FLOWER BUD DIFFERENTIATION IN

Piper sp.

By
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THESIS

**Submitted in partial fulfilment of
the requirement for the degree of**

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COLLEGE OF HORTICULTURE
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1985.

DECLARATION

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

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We, the undersigned, members of the advisory committee of Shri. Rajan.P.S., a candidate for the degree of Master of Science in Horticulture with major in Horticulture, agree that the thesis entitled "Flower bud differentiation in Piper sp." may be submitted by Shri. Rajan. P.S. in partial fulfilment of the requirement for the degree.

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INTRODUCTION

In plants like pepper, in which the reproductive structure is the economically important part, every attempt should be made to enhance the flower bud differentiation activity.

Theoretically all the buds in a plant are capable of differentiating into flowers, if the conditions within as well as outside the plant are congenial at the time of differentiation. Hence, information on the role of various environmental, nutritional and hormonal factors in flower bud differentiation will provide valuable tips for successful crop production in flowering crop plants. These basic information form the scientific basis for determining the cultural, manurial and irrigational requirements and for scheduling them in such a way that the factors within as well as outside the plant are 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, etc.

The main yield components, in pepper as in any other flowering crop plant, are determined during flower

bud differentiation and development. Number of spikes per plant, average length of the spikes, number of berries per spike and the average weight of the berries are the important yield determinants in pepper. Some of these are known to be sequentially linked. A deficiency affecting a component early in the course of development may be partially compensated, if the conditions later improve. The scope for compensation decreases with ontogeny, the last component in the sequential chain offering the least opportunity to make good the effect of early deficiencies. Information on the ontogeny of flower development, the time lag between flower bud differentiation and visual emergence, the site and time of flower bud differentiation etc. will aid in scheduling the cultural operations, manuring, pruning, irrigation and hormonal applications, 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.

In pepper, flower bud differentiation studies have been initiated at the College of Horticulture Vellanikkara, Trichur and already some basic data have been collected (Nalini, 1983). The present study,

the second in the series, consisted of two parts, namely examination of the role of different factors on flower bud differentiation and investigation on the histological aspects of the process. Varietal differences in these respects between Panniyur-1 and Karimunda were also subjected to critical study.

2. REVIEW OF LITERATURE

The transition from vegetative to reproductive phase in plants is a functional activity which attracted several investigators. The factors influencing flower bud differentiation within as well as outside the plant, the site and time of flower bud differentiation and the duration from initiation to visual emergence are of particular importance with respect to crop production. The literature available on the above aspects has been reviewed here.

2.1. Factors influencing flower bud differentiation/ flowering.

2.1.1. Climatic factors

Analysis of weather data to examine their possible impact on flowering of plants was done by Pickering (1916) who observed that the weather of England tended to form a biennial cycle and he considered this, as the main reason for biennial bearing in apple. Macaun (1917) made observations on similar lines in Canada. According to Gibbs and Swarbrick (1930), the variation in time of flower bud differentiation depended upon climatic condition The role of the different weather parameters on flowering of important crops has been investigated by several workers.

2.1.1.1. Temperature

Apple flower buds formed more readily during warm dry weather than during cool weather (Gribanovskji, 1960). Suzuki and Tanno (1971) reported that the average minimum temperature in early March and the average maximum temperature in mid-April were closely correlated with the start of bud break. Mousdale (1983) stated that innate bud dormancy declined during winter bud burst and this could be artificially induced by transferring the plants to a growth chamber at 25°C. This low temperature hastened the decline of abscissic acid (ABA) in buds.

In apricot, Brown (1960) got a curvilinear relationship between temperature and development of flower buds. In coffee, a temperature drop following rain, plays a decisive role in breaking flower bud dormancy (Went, 1957; Rees, 1964). This has been reported earlier in another gregarious flowering plant Dendrobium sp. (Coster, 1926).

High mean temperature was found to induce differentiation of flower buds in different species of Citrus (Abbot, 1935; Randhawa and Disna, 1947; Singh and Dhuria, 1960; Bajpai and Mourya, 1963). On the other

hand Moss (1969) reported that more inflorescences were produced at lower temperature in sweet lime. Lenz (1969) found that flowering did not occur at high day/night temperature of 30°C/25°C, eventhough vegetative growth was evident. Hall et al. (1970) obtained more flower bud initiation at cooler air temperature of 20°C/15°C (day/night) than at temperature of 30°C/10°C.

In grapes, Perold (1927) found that warm and dry conditions in preceding season favoured flower bud initiation. Kolesnik (1953) recorded poor differentiation of buds under low temperature. Nikov (1964) opined that grapes do not require low temperature for the triggering of flower bud initiation. Baldwin (1964) and Dimitrieva (1969) obtained positive correlation between flower bud initiation and mean temperature.

Singh (1958) reported warm season to be conducive for flower bud differentiation in mango. Ravisankar et al. (1979) observed that a drop in night temperature and humidity increased flower bud initiation.

As the temperature rose, vegetative growth started and consequently the number of differentiated buds increased in karaunda (Mishra et al., 1968). In jamun, warm season

was found to be conducive for initiation of floral buds (Mishra and Bajpai, 1973).

In low bush blueberry Hall et al. (1970) found that the number of primordial meristems and the degree of development of floral primordia were enhanced by warmer conditions.

Studies on flower bud differentiation in pepper were carried out for the first time at the College of Horticulture, Vellanikkara in which Nalini (1983) found that mean temperature was high at peak periods of floral bud differentiation.

2.2.1.2. Rainfall, irrigation, humidity and water relations.

In apple, Wiggam (1918) observed that blossom showers were needed for obtaining good crop. According to Lees (1926) a wet summer was followed by less fruit bud formation and poor yield. Collison andHarlan (1927) and Degman et al. (1933) pointed out the influence of rainfall on flower bud formation in apple.

According to Aldrich and Work (1934) and Magness (1934), prolonged drought induced more flower buds in peach.

In grapes, Perold (1927) observed that warm and dry conditions in the preceding season favoured flower bud initiation. Balasubrahmanyam (1971) found a temporary

water stress prior to flower bud differentiation to be beneficial.

In case of mango, it has been observed that heavy rains during the period of flower bud initiation stimulated vegetative growth at the expense of fruit production (Chacko and Randhawa, 1971). Ravisankar et al. (1979) observed that drops in night temperature and humidity enhanced flower bud initiation.

In coffee, a gregarious flowering plant detailed studies have been made. Based on his studies in the coastal humid region of Peru, where internal water potential of the plant is not controlled by transpiration but by the available soil moisture, Alvim (1960) 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. Franco (1962) observed that coffee plants in nutrient solution blossomed at the same time as these in the surrounding fields. Rees (1964) could observe flowering after the first showers in coffee plants kept watered in dry season. Van der Veen

(1968) and Browning (1971) reported that conditions favouring constant high water potential within the plant such as rainfall, irrigation, low temperature etc. induced dormancy due to high concentration of abscissic acid as a result of enhanced translocation rates. Alvim et al. (1972) observed water potential of coffee plants to be controlled by humidity; but rainfall increased the relative humidity and influenced the water potential, thus affecting flowering indirectly. Alvim (1973) opined that moisture stress due to low water potential caused by high transpiration or low soil moisture reduced abscissic acid concentration due to reduced translocation from leaves to flower buds.

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. Rainfall was found to be the critical factor influencing flower bud differentiation in pepper.

2.1.1.3 Light

Shade greatly reduced the flower bud differentiation in apple (Kraybill, 1923; Autcher et al. 1926; Jackson and Palmer, 1977), in apricot (Jackson, 1969) and peach (Kraybill, 1923).

In grapes, it was found that artificial shading depressed the fruitfulness of the central buds and yields

were reduced due to retarded development of flower primordia (May and Anticliff, 1964 and Dikan 1976), Balasubrahmanyam (1971) opined that photosynthetic and photomorphogenic processes were dependent on light perceived by leaves. Variation in fruitfulness in grapes was thus attributable to the sunshine and intensity of light.

2.1.2 Nutritional factors

2.1.2.1 Carbohydrates, nitrogen and carbon/nitrogen ratio.

Kraus and Kraybill thepioneer workers on this aspect of flowering stressed the importance of carbon/nitrogen balance as early as in 1918.

In apple spurs possessing fruit buds had a greater supply of starch than unproductive spurs (Gourley, 1915). Chandler (1925) proposed that a poor crop may result from (i) a general deficiency of carbohydrates or deficiency of a particular carbohydrate, (ii) deficiency of a carbohydrate-nitrogen compound or (iii) an unfavourable C/N relationship. Hooker (1930) also found carbohydrate to be a determining factor in flowering. Archbold (1928) reported that the greatest demand for nitrogen was during blossoming and fruit setting period and of carbohydrate from there on until fruits were practically full grown. However, Sabulka (1962) could not get any correlation between flower bud initiation and nitrogen content.

Regarding C/N ratio, Kar and Randhawa (1968) observed that it was high in September flush, intermediate in June-July flush and low in March flush. The C/N ratio increased with the age of shoot. Carbohydrate level in non bearing shoots were found to be greater than that in bearing shoots.

In grapes, the axillary buds which developed after pruning differentiated into flower buds within 40 to 90 days and this was attributed to the existence of optimum C/N ratio (Thomas and Bernad, 1937; Shantha, 1965; Rao and Sathyanarayana, 1978). Chadha and Cheema (1971) observed that in 'Perlette' starch accumulation favoured flower bud initiation. However, Winkler et al. (1962), Khajuria et al. (1970) and Chitkara et al. (1972) could not obtain any correlation between flowering and C/N ratio.

A nitrogen supply that induced normal growth andgood foliage colour fostered fruitfulness in grapes. A temporary reduction in available nitrogen, also increased fruitfulness without affecting bunch size (Winkler, 1945). Baldwin (1966) also recorded similar findings from Australia. Bindra and Chohan (1974) reported that higher levels of nitrogen enhanced 'bud killing' and decreased differentiation causing barrenness in grapes.

Fayek et al. (1983) found that in Dwarf Cavendish banana, shortly before and at flower bud differentiation, carbohydrate content declined while nitrogen content showed a reverse trend.

Carbohydrates appeared to influence fruiting in 'Wilking' mandarin leaves (Lewis et al. (1952) and 'Valencia' orange (Jones et al. 1964). Smith et al. (1952) in 'Valencia' orange and Dugger et al. (1969) in lemon reported marked seasonal variation in the utilizable carbohydrate materials with peak accumulation prior to flowering. Jones et al.(1970) observed that carbohydrate accumulation in 'Valencia' orange leaves sampled in February was inversely related to fruit load on the tree at sampling; but directly related to the amount of fruit produced from flowering which followed the time of leaf sampling.

In 'Shoumati' orange nitrogen content of older leaves was depleted by blossoming andhence a negative correlation was observed (Ayalon and Monselise, 1960). Harding et al. (1962) in oranges and Aiyappa et al. (1965) in mandarin observed that the leaf nitrogen content was high in the non-fruiting branches than in the fruiting branches. In sweet orange, Millela (1968) observed high level of nitrogen before flower bud initiation, which declined later.

High starch reserves, total carbohydrates and C/N ratio in shoots favoured flower bud differentiation in mango (Naik and Shaw, 1937; Mallik, 1953; Singh 1960; Sen et al., 1963). Sai (1946) proposed that biennial bearing is conditioned by nutrient deficiency especially, that of nitrogen. Chacko (1968) found, high level of nitrogen prior to fruit bud differentiation, which was depleted during flowering. A similar trend was observed in the case of carbohydrates also.

Bai and Ramadasan (1982) found that the number of female flowers produced were maximum during March-April in coconut. The starch content in stem and leaves also was maximum during this period.

In pepper, total soluble carbohydrates, nitrogen content and C/N ratio of the two types of laterals and of the new shoots varied considerably during the growth cycle. Carbon-nitrogen ratio exhibited two peaks, the first synchronising with the commencement of the differentiation process and the second, with the step up of flower bud differentiation activity (Nalini, 1983).

2.1.2.2. Phosphorus and potassium.

Other two primary nutrients, phosphorus and potassium have also been studied in a number of crops vis-a-vis flower

bud differentiation. Elhinnawy (1956) opined that phosphorus and calcium play an important role in perceiving flowering response in plants.

Harding et al. (1962) in oranges and Aiyappa et al. (1965) in mandarins observed that at full bloom stage, leaf nutrient status of non-fruiting terminals in respect of phosphorus and potassium were high than those of fruiting terminals.

In grapes, Potter and Philip (1930) found that high level of phosphorus favoured flower bud initiation. Increase in fruitfulness due to the application of phosphatic fertilizers to grapes have been reported by Kolesnik (1953) and Arutjuna (1964). Havelka (1964) recorded an increase in fruitfulness due to the application of phosphatic fertilizers. Srinivasan and Muthukrishnan (1970) reported that early application of potassium (20 days after pruning) advanced the bud development and correspondingly increased the fertility of the basal buds.

Singh (1959) Kazarjan et al. (1965) and Thimmaraju (1966) reported that high level of phosphorus favoured flower bud initiation in mango. There was maximum accumulation of phosphorus and potassium before flower bud differentiation,

which declined as the tree passed through the different stages of fruit development (Avilan, 1971; Pathak and Panday, 1978). Singh and Singh (1973) found that high level of phosphorus favoured flower bud differentiation in mango. The low amount of the nutrient in 'on' trees reduced vegetative growth after harvest and also subsequent flower bud formation.

In strawberry, phosphorus application at fruit bud initiation stimulated the differentiation of buds (Hodzaeva, 1962).

In guava, Rodriguez (1967) reported that levels of nitrogen, phosphorus and potassium were high at full bloom stage in non-fruiting terminals than those in fruiting terminals.

2.1.3. Varietal influence

Gibbs and Swarbrick (1930) stated that the time of flower bud differentiation showed variation with variety.

In grapes, varietal difference has been observed as an important factor causing variation in fruit bud differentiation especially with respect to time (Bernad, 1932; Bernad and Thomas, 1933; Winkler and Shemsettin, 1937). In a study made in Italy, Khalil (1961) found that flower bud differentiation occurred by early June in cv. Barbera. Bindra (1981) studied the time of flower bud differentiation in different varieties of grapes and found that the peak differentiation was by 3rd April in 'Perlette', by 11th April in 'Beauty Seedless', by 18th April in 'Banquabad' and 9th May in

Anab-e-shahi.

Fujitha and Yagi (1956) reported that in Japan, blossom bud differentiation occurred by the middle of December in Washington Navel, by late January in Valencia and Fukuhara and by early February in New Summer. They found that blossom bud differentiation continued for about four months in Washington Navel. In North India, the time of flower bud differentiation has been reported to be the beginning of January in Blood Red and the end of January in Jaffa (Babu and Kaul, 1972).

In mango, varietal influence has been shown as an important factor causing variation in time of fruit bud differentiation (Singh, 1958).

Pathak and Singh (1977) observed that in 'Pusa Early Dwarf' variety of strawberry, the bud development was rapid after initiation and hence it flowered early as compared to Katrain Sweet variety.

2.1.4. Hormonal factors

Elaborate studies have been made in a number of crops to find out the relationship between action of growth substances and flower bud differentiation.

2.1.4.1. Auxin

Harada (1962) and Hilman (1962) reviewed the physiological action of auxins in flowering and concluded that the main effect might be either on pre-inductive vegetative growth or on

post-inductive floral development. Paulet and Nitsch (1964) and Nitsch and Nitsch (1967) opined that auxins had an inhibitory effect on formation of flower buds, both in short day and long day plants. Wardell and Skoog (1969) explained that low concentration of auxins promoted flowering, while higher ones were inhibitory.

In peach, Blommaert (1955) correlated the termination of rest in flower buds and vegetative buds with the disappearance of an ether extractable inhibitor and an increase in auxin type activity.

In an year round study, Ramsay and Martin (1970) could not detect any consistent auxin activity in apricot buds.

Alvim (1958) could not induce flowering in coffee by spraying hormones coming under auxin category.

Based on his investigations on alternate bearing in mango, Chacko et al (1972) reported that at Rf 0.3 - 0.6 the concentration of indole acetic acid equivalents was high in 'on' year than in 'off' year. The shoots of Dushehari 'on' (an irregular bearing variety) and Totapuri Red Small trees (a regular bearing variety) which were expected to flower during 1968 contained a higher level of growth promoting substances during the period of flower bud initiation than the shoots of Dushehari 'off' trees which remained vegetative. During October-December (the period of flower bud differentiation in North India), the level of growth promoters increased in shoots of 'Dushehari 'on' trees. In 'off' trees of

Dushehari the increase was negligible. Singh and Singh (1974) found that regular bearing varieties in general contained higher amounts of growth promoting substances than biennial bearing varieties.

2.1.4.2. Inhibitors

Evidences point to the role of inhibitors in the regulation of flower bud differentiation. Inhibitors might influence an essential phase of floral differentiation or development (Zeewast and Lang, 1963). Abscissic acid (ABA) is believed to be a growth inhibitor associated with mechanism of bud dormancy in plants (Eagles and Wareing, 1964; Millborrow, 1966). Lipe and Crane (1966) and Martin et al. (1969) found that levels of ABA correlated with the rest period of buds and seeds.

Alvim (1960) observed that water stress reduced a growth inhibitor responsible for bud dormancy in coffee. Van der Veen (1968) treated coffee flower buds with 200 ppm ABA in lanolin paste and found them dormant for several months. Watering subsequently was ineffective. Conditions favouring constant increase in water potential (frequent irrigation, low transpiration rates, etc.) kept flower buds dormant due to high concentration of ABA as translocation of it was easy from the leaves to the buds at higher water potential (Van der Veen, 1968; Browning, 1971). Alvim (1973) also

opined that moisture stress by low soil moisture status or by high transpiration rates, reduced ABA concentration in flower buds, possibly because of reduced translocation from the leaves to the flower buds, which resulted in the termination of true dormancy.

Browning (1971) estimated the content of ABA in coffee flower buds collected before and after bud break caused by rain. The yield of ABA, as estimated by bioassay, was 0.10 - 0.16 µg/g (dry weight) for the dormant flower buds and 0.04 - 0.09 µg/g for the buds collected two days after rain.

In his investigations on causes of alternate bearing in mango Chacko et al (1972) observed that the level of inhibitor was low during August (prior to flower bud differentiation) which increased subsequently in October-November and recorded a maximum in November-December (at the time of flower bud differentiation). The 'on' trees recorded more inhibitors compared to the 'off' trees which indicated the possibility of (vegetative) growth promoting activity of growth substances like gibberellic acid and auxin, counteracted by inhibitors. Singh and Singh (1974) stated that regular bearing mango varieties, in general, contained higher amounts of inhibitors than biennial bearing varieties.

The termination of rest in bud was correlated with decrease in the level of inhibitor activity in peach (Blommaert, 1955) and apricot (Ramsay and Martin, 1970).

Iwasaki and Weaver (1977) and Emerson and Powel (1978) found that ABA decreased during chilling by which bud growth was promoted in grapes.

Mousdale (1983) found that innate bud dormancy declined during winter bud burst in apple and this can be artificially induced by transferring the plants to a growth chamber at 25°C. According to him this temperature hastened the decline of ABA in buds.

2.2. Extraction, purification and estimation of growth substances.

Apart from the sophisticated instrumental methods, sensitive bioassays have been standardised for estimation of growth substances in plants. Minor modifications may become necessary with respect to the crop, the plant part, the environmental conditions and infrastructural facilities available.

2.2.1. Extraction

Organic solvents like methanol, ethyl alcohol, acetone, chloroform etc. are generally used for initial extraction in which plant tissue is freezed or chilled and mascerated repeatedly. Of all the solvents, methanol is the widely used one. Ramsay and Martin (1970) used it for the extraction of growth substances from apricot leaves, Ravinankar (1983) from ginger leaves and Chellappan (1983) from banana leaves.

