentiation showed significant positive correlation with the number of buds differentiated. So from these observations it could be elucidated that relatively high levels of temperature that prevailed during the period from the second fortnight of March to the second fortnight of May might have triggered the process of flower bud differentiation. The temperature recorded during the second fortnight of April (34.19°C), the highest recorded temperature during the study period showed the maximum correlation with flower bud differentiation. Singh (1953) reported that a warm season was conducive for flower bud differentiation in mango. Mishra and Yamadagni (1958) found that a warm season favoured blossom bud diffe-

rentiation in grapefruit and as the temperature rose, the number of buds differentiated proportionately increased. In jamun also, a warm season was found to be conducive for the initiation of floral buds (Mishra and Bajpai 1973).

Studies conducted in pepper showed a more or less similar trend with regard to flower bud differentiation. Nalini (1983) observed a rise in mean temperature at the peak period of flower bud differentiation in black pepper. Subsequently, Rajan (1985) reported that the maximum and minimum temperatures in the preceding summer, and the subsequent showers played major roles in triggering flower bud differentiation activity. Vasanthamular (1986) found that a period of high temperature promoted the flowering mechanism in cardamom. So in clove also, a similar possibility could be expected since the temperature that prevailed during six to ten fortnights prior to peak differentiation was relatively high.

5.1.1.2 Minimum temperature

Minimum temperature recorded during the study period ranged from 22.29°C during the second fortnight of May to 24.44°C during the first fortnight of September. A negative correlation was found for minimum temperature (Table 4) during

the second fortnight (July second half 1987) prior to maximum differentiation (22.60°C) and also during the 13th fortnight (February first half 1987) prior to peak differentiation (32.12°C) and the 14th fortnight (January second half 1987) prior to maximum differentiation (21.95°C). Alvim (1981) observed that low temperature had a depressing effect on flowering in cocoa.

5.1.2 Rainfall

From the data, a step up of flower bud differentiation could be seen with an increase in the rainfall. Flower bud differentiation reached the maximum level (90%) when the rainfall received was maximum (237.4 mm). Following the dry spell from January 1987 to April 1987, pre-monsoon showers were obtained during which period flower buds were initiated. The absence of rain during the above period might have brought about a physiological ripening of the shoots, which is a pre-requisite for initiation of flower buds.

The commencement of flower bud differentiation immediately after the summer showers and the maximum differentiation of flower buds recorded during the period of maximum rainfall indicate that rainfall could be a predominant factor in influ-

encing flower bud differentiation in clove. This is further confirmed by the correlation analysis wherein a significant negative correlation was obtained for rainfall received during 10 to 13 fortnights preceding flower bud differentiation. From Table 3, it could be seen that during 10 to 13 fortnights (the second fortnight of March to the first fortnight of February 1987) prior to the fortnight of maximum flower bud differentiation, rainfall was rather scanty or nil.

Based on the studies on coffee in the coastal humid regions of Peru, Alvim (1960) opined that flowering primarily depended on rainfall, followed by a period of stress.

Veeraraghavan and Vasavan (1979) stated that a well-distributed rainfall during October and November months was required for optimum flowering and fruit set in cashew. In pepper, Nalini (1983) observed flower bud initiation to be triggered by the receipt of pre-monsoon showers after a long spell of dry weather. Subsequently, Rajan (1995) confirmed the beneficial effects of rainfall on flower bud differentiation process in pepper.

### 5.1.3 Relative humidity

Relative humidity recorded during the period of maximum

flower bud differentiation (August, 1997) was comparitively high (94.63%) indicating a favourable effect of humidity on flower bud differentiation. Relative humidity during 6th fortnight (May second half 1997) prior to peak differentiation (74.31%) and 16th fortnight (December second half 1996) prior to peak differentiation (69.21%) showed significant negative correlation with percentage of flower bud differentiation. This negative correlation could be attributed to the lower humidity during the above periods. It further establishes the positive relationship between humidity and flower bud differentiation in clove. Vasanthakumar (1996) found that a high status of relative humidity improved the capsule setting in cardamom.

5.1.4 Sunshine hours

During the period of observation, the mean duration of sun shine ranged from 3.73 hours per day during the second fortnight of August to 10.69 hours per day during the first fortnight of May (Table 1). There was a decline in the duration of sunshine received during June with the onset of South-West monsoon. Sunshine hours coinciding with flower bud differentiation did not show any significant correlation. The correlation between sunshine hours during 9 to 12 fort-

nights prior to differentiation and the percentage of flowerbuds differentiated was positive. Fruitfulness can also be attributed to the hours of sunshine received and the light intensity that prevailed during 9th to 12th fortnights prior to flower bud differentiation.

A similar observation was made by Rajan (1985) in pepper. Sunshine hours during four to six fortnights prior to differentiation was positively correlated with flower bud differentiation in black pepper. Balasubramoniyan (1971) found that fruitfulness in grapes was affected positively by sunshine hours during the period preceding flower bud differentiation.