2.2.2. Purification and separation

Fractionation method (based on differential solubility) was employed by Cogan and Payton (1970) and Sharma and Singh (1980) for extraction and purification of auxins from peach leaf extract. Chacko et al (1972), Rehman et al (1975), Chellappan (1983) and Ravisankar (1983) used the same method for extraction and purification of auxins from extracts of mango leaves, tomato, banana and ginger respectively.

Nitsch (1956) followed the acetonitrile method for purification of growth substances in which the plant extract was shaken with acetonitrile and hexane, and the acetonitrile layer was discarded. Hexane layer was collected and dried.

For further purification and separation, paper chromatographic methods are widely adopted. Nitsch (1956) proposed paper chromatography for purification of auxins. The plant residue was dissolved in distilled water and the resulting solution spotted in Whatman No.1 chromatographic paper and run in a chromatographic chamber in ascending or descending method using isopropanol, ammonia, water (10 : 1 : 1 v/v) solution as the running medium. Singh and Gurang (1982) employed descending paper chromatography using Whatman No.1 chromatographic paper. The running solution was isopropanol : ammonia : water (10 : 1 : 1 v/v) for auxins and isopropanol : n-butanol : ammonia : water (6 : 2 : 1 : 2 v/v) for inhibitors.

In chromatography, the different groups of growth substances are separated at different Rf positions depending on the running solution, the chromatogram paper, running time, method employed (ascending/descending) etc.

Gur and Samish (1966) reported growth accelerators in mango at Rf 0.57 in bark extract of seedlings. Ramsay and Martin (1970) could not obtain auxin type activity consistently in any fraction of chromatogram of fresh peach leaf extract. In mango shoot extract, Chacko et al (1972) found growth promoting activity at two zones (Rf 0.4 - 0.5 and 0.8 - 1.0). The factor present at Rf 0.8 - 1.0 was not consistent. Therefore, the factor at Rf 0.3 - 0.6 only was reckoned as indole acetic acid (IAA) equivalent. Mainland and Eck (1974) reported active growth substances between Rf 0.3 - 0.6 in the extracts of flowers and one week old fruits of blueberry. Singh and Singh (1974) observed auxin like activity at two Rf positions (0.4 - 0.5)and(0.8 - 1.0) in mango shoot extract. The activity of auxin like substances was confined to Rf 0.2 - 0.4 in banana leaves (Chellappan, 1983) and ginger leaves (Ravisankar, 1983).

Millborrow (1966) as well as Iwahaki and Weaver (1977) repoted that ABA was associated with Rf 0.6 - 0.8 in grape. In peach, Ramsay and Martin (1970) reported peak inhibitory activity at Rf 0.6 - 0.7. Gur and Samish (1966) reported an inhibitor complex in mango at Rf 0.70 - 0.93 in bark extract of mango seedlings. Chacko et al (1972) obtained inhibitor activity in mango shoot extracts at Rf 0.6 - 0.9

and Singh and Singh (1974) at Rf 0.7 - 0.8. The activity of inhibitors was confined to Rf 0.5 - 0.7 in banana leaves (Chellappan, 1983) and ginger leaves (Ravisankar, 1983).

2.2.3. Estimation

Several biological agents are employed for detection and estimation of growth substances. Mer et al. (1962) employed wheat coleoptile bioassay for detection of auxins. "Wheat coleoptile section straight growth bioassay" has been reported as a simple and sensitive method for estimation of auxins (Mitchel and Livingston, 1968; Rehman et al., 1975; Chellappan, 1983; Ravisankar, 1983). "Rice second leaf sheath bioassay" was perfected by Ogawa (1963) for both auxins and inhibitors.

For inhibitors also a number of biological tests are available. Chacko et al., (1972) and Singh and Singh (1974) employed "cress seed germination inhibition bioassay" for abscissic acid (ABA). Eidelnant et al. (1980) perfected "mustard seed germination inhibition bioassay" for ABA. Rehman et al. (1975) employed this method in tomato, Chellappan (1983) in banana and Ravisankar (1983) in ginger.

2.3. Time of flower bud differentiation

Wide variations have been reported on the time of flower bud differentiation in grapes, depending on the variety,

location and environmental factors (Bernard, 1932; Bernad and Thomas, 1933; Winkler and Shemsettin, 1937; Rajaram et al. 1964, Nayana et al. 1968; Chadha and Cheema, 1971; Rao and Sathyanarayana, 1978; Bindra, 1981).

Bernard (1932) established that the cluster primordium was terminal in origin; but appeared lateral to the apex during the subsequent developmental stages ofthe primordial shoots.

Flower bud differentiation in grapeshas been reported to take place during the period preceding the fruiting season (Bernard, 1932; Rajaram et al. 1964 and Chadha and Cheema, 1971). Constantine (1958) reported that flower bud initiation in grapes began soon after the appearance of the 17th or 20th leaf and this character was reckonedas a biological method for determining the time of fruit bud initiation.

In mango also, wide variations have been reported in thetime of flower bud differentiation from year to year, place to place andvariety to variety (Singh, 1958). In Florida, it was in October (Sturrock, 1934; Mustard and Lynch, 1946); but delayed to the first week of November in late season varieties (Sturrock, 1934). In India also,

reports indicated that the time of flower bud initiation vary from place to place - October to beginning of November in Bihar (Sen and Mallik, 1941); middle of August to end of October in Punjab (Musahib-ud-din, 1946); December in Saharanpur (Singh, 1960); first fortnight of August in Vengurla and September in Poona (Sawant, 1969).

In Citrus, differentiation occurred at the initiation of growth in the sping or upon the resumption of growth at any other season of the year subsequent to a period of environmental conditions favourable for the accumulation of food reserves (Abbot, 1935; Randhawa and Disna, 1947; Ahamed and Khan, 1951; Ayalon and Monselise, 1960; Randhawa and Chopra, 1963; Mishra and Yamdagni, 1968). In sweet orange, differentiation was observed by January 20th while in grape fruit it was by February 13th (Abbot, 1935).

In fig, initiation and differentiation of flower buds occurred throughout the growing season, which was from the beginning of April to the 15th of July. The first crop 'Breba' was produced from the buds that differentiated in the previous year and the second crop, either from the buds that differentiated in the previous year or from the buds differentiated during the season. (Rane and Singh, 1965)

In pepper, studies conducted at the Kerala Agricultural University in variety Panniyur-1 by 'Nalini (1983) have revealed that flower bud differentiation occurred only in the new shoots arising from the laterals. A spurt of flower bud differentiation activity was observed immediately after the receipt of pre-monsoon showers and maximum flower bud differentiation occurred during June-July. The process of flower bud differentiation was completed within about 20 days of its commencement.

2.4. Histology of flower bud differentiation

Reports indicate that the histological features of the developing flower buds are more or less similar in all plants at initial stages of development. But later, the shape, the pattern of development and the duration vary considerably in different species of plants depending on the type and nature of inflorescence produced.

2.4.1. Vegetative apex

The vegetative apex follows the tunica-corpus concept of apical meristem arrangement. It is generally conical and surrounded by pointed leaf primordia. The conical nature of vegetative primordium has been observed in crop plants such as grapes (Chadha and Cheema, 1971) mango (Singh 1960), jaman (Mishra and Bajpai, 1973); andstrawberry (Pathak and

Singh; 1977). Shukla and Bajpai (1974) found that the vegetative apex was dome shaped in litchi. Einert et al. (1970) made similar observations in Lilium longiflorum,. In cauliflower, the young leafy plant was observed to possess small pointed shoot apex surrounded by narrow leaf primordia which arose in spiral succession around the shoot apex. The apex continued to differentiate foliage as long as the plant was not cold treated; (a pre-requisite for "flowering".) (Sadik, 1966). In mango, Singh (1960) found that the vegetative apex was conical. He could not observe any distinct stages in the developmental process.

In pepper Nalini (1983) reported three distinct stages in the developmental process of the vegetative bud. At the beginning of initiation the vegetative primordium was conical, undifferentiated and surrounded by leaf sheaths which elongated in the further stages.

2.4.2. Transition stage

The occurrence of a transition stage in differentiating flower buds has been described by Janick (1972). On transition, growth of the central portion was reduced or inhibited and the meristem was flattened in constrast to the concical vegetative meristem. Another basic difference was that

there was no elongation of the axis between successive floral primordia as there was between leaf primordia. Esau (1962) stated that the small depth and comparatively broad expanse of the meristematic tissue were the common histological features of floral meristem.

Broadening and flattening of the apical meristem just before flower bud initiation have been observed in citrus (Abbot, 1935; Randhawa and Disna, 1947; Mishra and Yamdangi, 1968; Babu and Kaul, 1972), litchi (Shukla and Bajpai, 1974), Strawberry (Pathak and Singh 1977), karaunda (Mishra et al., 1968), coffee (Alvim, 1973) and in jaman (Mishra and Bajpai, 1973).

Indications are available in a number of crop plants on the occurrence of a transition stage during bud differentiation. In grapes formation of bract primordium was the first indication of the formation of cluster primordium (Winkler and Shemsettin, 1947). Chadha and Cheema (1971) reported that the leaf primordium was pointed whereas cluster primordium was broad.

In mango, high meristematic activity marked by the production of braod conical protuberances in the axils of scales, has been reported as the first sign ofblossom bud differentiation (Gunjate et al, 1977; Ravisankar et al. 1979).

In fig, bud primordium appeared to be roundish and convex (Rane and Singh, 1965).

Nalini (1983) described the appearance of two undifferen tiated conical primordia surrounded by leaf sheath as the first sign of flower bud initiation. These primordia could not be distinguished from the vegetative primordia.

2.4.3 Development of floral primordia

Further development of differentiated floral primordium follows variant patterns and is determined mainly by the type and nature of inflorescence produced.

In Perlette grapes Chadha and Cheema (1971) described the process of development of inflorescence primordia. After differentiation, the cluster primordium produced numerous growing points. The cluster primordium consisted of a complex branching system and the elongation of cluster branches occurred gradually with continued rapid division. With the advancement of season, the cluster primordium increased in size with numerous growing points.

In mango, four staged have been identified in the development of the fruit bud, (Sen, 1943; Gunjate et al., 1977; Ravisankar et al., 1979). After differentiation the buds became plump and conically protruded out of the scales. The main axis elongated and became multilobed due to the development of primary branches of flower panicle. Some of the side protuberances also became multilobed due to

the presence of primordia of secondary branches. In the third stage, the flower buds which became conical and plump and emerged out of the scales. During the fourth stage, the scales started loosening, indicating bud break. The floral organs developed in the succession - sepals, petals, stamens and carpels.

In coffee, there was a flattening of the apical growing point with its subsequent division into two flower bud primordia. They developed into two lateral dome shaped growing points which produced additional lateral flower buds resulting in an opposite decussate inflorescence (Alvim, 1973).

In fig, Rane and Singh (1965) identified five stages in the differentiation of flower buds. As the differentiation proceded the apex turned completely concave and was lined, with floral primordia.

Sharma and Singh (1980) observed four stages in the development of flower primordia in Pusa Early Dwarf strawberries. The conical undifferentiated primordium broadened and flattened on initiation. Subsequently, the primordium elongated and new growing points appeared at the base, just below those of the primary flowers. Sepals and petals developed in the primary flowers in the next stage. Later, rudimentary stamens, and pistils appeared in the primary flowers.

In pepper, five stages were identified in the development of flower buds (Nalini, 1983). During the first stage, two undifferentiated conical primordia surrounded by leaf sheath were observed indicating the commencement of flower bud differentiation process. Towards the latter half of the first stage, one of the primordia was found to be broadened and elongated. Appearance of a dome shaped structure at the apex of the broadened primordium in the second stage denoted spike initiation stage. The third stage indicated floral initiation and a structure resembling the pepper spike couldbe clearly observed. During the fourth stage differentiation of floral partswere observed. Stamen and pistil premordia could be seen towards the end of the fourth stage. Completion of the differentiation process was indicated by the appearance of the stamens and the ovary during the fifth stage.

2.5. Microtechnique

Microtechniques for histological examination of pepper shoot tissues were standardised by Nalini (1983) who concluded that formalin - aceto-alcohol (FAA) is the best killing and fixing agent and tertiary butyl alcohol (TBA) series, the best for dehydration. The embedding medium used was Hance's paraffin mixture without ceresin and the staining methods adopted were saffranin, single staining and saffranin-fast green double staining.

Johanson (1940) described FAA as the most widely used fixative in which plant specimens could be kept idenfinitely without appreciable damage. Among the dehydration methods, graded series of TBA: ethyl alcohol : water mixture was found to be satisfactory. Sass (1951) opined that isopropyl alcohol, which can be purchased without restrictions, could be used in exactly the same manner as scanty ethyl alcohol for dehydration purposes along with TBA.

To facilitate easy sectioning of the specimens and to obtain the desired thickness, the embedding media must be modified to suit the experimental material. Sass (1951) suggested that the texture and cutting property of soft paraffin could be modified by addition of other materials like rubber, bees wax or other hard waxes like ceresin.

A section must be so stained that contrasting colours are exhibited by the different parts as cell wall, protoplasm and nuclei. Thus even triple or multiple staining may become necessary. Sass (1951) opined that the real test for the desirability ofa multiple stain is its specific selectivity of the colour components for defenite morphological/chemical entities in the cells.

Haidenhan's Azan staining has been reported as a very promising triple stain combination for animal tissue (Mallory, 1961) in which the dyes used were azooarmin, Orange G., and anilin blue. Reports on its use for plant material are not available. Gray (1958) described carmin stains as giving bright red colour to the nuclei. Orange.G. is one of the most important cytoplasmic counterstains and is specified in innumerable staining schedules (Johanson, 1940; Sass, 1951). Johanson (1940) described anilin blue as a good counterstain for plant tissues where it stains the cellulose cell walls and achromatic figures.

3. MATERIALS AND METHODS

The studies on "Flower bud differentiation in *Piper sp.*" were carried out at the College of Horticulture, Vellanikkara in Panniyur-1 and Karimunda varieties of black pepper. The seven years old vines were under uniform cultural and manurial treatments as per the package of practice recommendations of the Kerala Agricultural University (Anon., 1982). The experimental standards of Panniyur-1 were irrigated during the summer months.

3.1 Factors influencing flower bud differentiation/ flowering

3.1.1 Climatic factors

From the weather data collected at the 'B' class meteorological observatory in the campus, fortnightly averages of daily maximum temperature, daily minimum temperature, rainfall, daily maximum relative humidity, daily minimum relative humidity and daily sunshine hours were computed. These parameters were examined for their possible role in flower bud differentiation.

3.1.2 Nutritional factors

Plant samples were analysed at fortnightly intervals to determine the levels of carbohydrates, nitrogen, phosphorus and potassium. For this purpose,

five Panniyur-1 standards were selected at random and laterals were collected at fortnightly intervals, starting from 1st August, 1983 to 31st July, 1984. The samples were labelled, dried in an oven at 80°C for 48 hours and powdered using a grinder (Multiplex) to a fineness of 14 mesh.

Total soluble carbohydrates in the samples were determined by the method suggested by Deiraz (1961). Nitrogen in the samples was estimated by colorimetric method as suggested by Snell and Snell (1967). To estimate the phosphorus and potassium contents the powdered plant material was digested in a mixture of nitric acid, sulphuric acid and perchloric acid in the ratio 9 : 2 : 1. The phosphorus content was determined colorimetrically by the Vanadomolybdate yellow colour method in nitric acid medium and potassium, photometrically (Jackson, 1958).

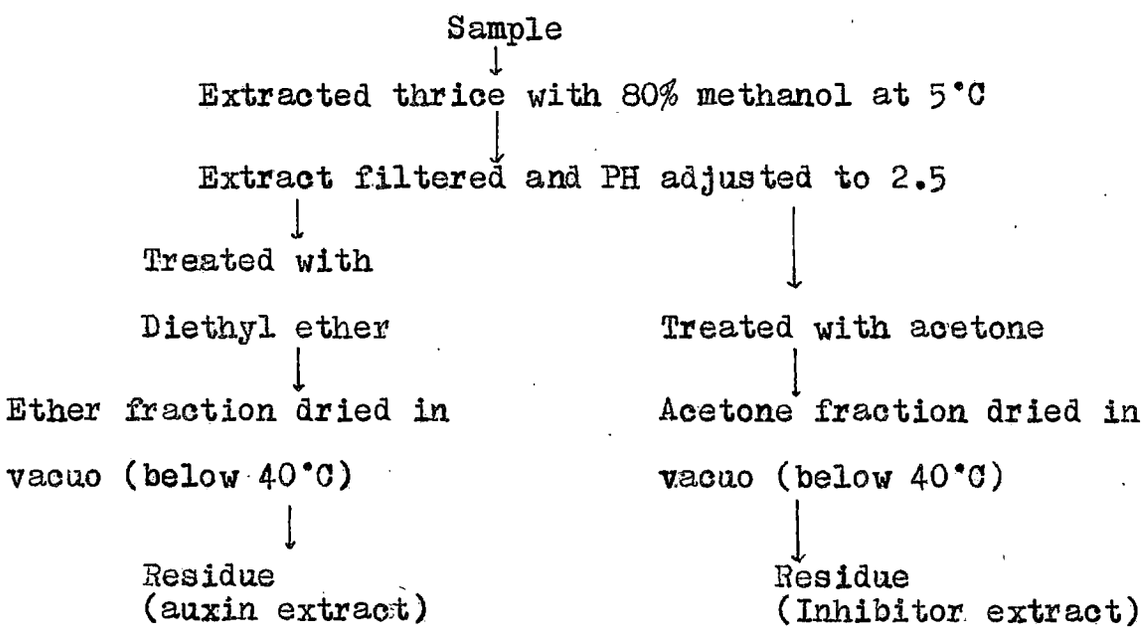
The levels of different nutritional factors thus obtained were correlated with the data on flower bud differentiation.

3.1.3 Hormonal factors

Endogenous levels of growth substances coming under the groups auxins and inhibitors, in the plant samples were analysed at fortnightly intervals.

3.1.3.1 Extraction and purification

A known weight of the sample (buds with nodal region), collected early in the morning, was extracted with 80 per cent pre-chilled methanol for 24 hrs, at 5°C. After re-extraction twice the extracts were bulked. The extract was filtered through glass wool and divided quantitatively for separation andpurification of auxins and inhibitors as detailed below (Murakami, 1970; Rehman et al., 1975)



3.1.3.2 Chromatographic separation

For separation of both auxins and inhibitors the residue obtained was dissolved in a known volume of distilled water and subjected to ascending paper chromatography using Whatman No.1 chromatographic paper. The running solution used was isopropanol : ammonia : Water

(10 : 1 : 1 v/v). The chromatograms so developed were dried at room temperature and stored below 50°C.

3.1.3 Estimation

"Wheat coleoptile section straight growth bioassay" (Mitchell and Livingston, 1968) was employed for estimation ofauxins. The quantity of auxins was worked out from individual Rf positions which showed significantly more response than the control, by referring to the standard curve obtained from the bioassay of authentic IAA. Authentic IAA gave significantly more response than the control at Rf position 0.2 - 0.4. As it was observed that in pepper bud extract, the auxin activity was not very consistent at these Rf positions or at any other point, the regions shown by authentic IAA (Rf 0.2 - 0.4) were selected for bioassay of auxin activity. Then individual values were posted and presented as IAA equivalents µg/g fresh plant sample.

"Mustard seed germination inhibition bioassay" (Eidelnant et al., 1980) was employed for estimation of inhibitor content. The inhibitor content was worked out for the Rf 0.6 - 0.8 referring to the ABA bioassay standard curve and expressed as ABA equivalent µg/g fresh weight of plant sample. The inhibitor activity was found to be very consistent at Rf 0.6 - 0.8.

3.1.4. Correlation of different factors on flower bud differentiation

To examine the influence of environmental, nutritional and hormonal factors on flower bud differentiation in Panniyur-1, the factors in a fortnight were correlated with the data on flower bud differentiation during the fortnight (simultaneous or lag 0). In addition, the factors during the preceding fortnight were correlated with the data on flower bud differentiation during a particular fortnight (lag 1). Similarly the factors during two fortnight before and data on flower bud differentiation during a particular fortnight also were correlated (lag 2). Likewise, analyses were done upto lag 6.

In Karimunda this analysis was done only with respect to environmental factors.

3.2 Histological studies

3.2.1 Collection and storage of plant sample.

For histological studies on flower bud differentiation twenty standards of Panniyur-1 were selected and from each standard, two buds emerging out of the laterals were

collected at fortnightly intervals starting from 15th June, 1983 to 31st July, 1984. In case of Karimunda, five standards were selected and from each standard five buds were collected at random at fortnightly intervals starting from 1st August 1983 to 31st July, 1984. The buds scooped out without injury to the apex or lateral parts during morning hours were kept in FAA* as suggested by Sass (1951).

For the purpose of estimating the total time taken for completing the different stages fifteen buds were taken daily from a single standard of Panniyur-1, starting from 4th June, 1984. These were processed and examined for the different stages.

3.2.2 Processing of the specimens

3.2.2.1 Killing and fixing

FAA was used for killing and also as a fixative in which specimens were kept immersed in corked specimen tubes. Specimens could be stored in FAA throughout the

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* Formalin-Aceto-Alchol - (FAA)
Ethyl alcohol - 50 ml
Glacial acetic acid - 5 ml
Formaldehyde (37-40%) - 10 ml
Distilled water - 35 ml

period of study of about one year without any damage to tissue organisation or shrinkage of the cells.

3.2.2.2. Dehydration.

Specimens were dehydrated after a minimum storage period of one week in FAA. For this two methods were tried. In the first method, suggested by Johanson (1940) and standardised for pepper buds by Nalini (1983), ethyl alcohol was used along with teritary butyl alcohol (TBA) as the dehydrating agent. (2) In the second method, isopropyl alcohol was used along with TBA. The schedule followed in the second method is given below:-

Specimens in FAA

↓

decanted and flooded with 50% isopropanol
(3 hrs)

↓

decanted and flooded with solution I*
(3 hrs)

↓

decanted and flooded with solution II
(12 hrs)

↓

decanted and flood with solution III
(3 hrs)

↓

decanted and flooded with solution IV
(3 hrs)

↓

decanted and flooded with solution V
(12 hrs)

↓

Rinsed with 3 changes of TBA and flooded
in the last change
(12 hrs)

↓

Paraffin infiltration.

* Grade No.	95% Isopropanol (ml)	Absolute Isopropanol (ml)	TBA (ml)	Water (ml)
I	50	-	10	40
II	50	-	20	30
III	50	-	35	15
IV	50	-	50	-
V	-	25	75	-

As the second method was found to facilitate good paraffin infiltration and provide other desirable features to the specimens this was adopted for further studies.

3.2.2.3. Paraffin infiltration

After dehydration infiltration was done using paraffin with ceresin (M.P. 60°C) as described by Johanson (1940).

3.2.2.4. Embedding

The following four types of media were used for embedding.

Soft paraffin (M.P. 60-62°C)

Soft paraffin (M.P. 58-60°C)

Hance's mixture* without ceresin

Paraffin with ceresin (M.P. 60°C)

Paraffin with ceresin (M.P.60°C) which was found to be superior to the other media by virtue of its excelle cutting property at room temperature and its ability to form good ribbons of desired thickness on sectioning, was selected and used for further studies.

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* Hance's mixture without ceresin contains 100.0 g paraffin, 4.5 g rubber paraffin mixture and 1.0 g bee's wax.

3.2.3 Microtome sectioning

Sections were taken at a thickness of 10-12 μ using a junior rotary microtome machine as per standard microtomy (Johanson, 1940).

3.2.3 Staining

The sections affixed to slides were stained with Haidenhan's Azan as per the method suggested by Mallory (1969), the schedule of which is given below:

Sections affixed to slides
↓
Heated to 50°C
↓
Xylene I (5 Min)
↓
Absolute Isopropanol (2 min)
↓
95% isopropanol (2 min)
↓
70% isopropanol (2 min)
↓
50% isopropanol (2 min)
↓
30% isopropanol (2 min)
↓
Distilled water (2 min)
↓

Azocarmin.G. (1 hr at 60-70°C)
↓
cooled and differentiated in dilute ammonia solution
↓
Arrested differentiation in 1% acetic acid solution
↓
Mordanted in phosphotungstic acid 5% (1 - 3 hours)
↓
Rinsed with distilled water
↓
stained with Anilin blue-orange G mixture (30 min)
↓
50% isopropanol (30 sec)
↓
90% isopropanol (30 sec)
↓
Absolute isopropanol (30 sec)
↓
Xylene (1 min)
↓
Dried & mounted in canada balsam.

---

* Azocarmin G

Azocarmin G. - 0.1 g
Glacial acetic acid - 1 ml
Distilled water - 100 ml

** Anilin blue - Orange G mixture

Anilin blue (water soluble) - 0.5 g
Orange G. - 2.0 g
Glacial acetic acid - 8 ml
Distilled water - 100 ml

3.2.4 Microscopic examination

The slides were examined through a binocular mono-objective microscope (Olympus KICBI) with 10 X/5 X objective and 10 X eye piece. Critical examinations were done at higher magnifications using a binocular 'Nikon optiphot' microscope available at the Central Instruments Laboratory, National Agricultural Research Project, (Southern Region) College of Agriculture, Vellayani.

3.2.5 Photomicrography

Photomicrographs of the selected sections were taken using a photomicrography system (Nikon Optiphot with Fx - 35A) available at the Central Instruments Laboratory, NARP (SR), College of Agriculture, Vellayani. ILLFORD black and white negative film of 120ASA, Sakura colour SR-100 negative film of 100 ASA and Kodachrome colour positive film of 64 ASA were used for taking the photomicrographs.

4. RESULTS

The results of the investigation conducted on "Flower bud differentiation in *Piper sp*" are presented in this chapter. The studies conducted in two varieties of pepper, Panniyur-1 and Karimunda, consisted of two parts, one on the factors influencing flower bud differentiation and the other on the histological aspects. The observations were made over a period of one year from 1st August, 1983 to 31st July, 1984.

4.1. Factors influencing flower bud differentiation/ flowering

4.1.1. Climatic factors

4.1.1.1. Temperature

During the period of observation, the maximum temperature rose from 28.14°C at the beginning of August to 33.38°C at the middle of January and reached the peak of 37.67°C in the second fortnight of March. Thereafter, the maximum temperature declined to 34.5°C in the latter half of May and came down to 28.75°C by the end of July. The minimum temperature fluctuated between 21.54°C and 26.43°C. The months of October, November, June and July recorded the lowest readings for minimum temperature (Fig.1, Appendix I).

4.1.1.2. Rainfall

The total rainfall during the period of study was

3393 mm which showed wide variation from nil (during the first half of December and during the months of January and March) to 494 mm (during the first fortnight of June). The amount of rainfall available from the first fortnight of December to the second fortnight of March was negligible. Pre-monsoon showers arrived by the beginning of April. In May, precipitation was negligible. South West monsoon commenced by the first fortnig of June (Fig1, Appendix.I).

4.1.1.3. Sunshine hours

The daily sunshine hours ranged from 0.95 (June 1st fortnight) to 10.42 (March 2nd fortnight). There was a drastic reduction in bright sunshine hours in June and July consequent on the onset of South West monsoon. During August-September months also, it was minimum (Fig.1, AppendixI).

4.1.1.4. Relative humidity

The relative maximum humidity which was above 90.0 per cent in August-September months declined gradually and the minimum of 67.8 per cent was recorded in December. There was a slight increase in relative maximum humidity, subsequent to the pre-monsoon showers in May, followed by a sudden increase as a result of the monsoon rains in June and July months. The relative minimum humidity also followed a similar trend (Fig.1, Appendix.I).

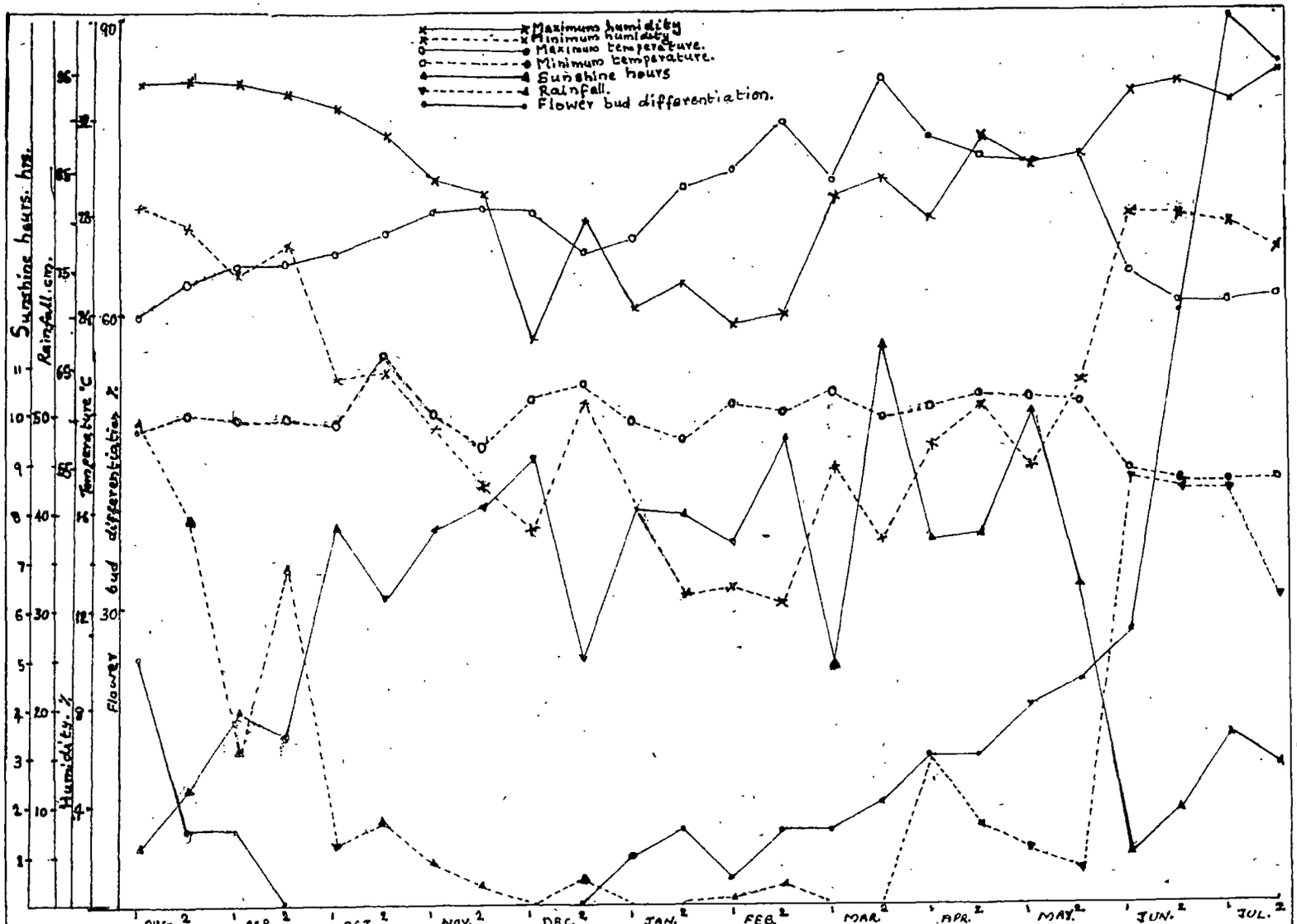

Fig.1. Relationship between climatic factors and flower bud differentiation (in Panniyur-1)

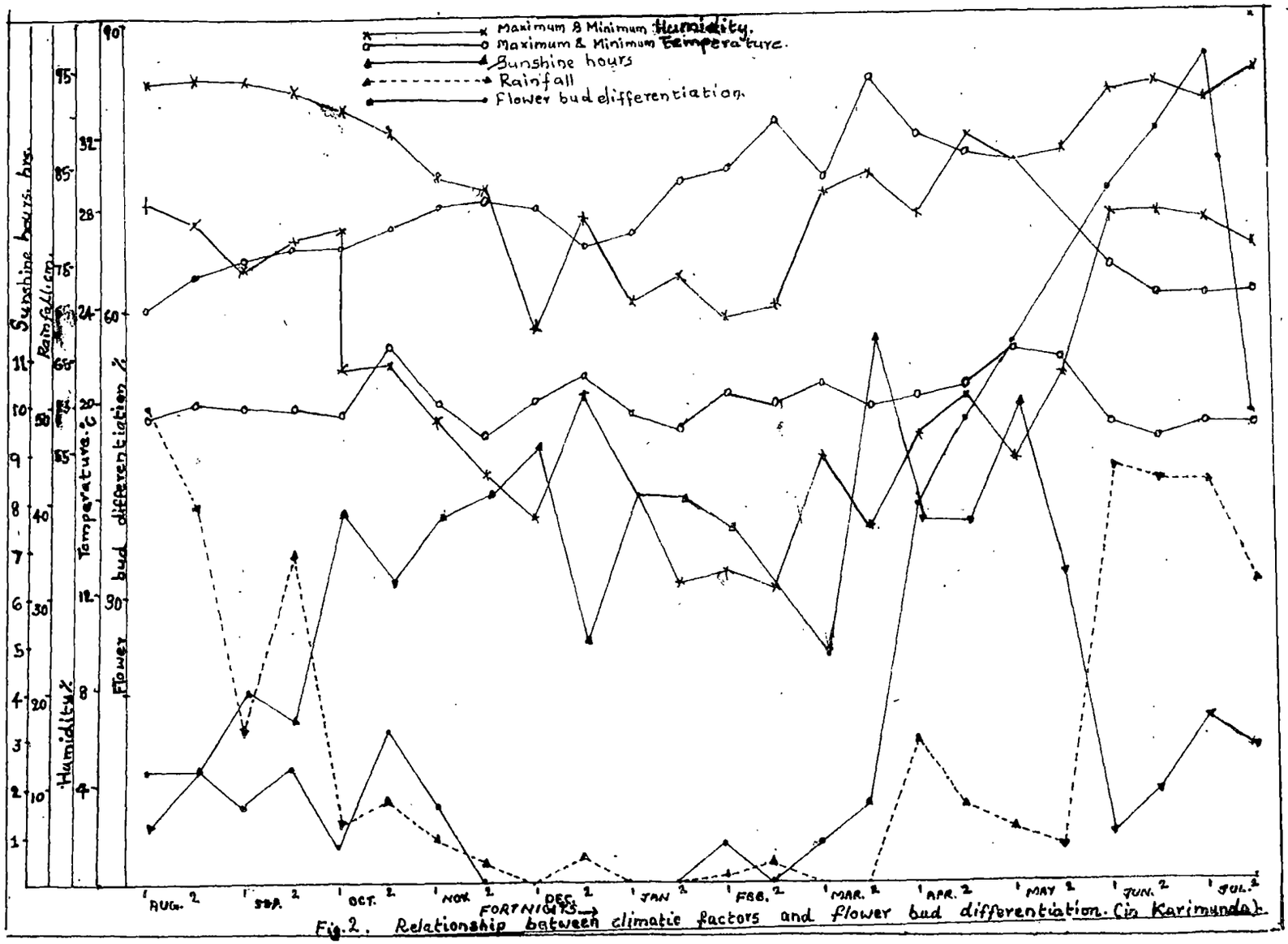

Fig. 2. Relationship between climatic factors and flower bud differentiation. (in Karimunda).

4.1.2. Nutritional factors

4.1.2.1. Carbohydrates, nitrogen reserves and C/N ratio

Data on fortnightly variation of total soluble carbohydrates and nitrogen in Panniyur-1 nodal regions are presented in Table 1, along with the C/N ratio.

During the period of observation, the total soluble carbohydrates varied from 3.04 to 5.99 per cent and nitrogen from 1.63 to 3.14 per cent. In both the cases, the trends of accumulation/depletion were erratic and did not show any definite pattern. However, a slight increase in the nitrogen content was observed in June-July months (Fig.3).

The C/N ratio ranged between 1.22 and 2.86 with a mean of 2.07. From the beginning of October to the middle of November, the middle of December to the middle of February, April first half and from the middle of May to the middle of June, the C/N ratio remained above the mean. The maximum C/N ratio of 2.86 was recorded during May 2nd half. During the other periods, the ratio fell below the mean.

4.1.2.2. Phosphorus and potassium

The content of phosphorus and potassium in the plant samples of Panniyur-1 are given in Table 2. During the period of study, phosphorus content varied from 0.102 per cent in February 1st fortnight to 0.143 per cent in September 2nd and December 2nd fortnights. No definite

pattern could be observed in the accumulation of phosphorus (Fig.3).

The potassium content ranged from 1.871 per cent in February 2nd fortnight to 2.921 per cent in August 1st fortnight. It remained high from August to the middle of January. Thereafter, it decreased and the low level persisted till May when it again increased (Fig.3).

4.1.3. Hormonal factors

Data on the auxin and inhibitor contents in Panniyur-1 shoots are presented in Table 3 and Fig.4.

4.1.3.1. Auxins

In paper chromatography ofpurified pepper shoot extract, auxin like substances were not consistent at a particular Rf position. As authentic indole acetic acid (IAA) was separable at Rf 0.2-0.4, this portion was selected for estimation of auxin like substances.

During the period of study, the endogenous level of auxin like substances varied from 0.01-0.02 µg/g of fresh plant material (expressed as IAA)

4.1.3.2. Inhibitors

Inhibitors were found to be located at Rf position of 0.5-0.7 in the paper chromatograms of pepper shoot extracts and this was found to be consistent throughout the period of study.

Table 1. Total soluble carbohydrates, nitrogen and C/N ratio in the shoots of Panniyur - 1.

Date of observation	Fortnight No.	Carbohydrates %	Nitrogen %	C/N ratio
1.8.1983	I	4.21*	2.89	1.45
15.8.1983	II	4.41	2.92	1.51
1.9.1983	III	3.91	2.13	1.83
16.9.1983	IV	4.09	2.04	1.54
1.10.1983	V	3.41	1.63	2.09
15.10.1983	VI	5.40	1.95	2.76
1.11.1983	VII	5.25	1.87	2.81
15.11.1983	VIII	3.69	2.19	1.68
1.12.1983	IX	3.04	2.49	1.22
15.12.1983	X	5.04	1.99	2.53
1.1.1984	XI	5.03	1.94	2.59
15.1.1984	XII	4.63	2.13	2.17
1.2.1984	XIII	4.71	2.01	2.31
15.2.1984	XIV	3.93	1.91	2.05
1.3.1984	XV	3.81	2.00	1.91
15.3.1984	XVI	3.80	2.10	1.81
1.4.1984	XVII	5.63	2.06	2.73
15.4.1984	XVIII	4.75	2.40	1.98
1.5.1984	XIX	3.83	1.91	2.01
15.5.1984	XX	5.12	1.79	2.86
1.6.1984	XXI	5.43	2.03	2.67
15.6.1984	XXII	4.65	2.72	1.71
1.7.1984	XXIII	4.63	3.14	1.47
15.7.1984	XXIV	5.99	3.05	1.96

* Mean of five standards each with four shoot samples.