### 5.1.5 Nutritional factors

Data on the levels carbohydrate and nitrogen in the leaves and the C/N ratio (Table 1) revealed that accumulation of carbohydrates and nitrogen were erratic. Carbohydrate content showed an increase from the first fortnight of May to the second fortnight of June with the exception of the second fortnight of May. Nitrogen content of the leaves did not show any definite pattern with regard to flower bud differentiation. It showed alternate peaks and falls (Table 1).

The C/N ratio also showed a similar trend. But there was an increase in C/N ratio during the fortnight preceding maximum differentiation. Then it showed a decline. The C/N ratio did not show any correlation with flower bud differentiation. Peak accumulation of carbohydrates has been reported in mango (Naik and Shah, 1937; Singh, 1960; Sen et al, 1963), grapes (Chadha and Cheema, 1971) and coconut (Bai and Ramadasan, 1982) prior to or at the time of flower bud differentiation. These reports further indicated that C/N ratio will be lowered after the flower bud differentiation process.

Nalini (1983) observed that C/N ratio exhibited two peaks, the first synchronising with the commencement of vegetative growth and the second with the step up in flower bud differentiation activity. Rajan (1985) observed an accumulation of carbohydrates and a build up of C/N ratio prior to peak differentiation in pepper.

Winkler et al (1937) and Chitkara et al (1972) in 'Anab-e-Shahi' and Khajuria et al (1979) in Gulabi varieties of grapes could not find any significant correlation between C/N ratio and flowering.

Vasanthakumar (1996) observed high status of carbohydrate at the panicle initiation and flower bud development stages of cardamom.

## 5.2 Histological aspects

In clove, the inflorescence is a terminal panicle. The number of flowers produced in each panicle vary from three to fifty.

### 5.2.1 The vegetative meristem

The vegetative apex was found to be more or less dome-shaped and surrounded by leaf primordia that in turn initiated from it (Plate 1). In many crop plants the vegetative apex is characterised by a conical shape as observed in mango (Singh, 1960), grapes (Chadha and Cheema, 1971), strawberry (Pathak and Singh, 1977) and in jasmine (Subramonian and Shanmugavelu, 1990).

During leaf initiation, the size and shape of the apex vary considerably from dome-shaped to nearly conical (Plates 1 and 2). It can be presumed that leaf initiation would continue from the apex, as long as it remained in the vegetative stage. Nalini (1983) observed in black pepper

that in the initial stage, the vegetative primordium was conical undifferentiated and surrounded by leaf sheaths. Rajan (1985) also found the vegetative apex to be conical in black pepper, surrounded by leaf primordia.

Transition stage

As growth advances, the apex is seen slightly more raised above the primordial site (Plate 2). Following this stage, the shape of the apex changes and become somewhat elliptical (Plate 3). Broadening and flattening of the apex is also noticed at this stage (Plate 4). Broader expanse of the meristematic tissue and flattening of the apex have been reported as the histological features of the apical meristem, on transition from vegetative to floral phase (Janick, 1972).

Development of secretory spaces were observed during these stages in clove (Plates 2 & 3). The number of secretory spaces were more in the bracts towards the periphery and fewer towards the lower region. The secretory spaces are schizogenous in origin and roundish (Plate 5). Esau, as early as in 1953, reported that the glandular canals of some members of Myrtaceae are schizogenous in origin with round shape.

In clove, the inflorescence is a terminal corymbose trichotomous panicle. Three flowers on a simple three-forked peduncle is the simplest unit (Plate 6). Among these three flowers, the central bud initial arises earlier and thus is seen much raised above the two lateral buds (Plate 7). In the magnified view of the lateral bud, it can be seen that the outer layer of cells, constituting the mantle are small and densely stained while the cells at the central zone constituting the core are larger in size and lightly stained (Plate 8). This phenomenon indicates that the mantle region was in an actively dividing stage. Popham and Chan (1950) introduced the term mantle for all the outerlayers of the apex which can be distinguished histologically from the inner cell mass, the core, without taking into accout the plane of divisions in these layers. Rajan (1985) made similar observation in black pepper where a few deeply stained outer layers of cells and comparatively less stained inner mass of cells could be distinguished.

Transition is clearly demarcated in the next stage (Plate 9) wherein the broad flat apex gives rise to two lateral protuberances on either side of it (Plate 9). Identical features of apical meristems with initiation of lateral

protuberances are well established phenomena associated with blossom bud differentiation in citrus (Abbot, 1935; Randhawa and Disna, 1947; Singh and Dhuria, 1960; and Mishra and Yamadagni, 1968). Anatomical investigations on jamun by Mishra and Bajpai (1973) revealed flattening and broadening of the crown during fruit bud differentiation stage. This was followed by a rapid elevation on both sides of the growing point. In litchi, Shukla and Bajpai (1974) observed that the growing point flattens and broadens with a rapid elevation on both the sides.

Transition from vegetative to floral phase is immediately followed by initiation of the non-essential whorls of flower (Plate 10). In the outer whorl, sepal primordia are seen and just below this, the initials of petals. Stamens developed during the next stage (Plates 11 and 12). Development of stamens was followed by the development of ovary in the succeeding stage (Plate 15). The ovary could be observed below the androecium. From the sequence of floral ontogeny observed, it may be presumed that differentiation of floral organs took place in the order of sepals, petals, androecium and gynoecium.

# SUMMARY

# 6. SUMMARY

Investigations on flower bud differentiation in clove were carried out at the College of Agriculture, Vellayani from August, 1986 to December, 1987. Studies were conducted to find out the role of climatological factors on flower bud differentiation and the histological basis of flowering in clove.

The weather parameters and the nutritional factors recorded during the period of study were correlated with flower bud differentiation. In addition, correlation was worked out between the weather parameters recorded during sixteen fortnights preceding the period of flower bud differentiation observed during a particular fortnight of the study period.

6.1 During the lag zero, the fortnight of study, neither the climatic factors nor the nutritional factors showed significant correlation with flower bud differentiation.

6.2 Maximum temperature during the sixth fortnight prior to differentiation to the tenth fortnight prior to differentiation showed significant positive correlation with flower bud differentiation.

6.3 Significant negative correlation was obtained for minimum temperature during the second, thirteenth and fourteenth fortnights prior to differentiation.

6.4 Rainfall showed significant negative correlation with the percentage of flower buds differentiated during the tenth, eleventh, twelfth and thirteenth fortnights prior to differentiation.

6.5 Percentage of flower bud differentiation was negatively correlated with the relative humidity during the sixth and sixteenth fortnights prior to differentiation.

6.6 Sunshine hours during the ninth fortnight prior to differentiation showed significant positive correlation with the percentage of flower bud differentiation. In the preceding three fortnights also (lag 10, 11 and 12), significant positive correlation was obtained between the sunshine hours and flower bud differentiation.

6.7 Neither carbohydrate content nor nitrogen content showed any significant correlation with flower bud differentiation. C/N ratio also did not reveal any significant correlation with flower bud differentiation.

6.8 Flower bud differentiation commenced during the month of May, increased during the months of June and July and attained a peak during the second fortnight of August. Thereafter, the flower bud differentiation steadily declined.

6.9 The vegetative shoot apex appears dome-shaped, surrounded by leaf primordia. The transformation from vegetative to reproductive stage in clove is marked by the appearence of two lateral protuberances on either side of the apical dome. In clove, the central bud initial is seen much raised above the two lateral initials, indicating that the central bud develops earlier than the lateral ones. Immediately after transformation, the initials of sepals and petals begin to appear. The androecium develops earlier than the gynoecium. The flower bud of clove which is green in the young stage, develops a pinkish tinge just before and after anthesis.

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*Originals not seen

# FLOWER BUD DIFFERENTIATION IN CLOVE, *Eugenia caryophyllus* (Sprengel) Bullock & Harrison

By

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ABSTRACT OF A THESIS
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## ABSTRACT

Investigations on flower bud differentiation in clove were carried out at the College of Agriculture, Vellayani from August, 1986 to December, 1987. Bearing clove trees (seven years old) at the Instructional Farm attached to the College were utilised for the study.

The plant specimens were stored in FAA (Formalin-aceto-alcohol) and then dehydrated through tertiary butyl alcohol - iso propyl alcohol series. The specimens were infiltrated and embedded in paraffin wax (m.p. 58-60°C) and sectioned in a rotary microtome. The sections were then de-waxed, stained and examined for the anatomical features and photomicrographed.

The weather parameters and the nutritional factors recorded during the period of study and those during sixteen fortnights prior to differentiation, were correlated with the data on flower bud differentiation.

Significant positive correlation was obtained between the maximum temperature during the sixth to tenth fortnights before differentiation and the percentage of flower buds differentiated. Sunshine hours during ninth to twelfth fortnight prior to differentiation showed a positive correlation with the percentage of flower bud differentiation.

Significant negative correlation was obtained for the minimum temperature during the second, thirteenth and fourteenth fortnights prior to flower bud differentiation and also for the relative humidity during the sixth and sixteenth fortnights prior to differentiation.

A negative correlation was obtained for the rainfall received during the tenth, eleventh, twelfth and thirteenth fortnights before differentiation and the percentage of differentiation.

Neither the carbohydrate content nor the nitrogen content showed any significant correlation with flower bud differentiation. The C/N ratio also did not reveal any significant correlation with flower bud differentiation.

Flower bud differentiation was maximum during the month of August.

The vegetative shoot apex which appeared dome-shaped initially was characterised by the appearence of two lateral protuberances during its transition to the floral phase. The floral organs appeared in the order of sepals, petals, androecium and gynoecium.