Table 2. Content of phosphorus and potassium in the shoots of Panniyur-1.

Date of observation	Phosphorus %	Potassium %
1.8.1983	0.123*	2.921*
15.8.1983	0.132	2.834
1.9.1983	0.119	2.658
16.9.1983	0.143	2.841
1.10.1983	0.104	2.653
15.10.1983	0.106	2.226
1.11.1983	0.109	2.358
15.11.1983	0.116	2.156
1.12.1983	0.137	2.347
15.12.1983	0.143	2.148
1.1.1984	0.134	2.092
15.1.1984	0.116	1.982
1.2.1984	0.102	1.991
15.2.1984	0.109	1.871
1.3.1984	0.111	1.923
15.3.1984	0.123	1.874
1.4.1984	0.112	1.935
15.4.1984	0.109	2.021
1.5.1984	0.131	1.981
15.5.1984	0.111	2.123
1.6.1984	0.142	2.392
15.6.1984	0.127	2.180
1.7.1984	0.137	2.428
15.7.1984	0.122	2.491

* Mean of 5 standards each with four shoot samples.

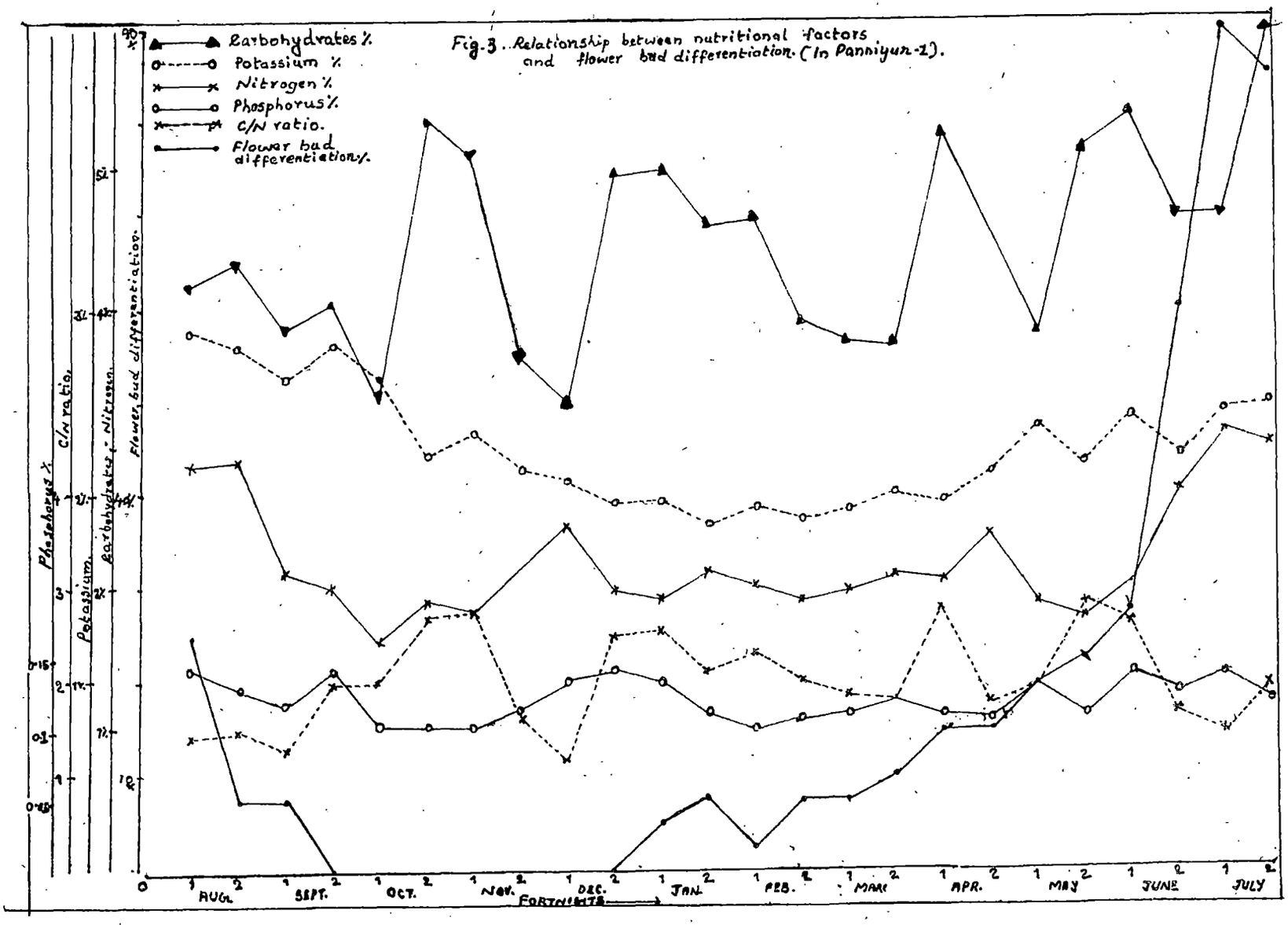

Fig.3. Relationship between nutritional factors and flower bud differentiation. (In Panniyur-1).

Table 3. Endogenous levels of auxins* and inhibitors** in the shoots of Panniyur-1.

Date of observation	Auxins µg/g	Inhibitors µg/g
1.8.1983	0.015	0.14
15.8.1983	0.015	0.13
1.9.1983	0.010	0.15
16.9.1983	0.020	0.14
1.10.1983	0.010	0.16
15.10.1983	0.010	0.14
1.11.1983	0.015	0.18
15.11.1983	0.015	0.20
1.12.1983	0.020	0.21
15.12.1983	0.010	0.21
1.1.1984	0.015	0.28
15.1.1984	0.015	0.19
1.2.1984	0.015	0.21
15.2.1984	0.015	0.22
1.3.1984	0.015	0.19
15.3.1984	0.010	0.15
1.4.1984	0.010	0.14
15.4.1984	0.010	0.14
1.5.1984	0.015	0.12
15.5.1984	0.015	0.13
1.6.1984	0.010	0.11
15.6.1984	0.015	0.12
1.7.1984	0.015	0.11
15.7.1984	0.010	0.12

* Expressed as indole acetic acid µg/g fresh plant sample.

** Expressed as abscissic acid µg/g fresh plant sample.

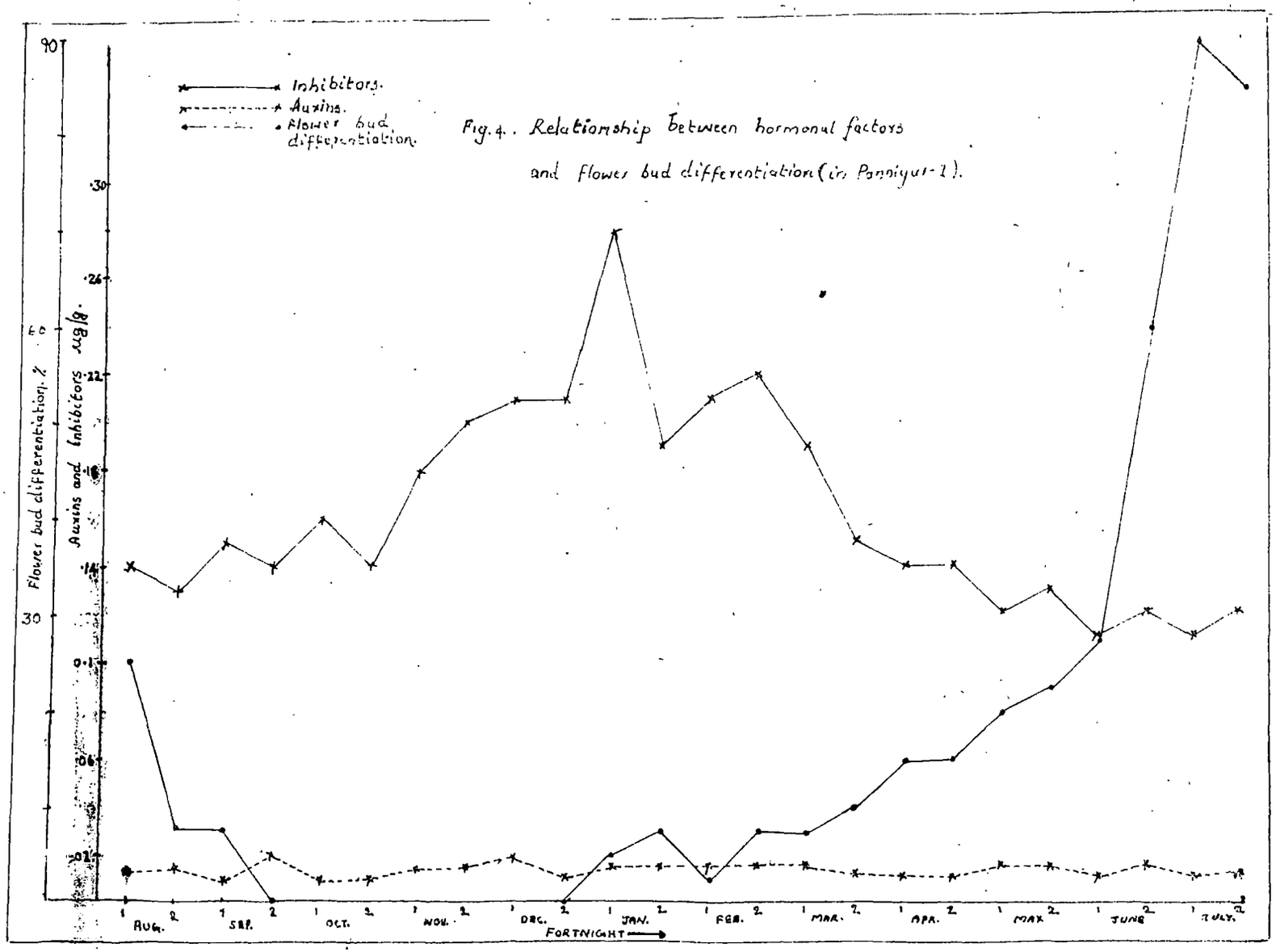

Fig. 4. Relationship between hormonal factors and flower bud differentiation (in Panniyur-1).

During the period of study, the level of inhibitors varied from 0.11-0.28 µg/g of fresh plant material (expressed in terms of abscissic acid). The inhibitor content increased from the start of the observations during the first fortnight of August to the middle of January. Then, it gradually decreased and reached the lowest level of 0.11 µg/g by June 1st fortnight. This low level persisted till the end of observations in second fortnight of July.

4.2. Correlation between environmental, nutritional and hormonal factors and flower bud differentiation

4.2.1. Panniyur-1

In Panniyur-1, the weather parameters as well as the nutritional and hormonal factors were correlated with the data on flower bud differentiation. The factors in a fortnight were correlated with the data on flower bud differentiation during the fortnight (simultaneous or lag 0). In addition the factors during the preceding fortnight were correlated with data on flower bud differentiation during a particular fortnight (lag 1). Similarly the factors during two fortnight before and data on flower bud differentiation during a particular fortnight also were correlated (lag 2). Likewise, analyses were done upto lag 6. The correlation co-efficients are presented in Table 4 and Fig. 5 and 6.

In the simultaneous analysis or lag 0, rainfall, relative maximum humidity, relative minimum humidity and

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nitrogen showed positive correlation (r=0.64**, 0.42*, 0.52** and 0.66**, respectively) with the number of flower buds differentiated. Maximum temperature, sunshine hours and inhibitors showed negative correlation (r=-0.45*, -0.49* and -0.55**, respectively).

Rainfall, relative mimimum humidity and nitrogen during the preceding fortnight (lag 1) were positively correlated (r= 0.61**, 0.48* and 0.48*, respectively) with the number of flower buds differentiated. Sunshine hours and inhibitors were negatively correlated (r= -0.46* and -0.53**, respectively).

Rainfall during the second fortnight prior to differentiation (lag 2) showed positive correlation (r=0.45*) while inhibitors recorded negative correlation (r = -0.50*).

The level of inhibitors during the third fortnight prior to differentiation (lag 3) showed significant correlation (r= -0.44*).

Maximum and minimum temperatures during the 4th and 5th fortnights prior to differentiation (lag 4 and lag 5) showed significant correlation with the number of buds differentiated (r= 0.55* and 0.62**, respectively during lag 4 and r= 0.56* and 0.47*, respectively during lag 5).

Maximum temperature during the 6th fortnight prior to differentiation (lag 6) showed significant correlation

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Table 4. Correlation between flower bud differentiation and factors influencing it at different fortnights (from lag 0 to lag 6) in Panniyur-1.

Factors	Correlation co-efficients						
	Simultaneous (lag 0)	Preceding fortnight (lag 1)	Prior to the 2nd fortnight (lag 2)	Prior to the 3rd fortnight (lag 3)	Prior to the 4th fortnight (lag 4)	Prior to the 5th fortnight (lag 5)	Prior to the 6th fortnight (lag 6)
Maximum temperature	-0.45*	-0.34	-0.11	0.27	0.55*	0.62**	0.74**
Minimum temperture	-0.24	-0.19	-0.02	0.31	0.56**	0.47*	-0.22
Rainfall	0.64**	0.61**	0.45*	0.11	-0.26	-0.15	-0.21
Sunshine hours	-0.49*	-0.46*	-0.39	-0.06	0.31	0.03	-0.40
Relative maximum humidity	0.42*	0.31	0.25	0.08	-0.02	0.05	-0.13
Relative minimum humidity	0.52**	0.48*	0.36	0.08	-0.18	-0.27	-0.32
Carbohydrate	0.37	0.26	0.34	0.28	0.14	0.15	0.31
Nitrogen	0.66**	0.48*	0.03	-0.31	-0.29	0.18	-0.05
C/N ratio	-0.22	-0.10	0.21	0.39	0.23	0.16	0.41
Phosphorus	0.22	-0.33	0.21	0.12	-0.12	0.45	-0.29
Potassium	0.12	0.05	-0.18	-0.29	0.05	-0.35	-0.16
Auxin	-0.12	0.04	-0.12	-0.12	0.04	-0.12	-0.02
Inhibitors	-0.55**	-0.53**	-0.50*	-0.44*	-0.38	-0.32	-0.20

* Significant at 5% level

** Significant at 1% level

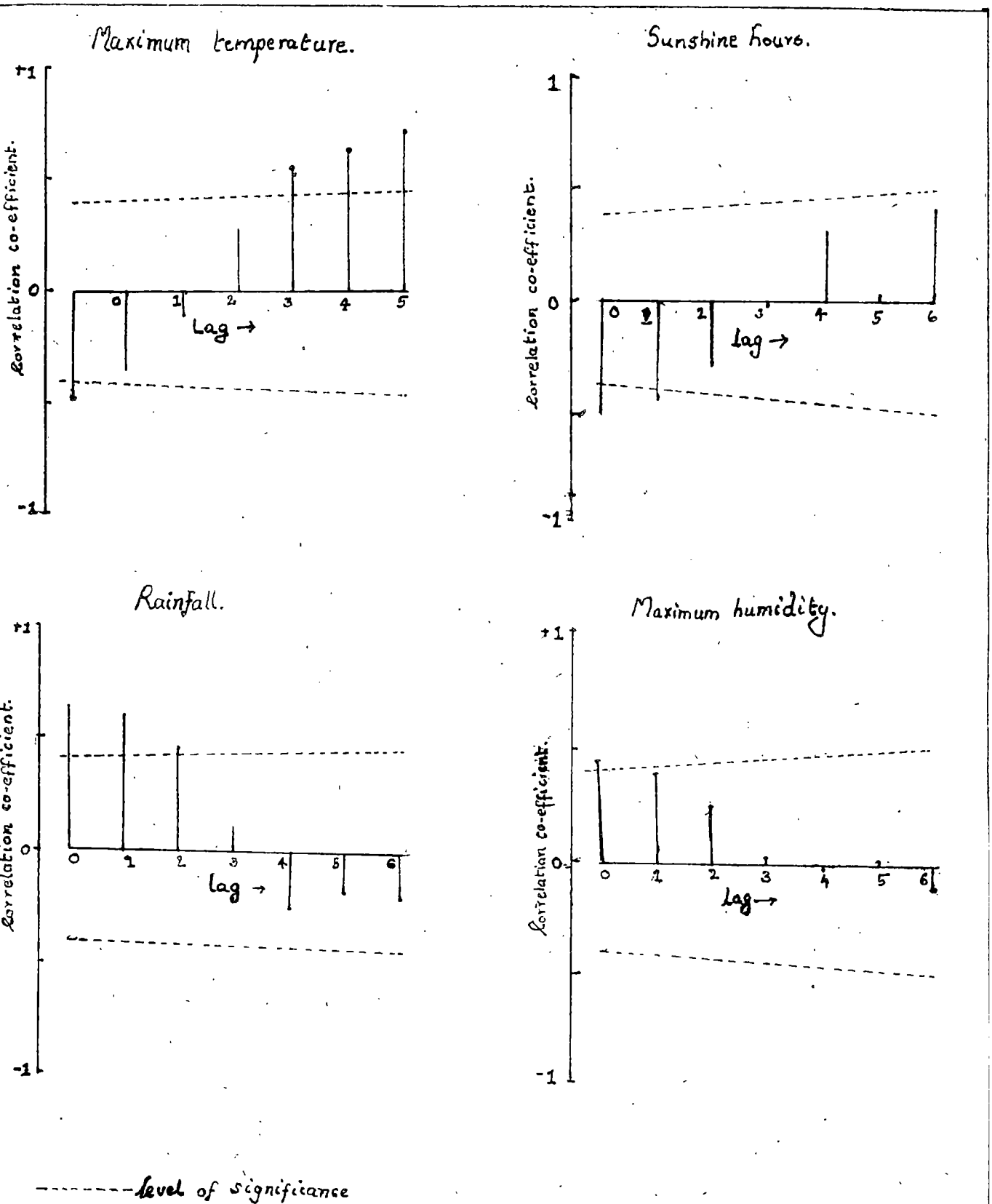


Fig. 5 Correlation of Climatic factors (lag 0 to lag 6) with flower bud differentiation, (In Pannigur-1)

(Scale - 4cm = ±1 (Y axis))

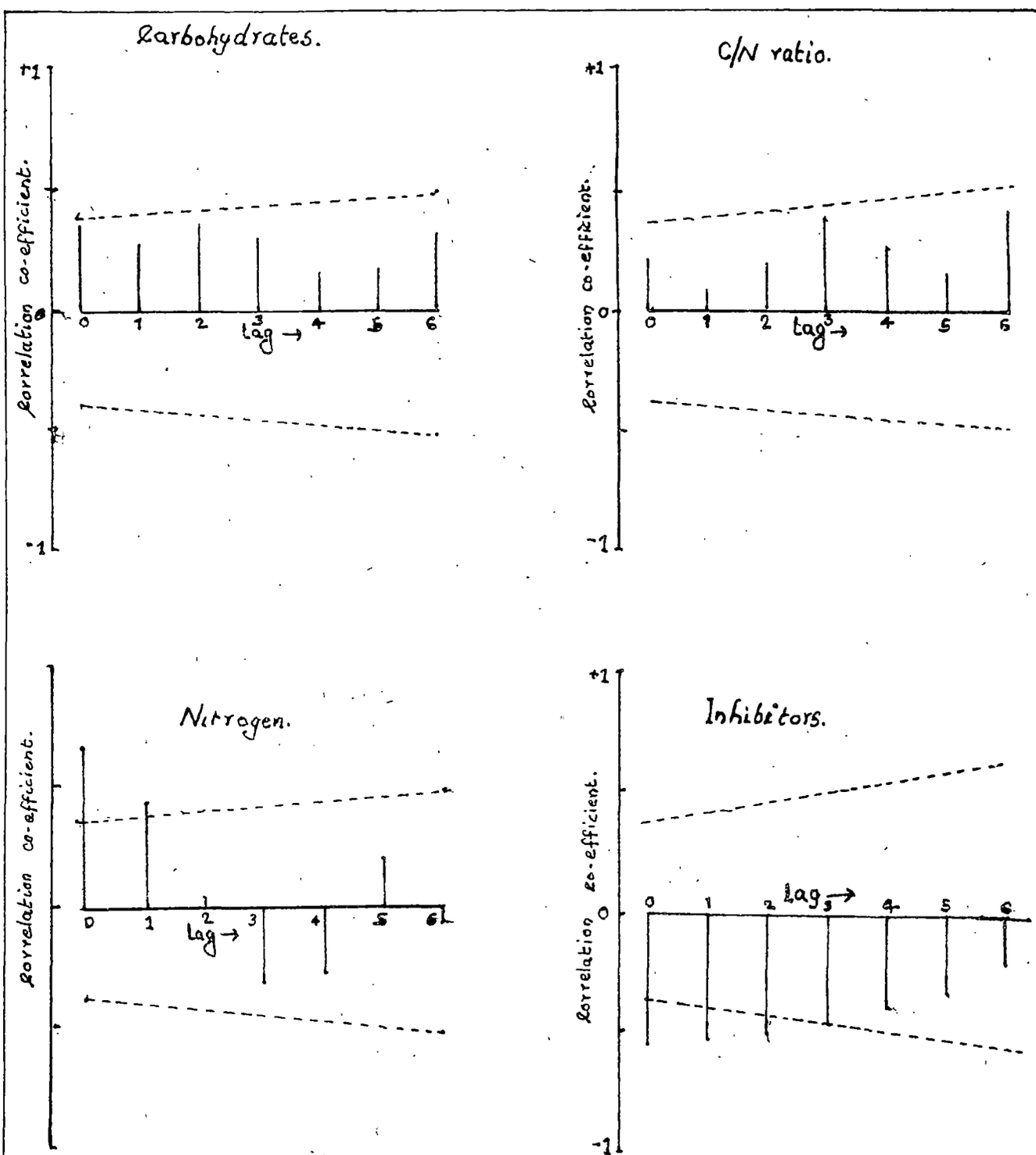


Fig. 6. Correlation of nutritional factors and inhibitors with flower bud differentiation (in Panniyur-1)

[Scale $_{y\text{-axis}}$ 4 cm = ±1]

(r= 0.74**). With the number of buds differentiated.

4.2.2. Karimunda

In Karimunda, the weather parameters were correlated (lag 0 to lag 6) with the data on flower bud differentiation. The correlation coefficients have been presented in Table 5 and Fig. 7.

In the simultaneous (lag 0) analysis rainfall, relative maximum humidity and relative minimum humidity were positively correlated (r= 0.61**, 0.52** and 0.55**, respectively) with the number of flower buds differentiated, while sunshine hours recorded negative correlation (r= -0.43*).

Rainfall and relative maximum humidity during the first fortnight prior to differentiation (lag 1) showed significant correlation which were positive (r= 0.43* in both the cases).

Relative maximum humidity was positively correlated (r= 0.44*) with the number of flower buds differentiated, during the second fortnight prior to the process (lag 2).

Maximum andminimum temperatures during the third fortnight prior to differentiation (lag 3) showed significant positive correlation (r=0.58** and 0.45*, respectively) with the number of flower buds differentiated.

Table 5. Correlation between flower bud differentiation and environmental factors influencing it at different fortnights (lag 0 to lag 6) in Karimunda.

Factors	Correlation co-efficients						
	Simultaneous (lag 0)	Preceding fortnight (lag 1)	Prior to the 2nd fortnight (lag 2)	Prior to the 3rd fortnight (lag 3)	Prior to the 4th fortnight (lag 4)	Prior to the 5th fortnight (lag 5)	Prior to the 6th fortnight (lag 6)
Maximum temperature	-0.31	-0.03	0.26	0.58**	0.25	0.11	0.78**
Minimum temperature	-0.01	0.03	0.23	0.45*	0.47*	0.23	0.22
Rainfall	0.61**	0.43*	0.19	-0.13	0.29	-0.28	-0.39
Sunshine hours	-0.43*	-0.26	-0.11	0.19	-0.03	0.44*	0.41
Maximum humidity	0.52**	0.43*	0.44*	0.29	0.11	0.12	-0.26
Minimum humidity	0.55**	0.37	0.15	-0.13	-0.33	-0.49*	0.57**

* Significant at 5% level

** Significant at 1% level

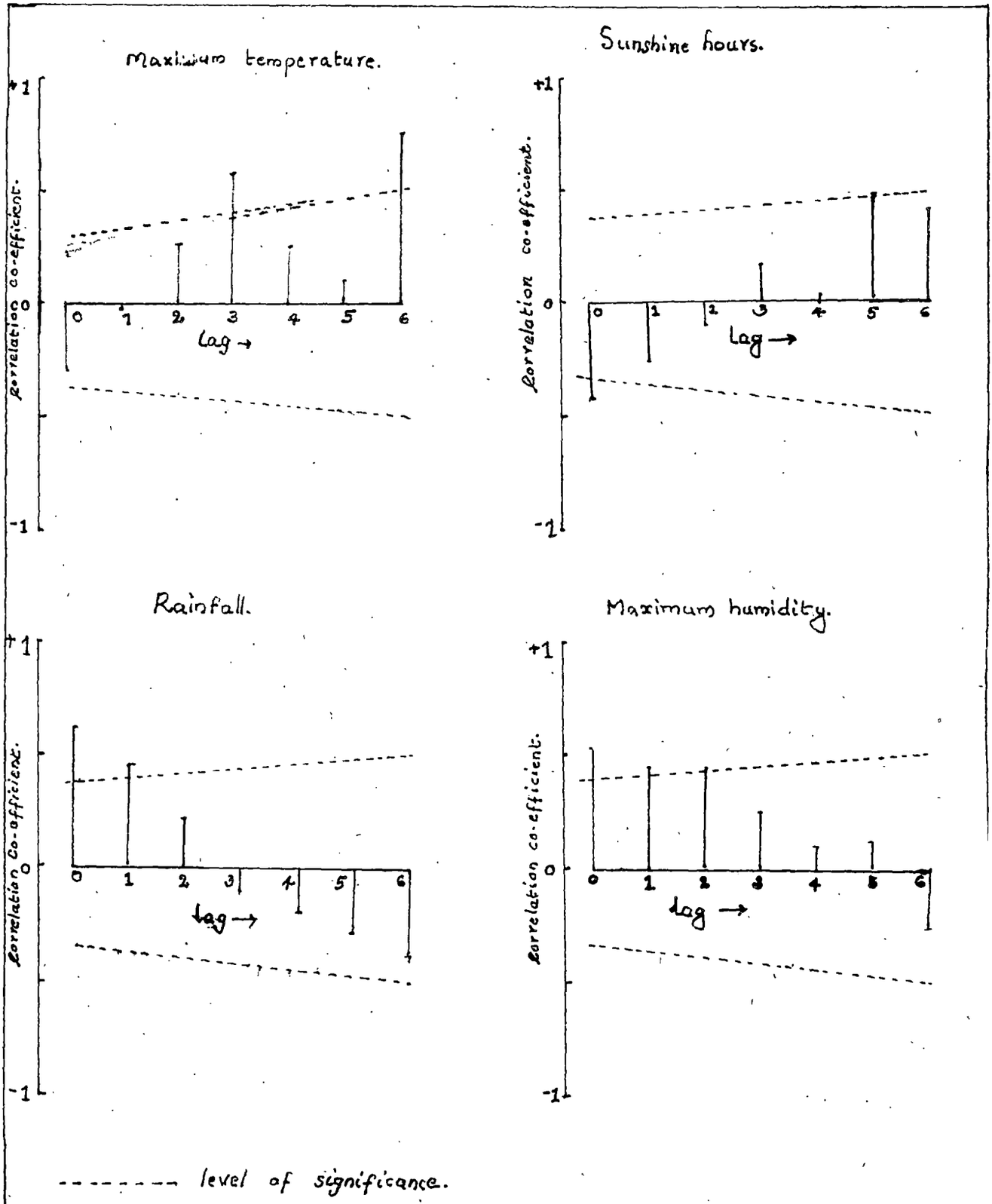


Fig. 7. Correlation of Climatic factors (lag 0 - lag 6) With flower bud differentiation (Karimunda)

(Scale - 4 cm = 1 unit (Y axis))

Only maximum temperature was significantly correlated (r=0.47*) with the differentiation process during the fourth fortnight prior to differentiation (lag 4).

Sunshine hours during the 5th fortnight prior to differentiation (lag 5) recorded positive correlation (r= 0.44*) with the process, while relative minimum humidity recorded negative correlation (r= -0.49*).

Maximum temperature and relative minimum humidity during the 6th fortnight prior to differentiation (lag 6), showed significant positive correlation (r= 0.78** and 0.57**, respectively) with the number of flower buds differentiated.

4.3. Histological studies

An year-round study was made in Panniyur-1 and Karimunda to assess the rate of flower bud differentiation during each fortnight and also to examine the histological features of the differentiating buds.

4.3.1. Rate of flower bud differentiation

4.3.1.1. Panniyur-1

The data regarding differentiation of flower buds have been presented in Table 6 and Fig.8. Differentiation could not be observed during the period starting from the middle of September to the end of December. Thereafter a gradual increase in flower bud differentiation activity was observed which

Table 6. Differentiation of flower buds in two varieties of pepper, Panniyur-1 and Karimunda.

Date of observation	Percentage of differentiation observed	
	Panniyur-1 %	Karimunda %
1.8.1983	* 25.0	** 12
15.8.1983	7.5	12
1.9.1983	7.5	8
16.9.1983	0	12
1.10.1983	0	8
15.10.1983	0	16
1.11.1983	0	8
15.11.1983	0	0
1.12.1983	0	0
15.12.1983	0	0
1.1.1984	5.0	0
15.1.1984	7.5	0
1.2.1984	2.5	0
15.2.1984	7.5	0
1.3.1984	7.5	4
15.3.1984	10.0	8
1.4.1984	15.0	40
15.4.1984	15.0	48
1.5.1984	20.0	56
15.5.1984	22.5	36
1.6.1984	27.5	72
15.6.1984	60.0	76
1.7.1984	90.0	88
15.7.1984	87.5	48

* 40 buds examined at the rate of two from 20 selected standards.

** 25 buds examined at the rate of five from five selected standards.

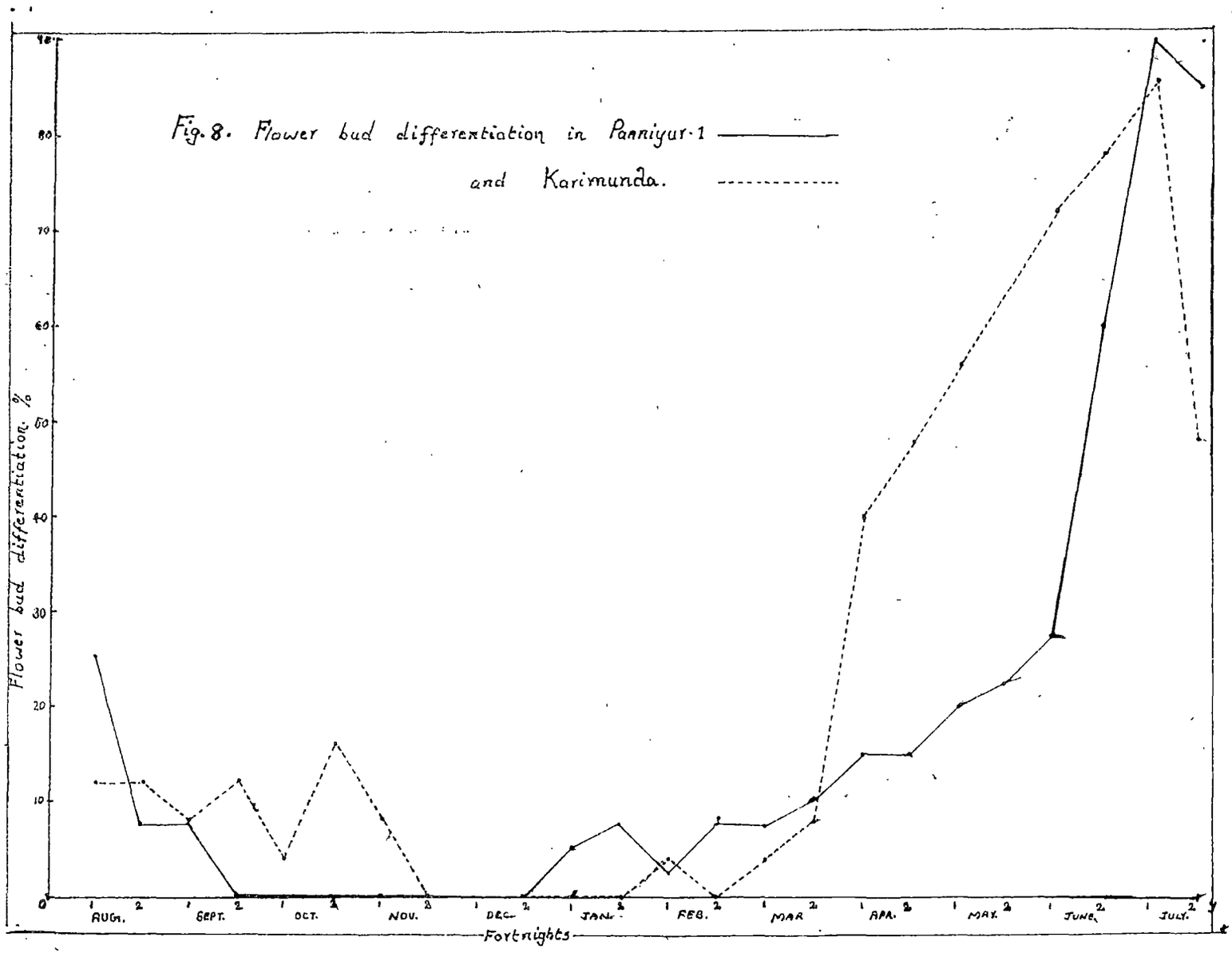


Fig. 8. Flower bud differentiation in Panniyur-1 ——— and Karimunda. ----------

reached the maximum (90.0 per cent) during the first fortnight of July. The second fortnight of July also recorded high degree of differentiation (87.5 per cent). During the month of August and the first fortnight of September, differentiation of flower buds was observed at a reduced rate (7.5 to 25.0 per cent).

4.3.1.2. Karimunda

The data on differentiation of flower buds in Karimunda are presented in Table 6 and Fig.8. Differentiation could not be observed during the period starting from the middle of November to the middle of February. A gradual step up in flower bud differentiation activity was observed after February. The first fortnight of July recorded maximum percentage of differentiation (88.0). By the end of July, the percentage of flower bud differentiation fell below 50.0. From the beginning of August to the middle of November, only very little differentiation could be observed (8.0 to 16.0 per cent).

4.3.2. Histology of apical meristem

Median longitudinal sections were examined microscopically to unravel the histological features of the differentiating buds in Panniyur-1 and Karimunda varieties of pepper.

Plates I-IV

In Plates I to IV, the apical meristem appears conical and is seen surrounded by leaf primordia. The size

and shape of the apex show variation. In Plates I and II the apical meristem is broadly conical with comparatively increased dimensions in width and height above the primordial site. The apex is sharply conical with reduced dimensions in Plates III and IV. The internal corpus cells of the apical meristem is seen covered by a two layered tunica. The plane of division in tunica appears to be anticlinal (Plates II and IV).

On one side of the apex, there is a wedge like protuberance in plates III and IV. This developes into a foliage like structure enlarged on the adaxial side, the enlargement being to a lesser extent at the basal portion (Plate I and II). From the conical apex, leaf primordia can be seen originating in succession (Plate I). The distal ends appear as united. However, independent layers of anticlinally dividing superficial cells (Plate II) separate the leaf primordia.

Plates V and VI

In Plates V and VI the conical apex can be seen transforme into a convex hemispherical structure which has a mantle-core configuration. The outer layer of the cells connstituting the mantle are small, less vacuolated and deeply stained. The cells at the central zones constituting the core are comparatively larger in size and lightly stained. The apex is seen covered by leaf primordia.

--------Leaf Primordia

---------Apex.

Plate-I, L.S. of vegetative bud showing the apex prior to the initiation of a leaf. X 12.5.

Plate-II, The vegetative apex in Plate-I, magnified - showing its conical nature and tunica-corpus layers X 100.

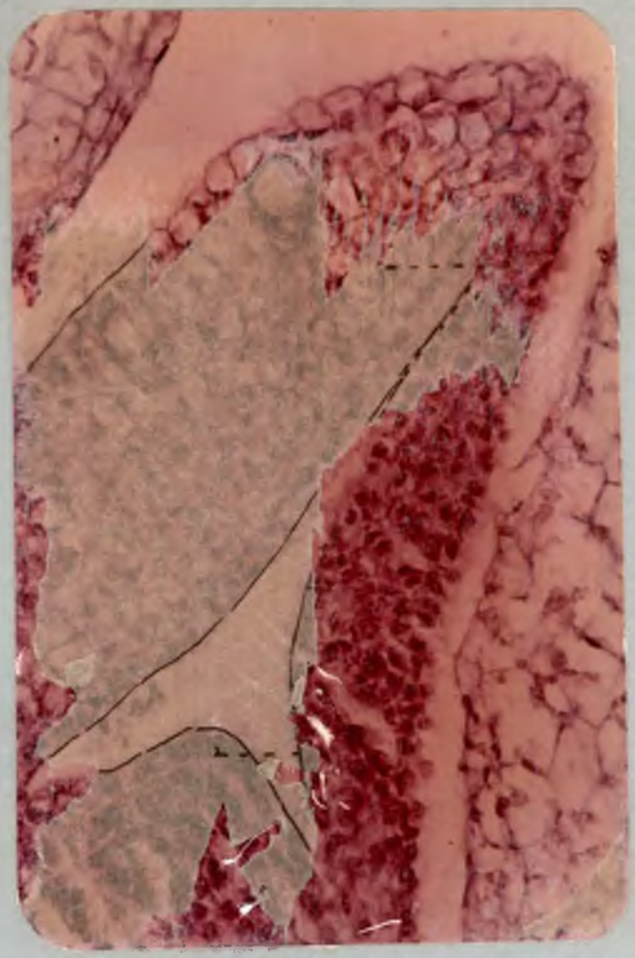


Plate-III, L.S. of vegetative bud showing the apex after the initiation of a leaf. X 25.

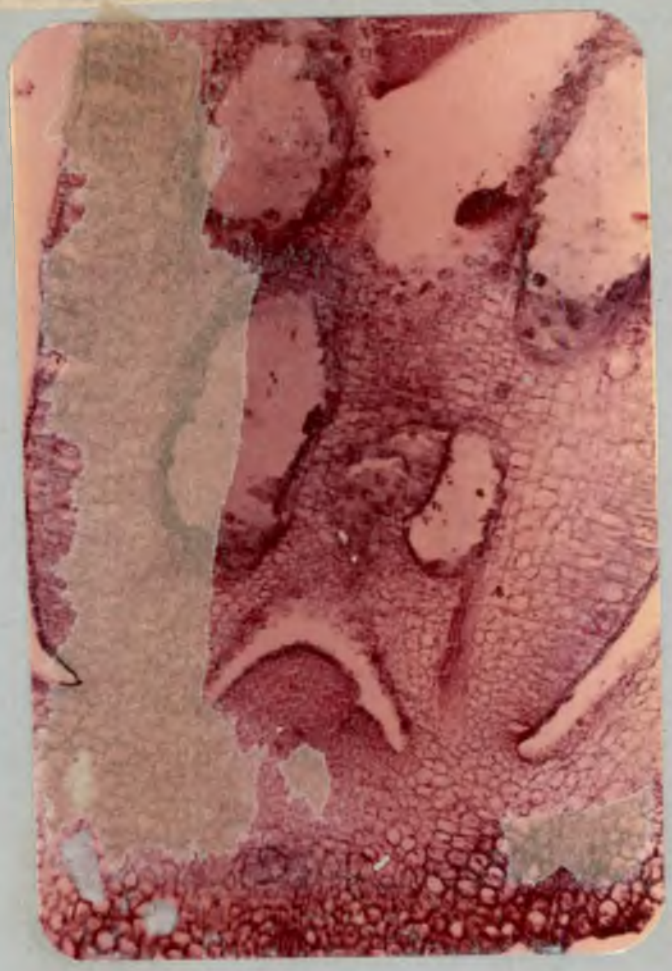


Plate-IV, The apex in Plate-III, magnified - showing its sharply conical nature. X 100.

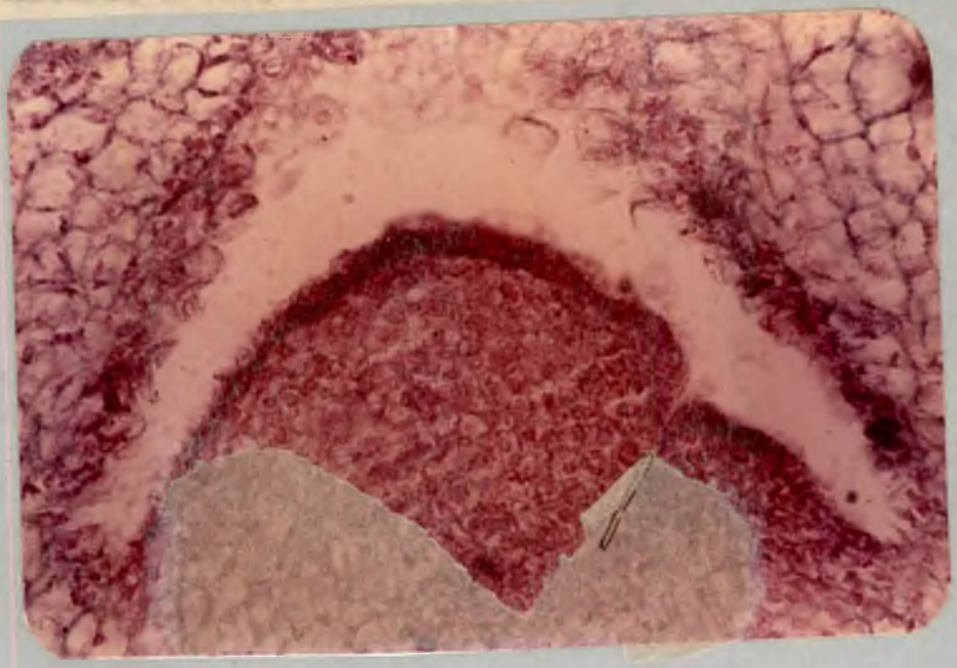


Plate-V, L.S. of a bud at the transition stage. X 25.

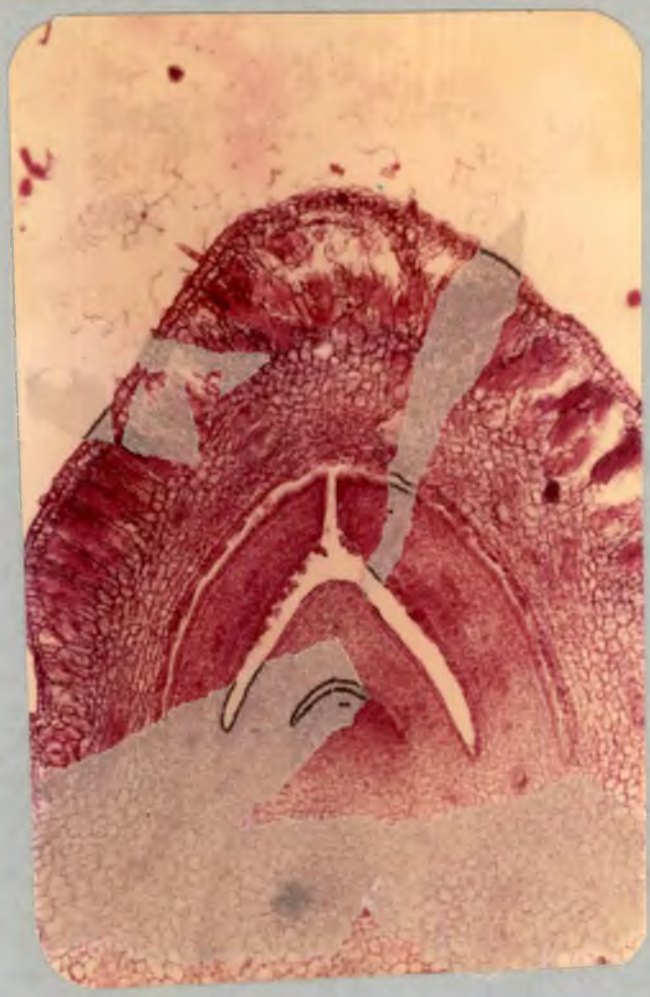


Plate-VI. The apex in Plate-V, magnified - showing the deeply stainable apical cells. X 100.

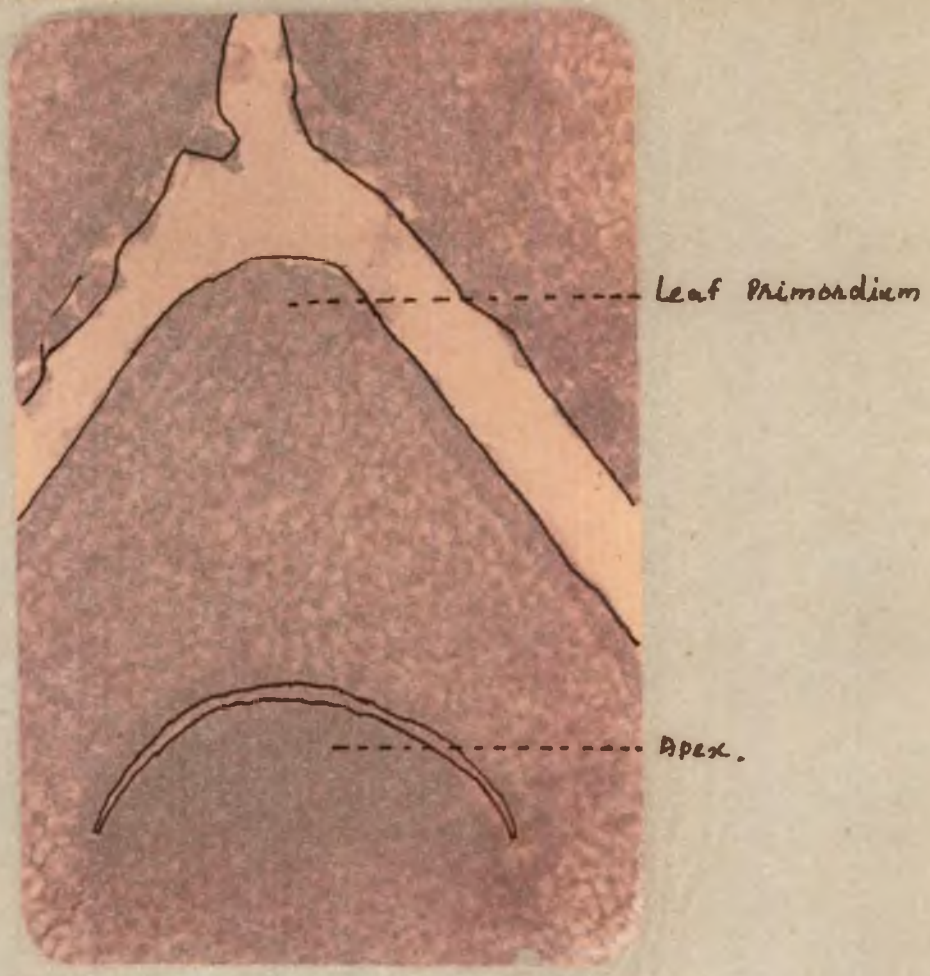


Plate VII

The hemispherical meristem seen in Plates V and VI, has grown into a dome shaped structure in Plate VII. The cells are densely stained. The initiation of a bract primordium (at the base of dome shaped structure) is also evident. Also distinguishable in primordial form adjacent to the dome shaped structure, is a narrow pointed leaf primordium.

Plates VIII to X

The dome shaped structure can be seen developed into a cylindrical structure in Plates VIII to X. The central rib-meristem of the cylindrical structure, characterised by vertical rows of cells, is stained only to a lesser degree (Plates IX and X).

Plates XI to XIV

On the periphery of the developing cylindrical structure, certain meristematic zones (in acropetal succession) can be observed from where finger like projections have developed. Small dome shaped structures alternate the finger like projections (Plate XI). The primordial spike can be easily recognised in Plates XI and XII. The bract primordia and the oval flower primordia, which are deeply stained within their axils are prominent in Plate XII. Under magnification

Plate-VII. L.S. of flower bud showing initiation of spike primordium. X 50.

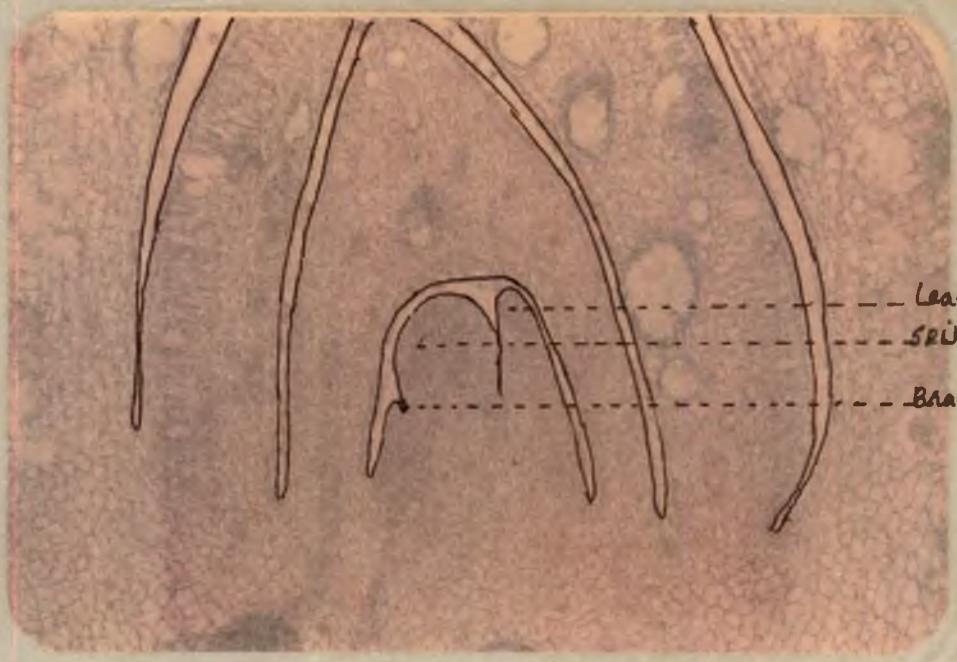


Plate-VIII. L.S. of flower bud showing enlargement of spike primordium. X 25.

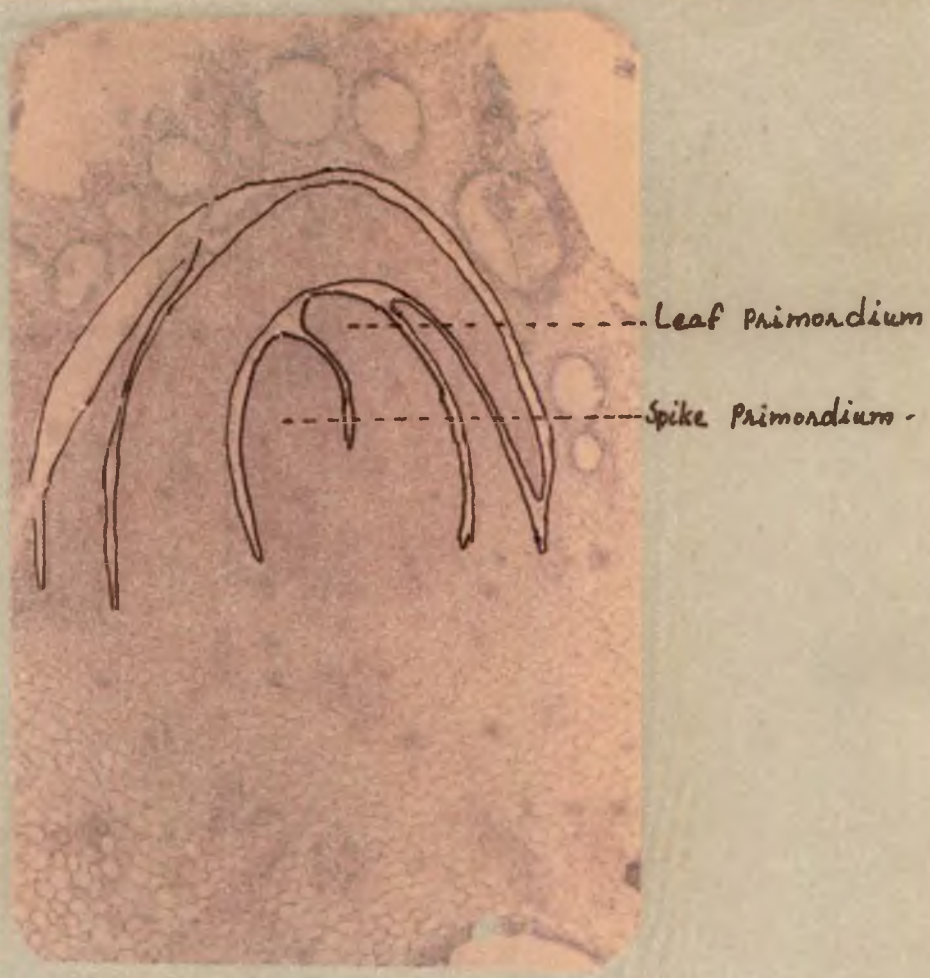


Plate-IX. The spike primordium in Plate-VIII, magnified - showing the deeply stainable peripheral layers. X 100.

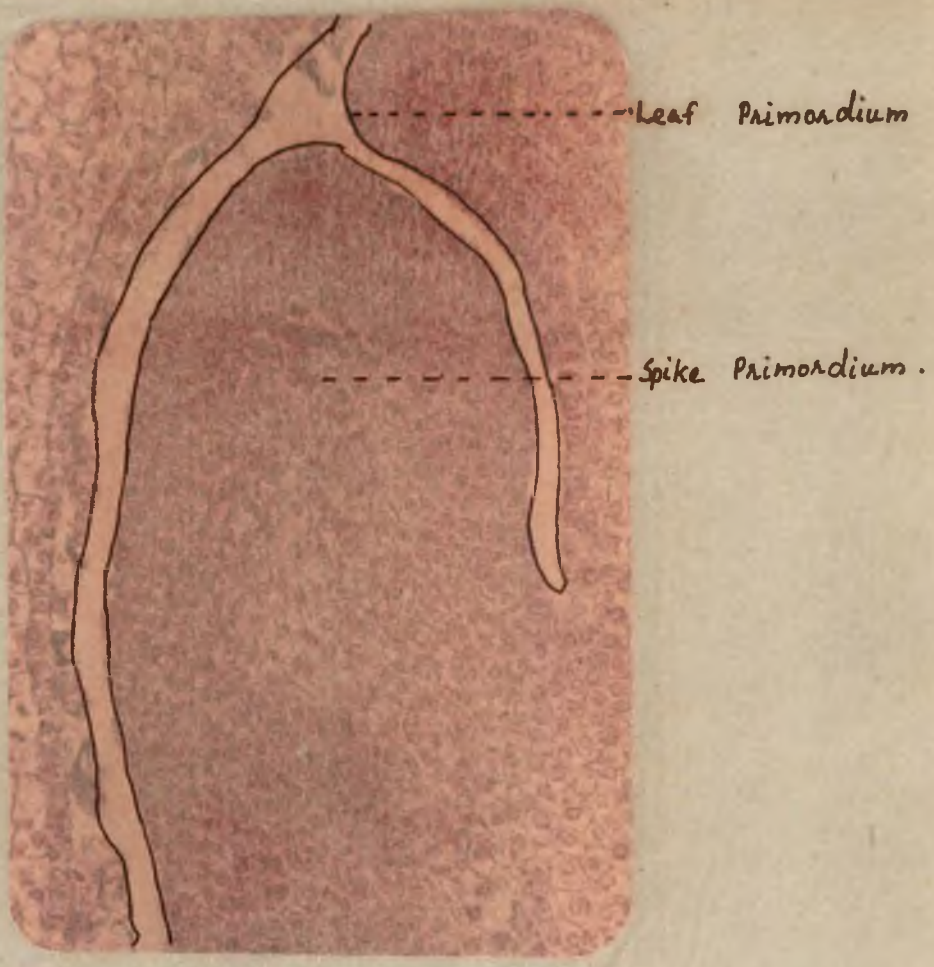


Plate X. L.S. of flower bud shewing the cylindrical spike primordium prior to bract initiation X 25.

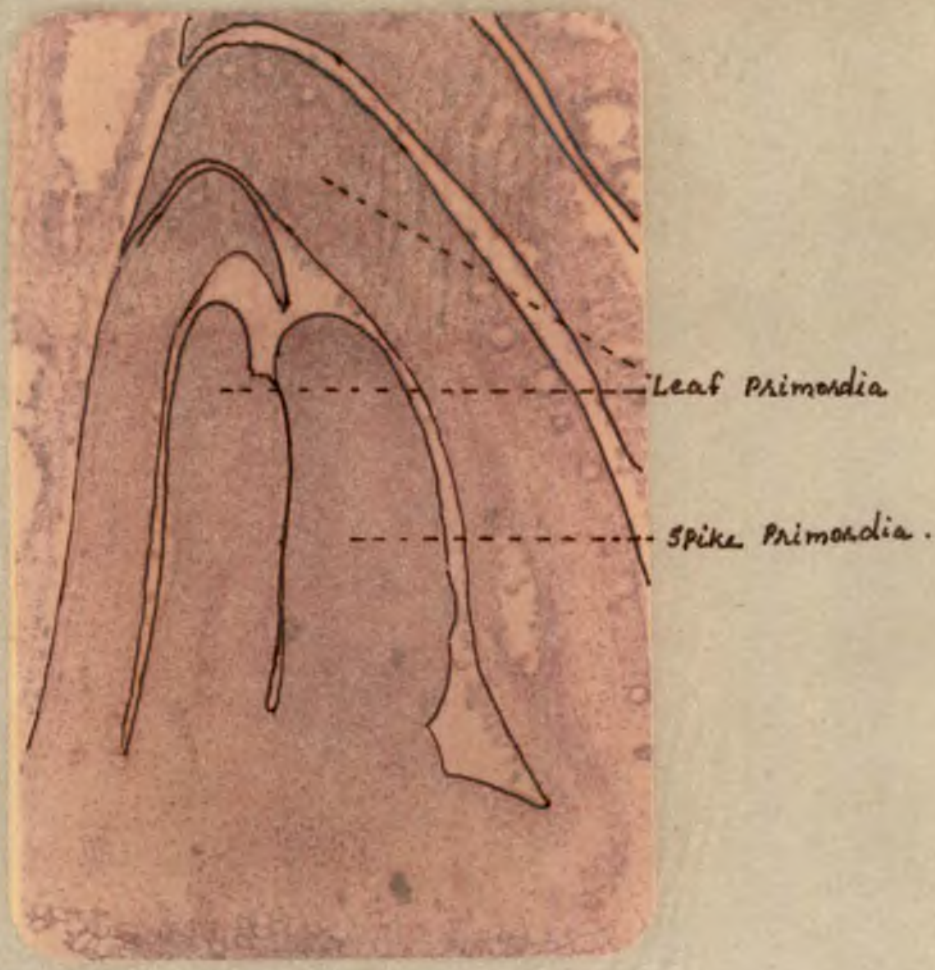


Plate-XI, L.S. of flower bud showing initiation of bract and flower primordia X 12.5.

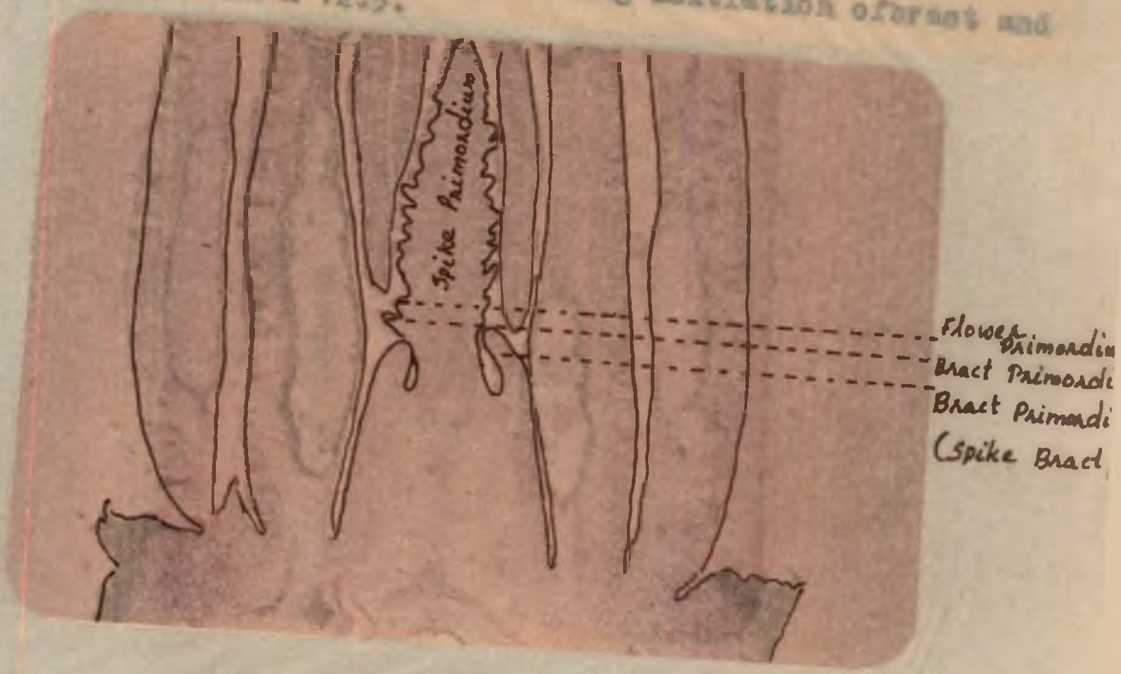


Plate-XII, L.S. of flower bud showing development of bract and flower primordia X 12.5.

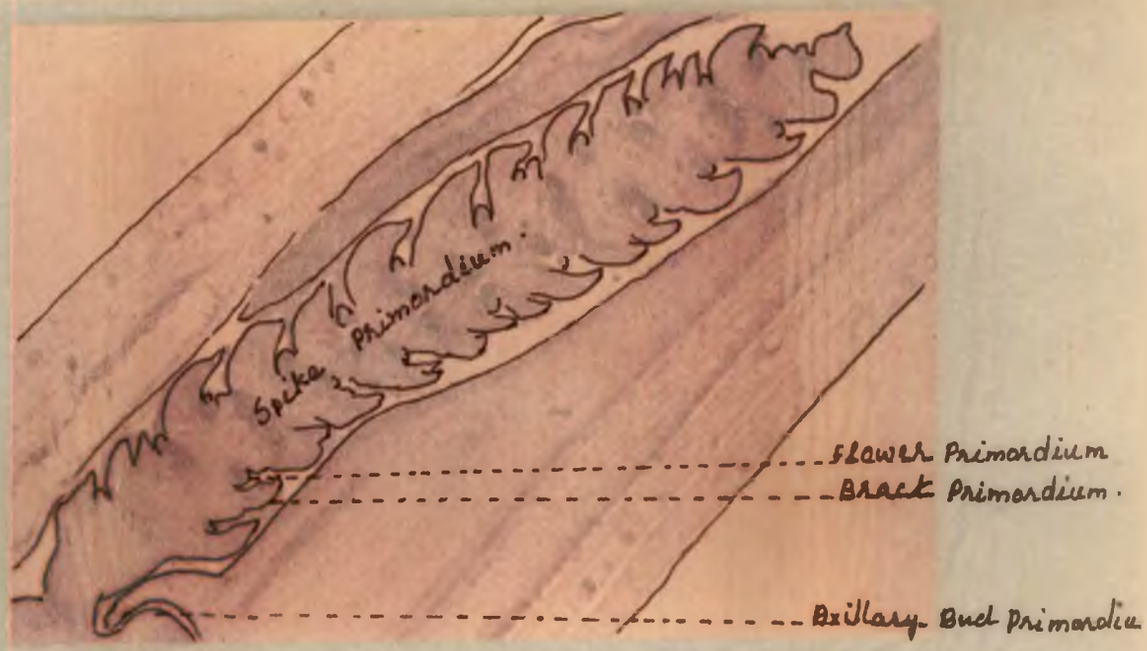


Plate-XIII, The bract and flower primordia in Plate-XII, magnified X 50.

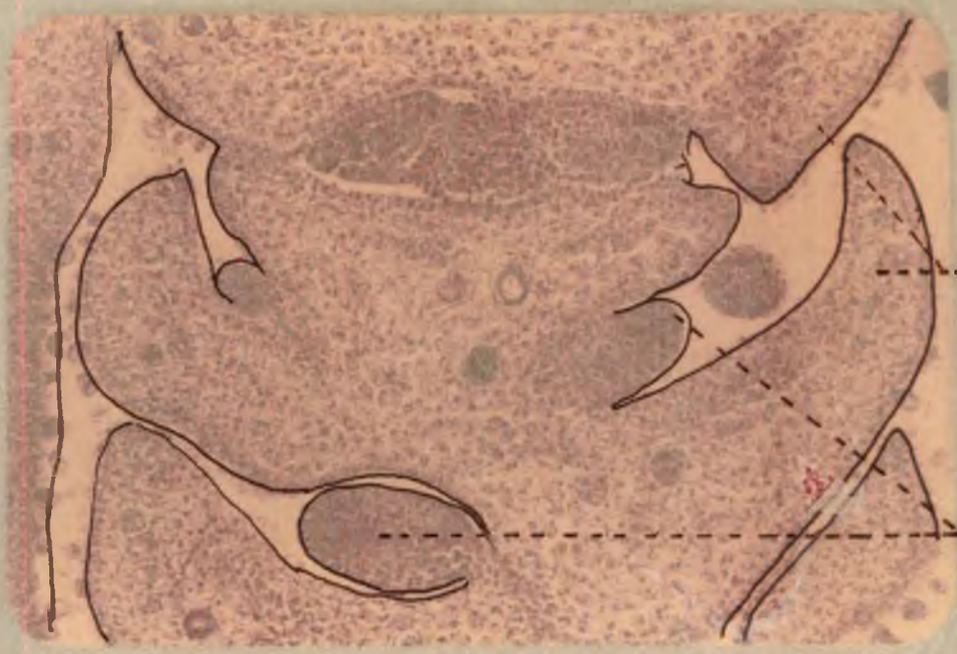
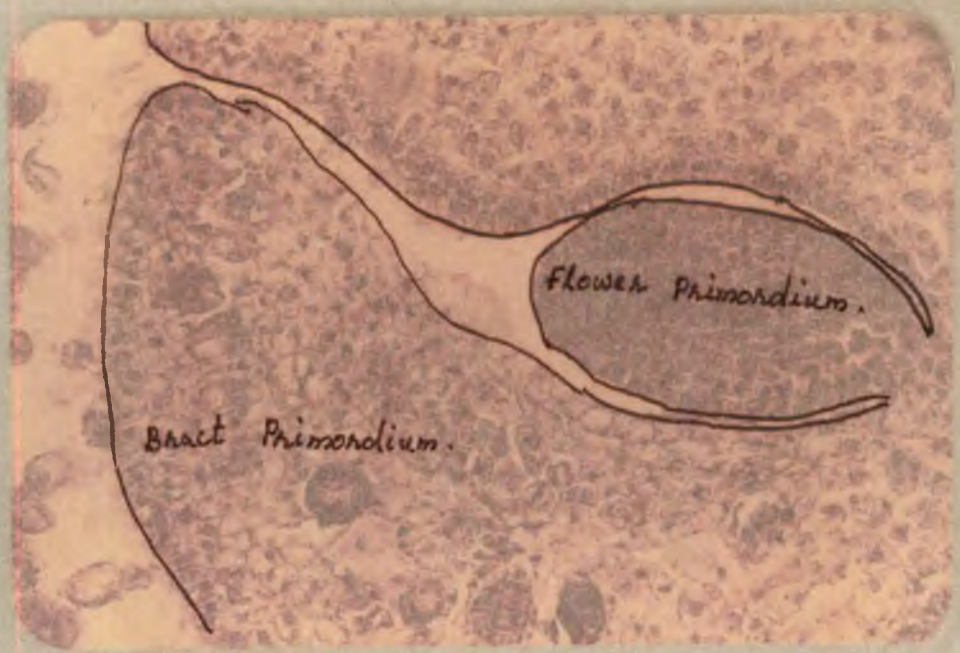


Plate-XIV. A flower primordium in Plate XII magnified - showing the row-wise arrangement of cells. X 100.



the flower primordia show row-wise arrangement of anticlinally dividing cells with nuclei.

Plates XV to XVII

In Plate XV, the ovary wall is seen fully developed, and inside it, the double layered integumentary tissues can be seen to be partially developed. In Plate XVI, the integuments are seen fully developed, and cover the nucellar tissue. Some space is seen in between the integuments and ovary wall. Plate XVII show a matured female flower with papillate stigma.

Plates XVIII and XIX

In hermaphrodite flowers two to four stamens can be observed on the sides of the ovary. In median longitudinal section only two stamens could be seen (Plate XVIII). Plate XIX is the magnification of a mature stamen with pollen grains inside the pollen sac.

4.3.3. Time taken for flower bud differentiation

To obtain information on time taken from the commencement to the completion of the different stages of the differentiation process, buds were extracted daily starting from 4th June, 1984, from a single standard of Panniyur-1.

The details of this analysis have been presented in Table 7. Internal developmental stages were completed

Plate-XV, L.S. of flower primordium (female) showing the ovary wall and developing integuments. X 25.

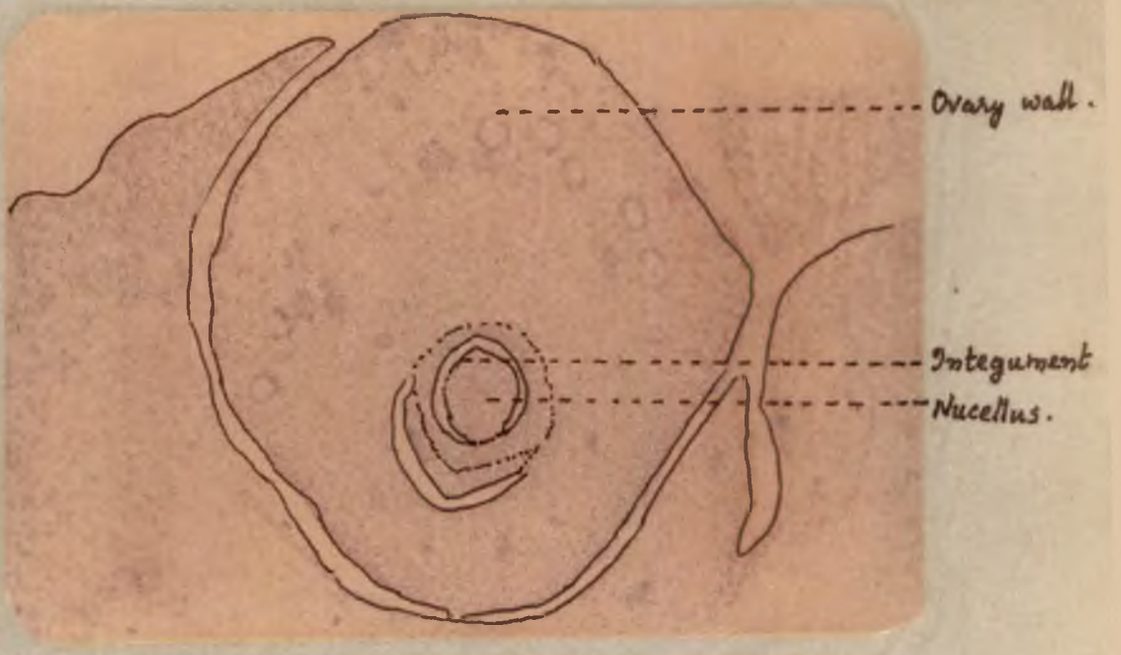


Plate-XVI. L.S. of flower primordium (female) showing ovary wall and fully developed integuments. X 25.

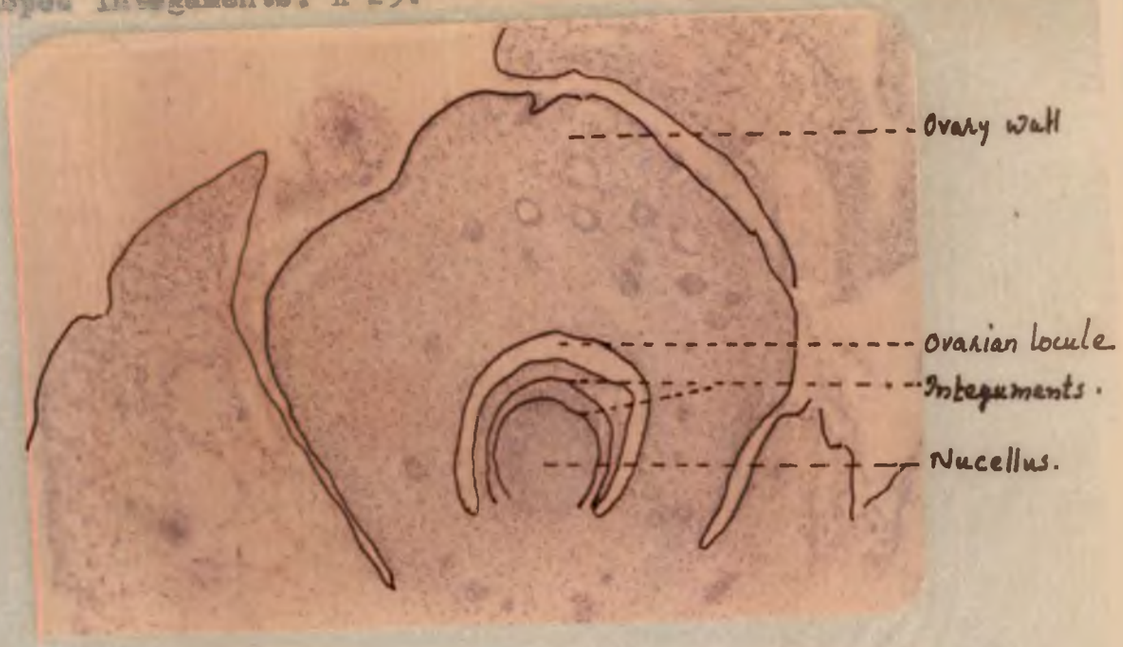


Plate-XVII. L.S. of mature female flower X 12.5

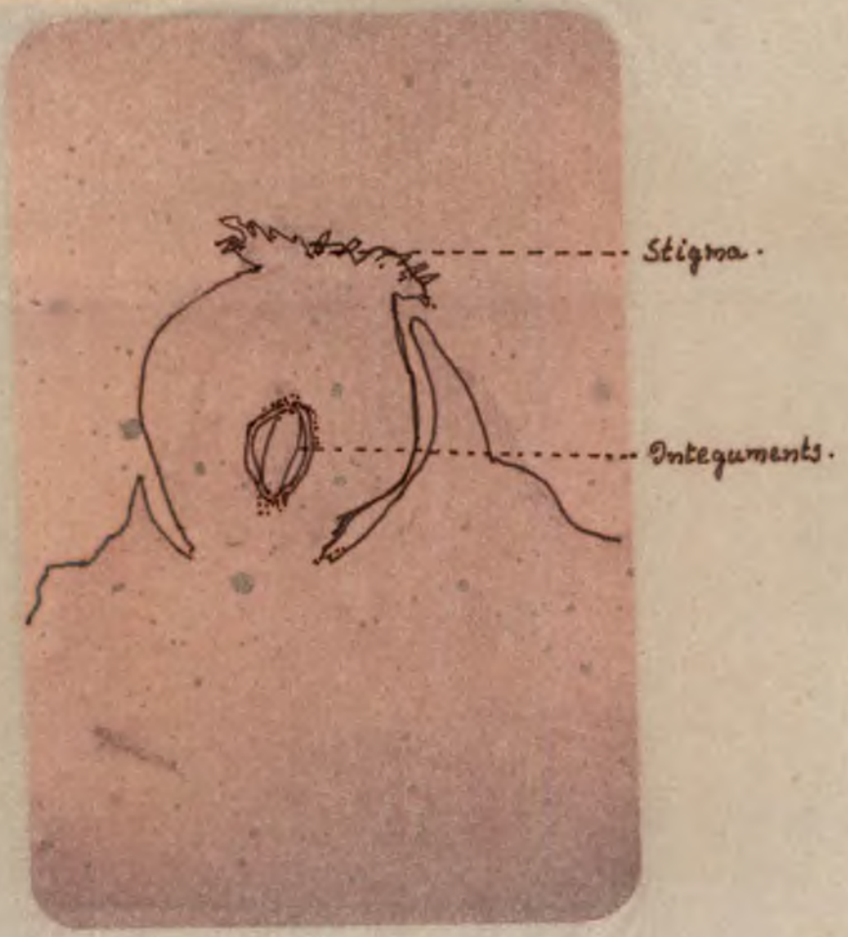
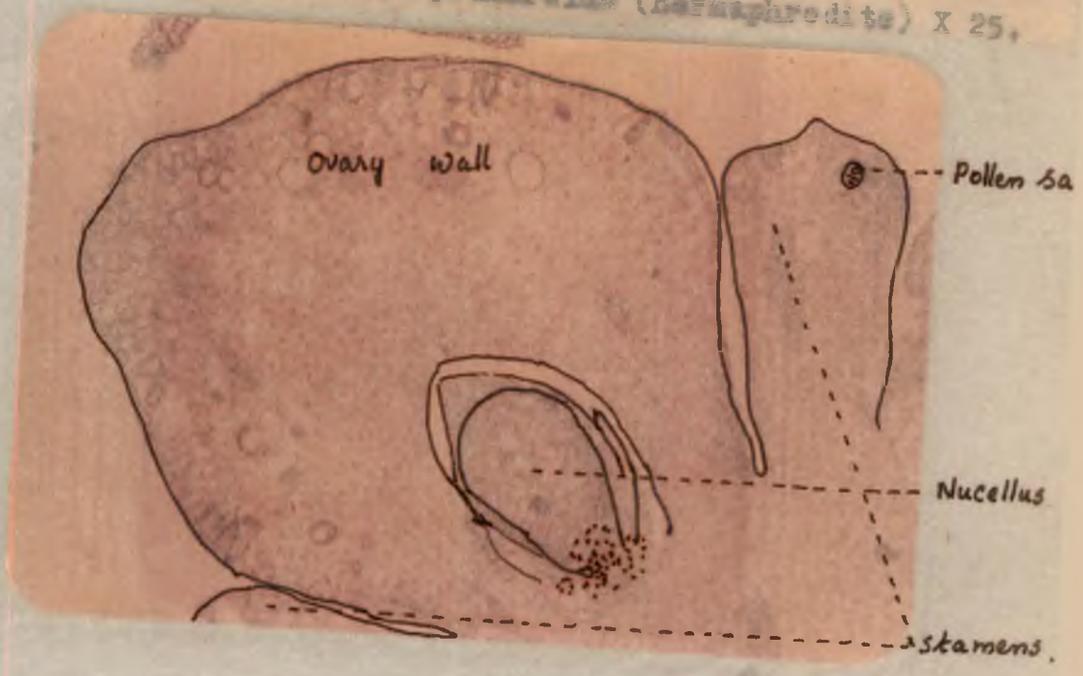


Plate-XVIII, L.S. of flower primordium (hermaphrodite) X 25.




Filament.

Plate-XII. L.S. of stamen showing microspores inside the pollen - sac. X 50.

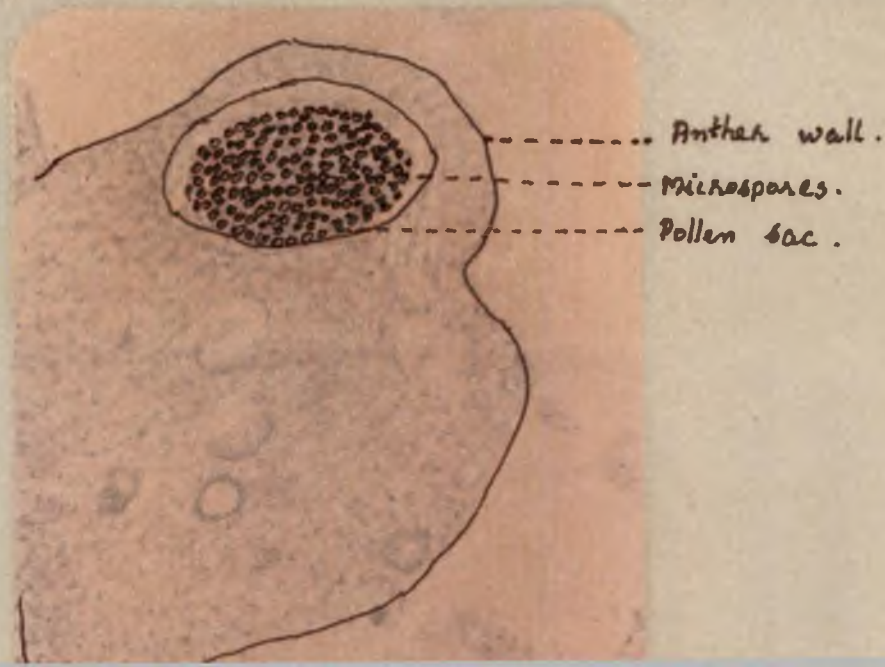


Table 7 - Number of days taken for completion of different stages* (in Panniyur-1)

Developmental stage	Days on which the stage was observed
Spike initiation stage	D_2 - D_6
Development of spike primordium	D_4 - D_{10}
Initiation of bract and flower primordium	D_7 - D_{18}
Initiation of flower primordium	D_{14} - D_{18}
Development of flower primordium	D_{15} - D_{26}

* Based on 15 shoot apices examined each day starting from 4th June, 1984.

within about 20 days after the initiation. After the bud break it took about five days for the complete development of flowers. Thus, the total time for completion of the different stages worked out to about 25 days.

5. DISCUSSION

Flower bud differentiation is an important event in the life of a flowering plant and is dependant on a host of factors. Information on the site and time of differentiation, the stages in the differentiation process and the factors influencing the process will provide invaluable tips for scheduling the package of practices for successful crop production.

Pepper exhibits simultaneous flowering over extended areas within a short period. From one day to the next, all the plants bloom, depending on the climatic pattern of the region (particularly, the rainfall distribution). This sudden blooming could be seasonal or non-seasonal. Alvim (1964) grouped this type of plants under gregarious flowering category. The other well known gregarious flowering plants include coffee (Alvim, 1964) and Dendrobium crumenatum (Coster, 1926). Information on the factors that trigger the process of flower bud differentiation in gregarious flowering plants would help to create/maintain favourable conditions for optimum crop production. A comprehensive analysis of the climatic as well as other factors was, therefore, attempted to ascertain the role of each in triggering flower bud differentiation activity in pepper.

5.1. Factors influencing flower bud differentiation/flowering

5.1.1. Climatic factors

5.1.1.1. Temperature

During the period of observation, the maximum temperature varied from 28.17°C during the first fortnight of August to 37.69°C during the second fortnight of March. The maximum temperature remained relatively high from the middle of February to the end of May, after which it showed a decline due to the South West Monsoon. The minimum temperature also was high during this period (Fig.1, Appendix.I).

The peak differentiation period was June-July in both the varieties studied. The maximum and minimum temperature which remained high during the summer season prior to the onset of the South West Monsoon might have been conducive for a step up in the flower bud differentiation activity, as indicated by the significant positive correlation (Table 4 and 5). A spurt in flower bud differentiation was reported in mango consequent on a drop in the night temperature and increase in humidity (Ravisankar et al., 1979). In apple, Suzuki and Tano (1971) found that the average maximum temperature in mid-April was closely correlated with the bud break. Warm and dry conditions in the preceding season favoured flower bud differentiation in grapes (Perold, 1927),

mango (Singh, 1958), karaunda (Mishra et al., 1968) and in jaman (Mishra and Bajpai, 1973). In gregarious flowering plants like coffee (Went, 1952; Rees, 1964; Alvim, 1973) and dendrobium (Coster, 1926) a drop in temperature following a stress caused by high temperature induced a sudden step up in flower bud differentiation. A similar possibility could be expected in this case also, as the relatively high maximum and minimum temperature from the middle of February to the middle of May was followed by a drop in both maximum and minimum temperatures consequent on the arrival of the South West monsoon.

5.1.1.2. Rainfall, irrigation, humidity and water relations

The weather data presented in Appendix 1 indicated that the total amount of rainfall was negligible from the first fortnight of December to the second fortnight of March. Summer showers were available in April; but again in May, precipitation was negligible. The South West monsoon commenced by the first fortnight of June. Table 4 and 5 show significant positive correlation between flower bud differentiation on the one hand and rainfall during the fortnight prior to flower bud differentiation on the other in both Panniyur-1 and Karimunda. The rainfall two fortnights before the flower bud differentiation exhibited

significant correlation only in the case of Panniyur-1. These findings are in conformity with those of Nalini (1983) who identified rainfall as a critical factor which triggered flower bud differentiation activity in pepper.

It may be seen that the spurt in the differentiation activity in Karimunda was immediate after the receipt of rainfall, compared to that in Panniyur-1. The amount of rainfall required to trigger flower bud differentiation activity seemed less in Karimunda, compared to that in Panniyur-1.

The relative maximum humidity which was above 90.0 per cent during August and September declined gradually to 67.8 per cent by December. There was a slight increase in the relative maximum humidity consequent on the receipt of the pre-monsoon showers in May followed by a sudden significant increase with the onset of South West monsoon in June-July. The relative minimum humidity also exhibited a similar trend. The correlation analysis (Table 4 and 5, Fig.5 in Panniyur-1 revealed that the relative humidity in the fortnight preceding flower bud differentiation exerted positive influence on the process. In Karimunda, increase in the relative humidity during the first andsecond fortnights prior to flower bud differentiation favourably influenced the process. Thus, in both the varieties, the increase in

the relative humidity prior to the differentiation period seemed to have influenced the process favourably. Nalini (1983) reported that the relative humidity was high during the peak differentiation period in Panniyur-1. In the present studies also, similar findings have been obtained.

The experimental standards of Panniyur-1 were under irrigation during the summer months and could not have been under moisture stress. Therefore, it appears that it is not the availability of water in soil; but the internal water potential that influenced the differentiation process. The high temperature and low relative humidity which prevailed during the summer months enhanced transpiration causing a reduction in water potential of the plant. However, subsequent to the arrival of pre-monsoon showers and commencement of South West monsoon by May-June, the internal water content of the plant increased, due to reduced transpiration at low temperature and high relative humidity. The fact that the summer rains were not effective in triggering the differentiation process also supported this argument.

In coffee, a gregarious flowering plant Alvim (1960) found that flowering is primarily dependant on rainfall following a period of stress. He opined that under conditions favouring high transpiration, the moisture stress cannot be

controlled by irrigation, but humidity can control transpiration and thus the internal water potential of the plant.

5.1.1.3. Light

During the period of observations, the mean daily sunshine hours ranged from, 0.95 at the beginning of June to 10.42 by the end of March. (Appendix I). There was a drastic reduction in bright sunshine hours in June and July consequent on the onset of South West monsoon. Sunshine hours, simultaneous to flower bud differentiation was negatively correlated with the process in both the varieties (Table 4 and 5; Fig. 5 and 7) whether or not the decrease in sunshine hours played any direct or independant role in the differentiation process can not be deduced from these data. However, an indirect effect of the cloudy days during the rainy days can be logically assumed. The correlation between sunshine hours during the 4^{th}, 5^{th} and 6^{th} fortnights prior to differentiation and the number of buds differentiated was positive in both the varieties but statistically significant only in Karimunda (during the fifth fortnight). Nearer to the peak flower bud differentiation period, the correlation became negative. This indicates the possibility that the photosynthetic and photomorphogenic process are dependant on light perceived by the leaves and fruitfulness is attributable to sunshine hours and light intensity during the fourth to sixth fortnights prior to flower bud differentiation.

In grapes, Balasubrahmanyam (1971) found that fruitfulness was affected by sunshine hours during the period preceding flower bud differentiation.

5.1.2. Nutritional factors

5.1.2.1. Carbohydrates, nitrogen and C/N ratio

Data on the content of carbohydrates and, nitrogen in shoot as well as C/N ratio are presented in Table 1. The trends of accumulation of carbohydrates and nitrogen were erratic. The carbohydrate content showed an increase from the second fortnight ofMay andthe high level persisted till the end of the observation period in the second fortnight of July. Nitrogen also showed gradual increase from the second fortnight of May onwards. Correlation co-efficients between the carbohydrate content during the pre-differentiation period (upto lag 6) and flower bud differentiation failed to show significance at any period. Nitrogen showed positive correlation during the time of differentiation (lag 0) and also during the preceding fortnight (lag 7). C/N ratio exhibited alternate peaks and falls (Table 1, Fig. 3). But prior to flower bud differentiation, (in the second fortnight ofMay and in the first fortnight of June) the C/N ratio exhibited peak values which declined during the succeeding fortnights. The C/N ratio was not found to be correlated with flower bud differentiation at any period. In pepper, Nalini (1983) also reported that

the soluble carbohydrates, nitrogen and C/N ratio of the laterals and new shoots varied considerably during the growth cycle. In her studies the 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. Peak accumulation of carbohydrates has been reported in mango (Naik and Shaw, 1937; Singh, 1960; Sen et al, 1969), grapes (Chadha and Cheema, 1971) and coconut (Bai and Ramadasan, 1982) prior to or at flower bud differentiation stage. The reports also indicate that the C/N ratio is lowered after the process. Nitrogen content also was reported to be high at flower bud differentiation/flowering in apple (Archbold, 1928) sweet orange (Milella, 1968) and mango (Chacko, 1968). In grapes higher levels of nitrogen decreased differentiation causing barrenness (Baldwin, 1966; Bindra and Chohan, 1974). High C/N ratio favoured flower bud differentiation in apple (Chandler, 1925) grapes (Thomas and Bernad, 1937; Rao and Sathyanarayana 1978) and mango (Naik and Shaw, 1937; Singh 1960, Sen et al., 1963). However, Winkler et al., (1962) and Chitkara et al. (1972) in 'Anab-e-shahi' and Khajuria et al. (1970) in 'Gulabi' varieties of grapes could not get any significant correlation between C/N ratio and flowering.

5.1.2.2. Phosphorus and potassium

Data presented in Table 2 indicate that during the period of study phosphorus content varied from 0.102 per cent in the first fortnight of February to 0.143 per cent in the second fortnight of September. Potassium content showed variation from 1.871 per cent in the second fortnight of February to 2.921 per cent in the first fortnight of August, The pattern shows that the variation in the content of these elements during the period are negligible especially in the case of phosphorus. Their contents were not seen correlated with flower bud differentiation at any period (Table 4). There was no defenite pattern of accumulation of these elements during the period of study, which is contradictory to the reports of Avilan (1971) and Pathak and Pandey (1978) in mango and Rodriguez, (1967) in guava, which show that before or at differentiation there was peak accumulation of phosphorus and potassium which declined after flowering. At full bloom stage phosphorus and potassium in the leaves of non-fruiting branches were high compared to that in fruiting branches of oranges (Harding et al., 1962) and mandarins (Aiyappa et al., 1965). The vines in the present studies were well managed and manured (as per the package of practices recommendations of the Kerala Agricultural University), and no deficiency of these elements was seen at any period.

A separate trial seems necessary to study the effect of these elements on flower bud differentiation. The experimental vines are to be applied with graded doses of elements and their roles have to be assessed.

5.1.3. Hormonal factors

5.1.3.1. Auxin

During the period of study, the endogenous level of auxin like substances varied from 0.01 - 0.02 µg/g of fresh weight of plant samples (expressed as indole acetic acid). In the paper chromatography of purified pepper shoot extracts auxin like substances were not consistent at a particular Rf position. The authentic indole acetic acid sample were separable at Rf 0.2 - 0.4 and the data given in Table 3 were estimated from this portion. It is to be admitted that this may not reflect the real status of auxin activity and for the purpose, studies employing sophisticated instrumental methods are necessary. Paper chromatography has failed to show consistent auxin type activity at a particular position in a number of occasions. Ramsay and Martin (1970) could not get any auxin type activity consistently at any fraction of chromatogram in peach leaf extract. In mango shoot extract Chacko et al.,(1972) found growth promotor activity at two regions, Rf 0.4 - 0.6 and 0.8 - 1.0. He found that

the activity at Rf 0.8 - 1.0 was not consistent. Hence the activity at other region only was utilized for estimation of auxins.

5.1.3.2. Inhibitors

Data presented in Table 3 show that during the period of study the level of inhibitors varied from 0.11 - 0.28 µg/g of fresh plant sample (expressed as abscissic acid). The inhibitor content gradually increased from the start of the observations in the first fortnight of August and reached the maximum level during the first fortnight of January. Thereafter, it decreased and the low level was reached by the end of observation in the second fortnight of July. In the correlation analyses (Table 5, Fig 6) the inhibitors showed significant negative correlation with differentiation during the corresponding fortnight (lag 0) and also during the period upto the third fortnight prior to differentiation (upto lag 3). This indicate that the low level of inhibitors during pre-differentiation period stimulated this. Inhibitors have been reported as one of the factors which control bud dormancy, differentiation and flowering in a number of crop plants like peach (Blommaert, 1955), coffee (Alvim, 1960 and 1973), apricot

(Ramsay and Martin, 1970) and grapes (Iwasaki and Weaver, 1977).

A critical examination of the data reveal temperature relative humidity, rainfall and inhibitors as possible factors influencing flower bud differentiation in pepper. The increase in maximum and minimum temperature by the end of January during a period when rainfall was scanty, seems to have caused a decline in the relative humidity of atmosphere. During this period, bright sunshine hours were also maximum. These environmental conditions which persisted till the onset of the South West Monsoon in the beginning of June, therefore, favoured high rate of transpiration reducing the internal water potential of the plant. The inhibitor content recorded an inverse relationship with these factors. Neither the moisture received from the scanty rainfall during the period (summer showers) nor that supplied through irrigation could maintain the internal water potential of the plant at a sufficiently high level. The low water potential of the plants could have reduced the translocation of the inhibitors from the leaves to the bud. Thus, the inhibitor level in the buds during the hot summer preceding the period of peak differentiation was found to be low. The low inhibitor level could have favourably influenced the

flower bud differentiation. Supporting evidences are available from the investigations on physiology of flowering in another gregarious flowering plant, coffee. Alvim (1960) found that flowering in coffee is primarily dependant on rainfall distribution following a period of stress. He stated that under conditions favouring high transpiration rates, the moisture stress cannot be controlled by irrigation. He observed that water stress reduced the translocation of a growth inhibitor from the leaves to the buds and was responsible for bud dormancy. Van der veen (1968) and Browning (1971) also made similar observations in coffee. According to them, the increase in water potential due to frequent irrigation, low transpiration rates etc. kept the flower buds dormant due to high concentration of abscissic acid as translocation of it was enhanced from the leaves to the buds at higher water potential. In another report Alvim, (1973)concluded that moisture stress either by low moisture status in the soil or by high transpiration rate, reduced the rate of translocation and lowered the inhibitor concentration in the flower buds, terminating the bud dormancy.

Further studies are required to determine whether or not a dormancy system as suggested in coffee (Van der Veen, 1968; Browning, 1971; Alvim, 1973) exists in pepper

also. There are grounds to believe that the buds in which the differentiation process has been triggered (due to favourable temperature, sun-shine hours, reduced relative humidity and low level of inhibitors) remain dormant or quiescent till the arrival of the monsoon showers.

5.2. Histological studies

5.2.1. Rate of flower bud differentiation

The data on the rate of flower bud differentiation in Panniyur-1 and Karimunda are presented in Table 7 and Fig.8

In Panniyur-1, the second half of June and the month of July recorded maximum percentage of flower bud differentiation while in Karimunda, the maximum percentage of differentiation occurred during the month of June and the first fortnight of July. Thus, Karimunda seems to be two weeks earlier than Panniyur-1. Gibbs and Swarbrick (1930) stated that the time of flower bud differentiation showed variation among the varieties of a number of crop plants. Bindra (1981) studied the time of flower bud differentiation in different varieties of grapes and found that flower bud differentiation period was 3rd April in 'Perlette', 11th April in 'Beauty seedless', 18th April in 'Banquabad' and 9th May in 'Anab-e-shahi'. Varietal influence has been shown as an important factor causing variation in the time of fruit bud differentiation in citrus (Fujitha and Yagi, 1956; Babu and Kaul, 1972), mango (Singh, 1958) and strawberry (Pathak and Singh, 1977) also.

The data (Table.6, Fig 8) also indicate that prior to the peak period (middle of June to end of July), differentiation activity was negligible in Panniyur-1. But Karimunda exhibited fair amounts of differentiation prior to the peak differentiation period (beginning of June to middle of July). The differentiation activity thus seems more spread out in Karimunda, while it is mostly confined to June-July and early August in Panniyur-1. The response of Karimunda to smaller quantities of rainfall might be cited as the reason for this spread of flowering. Flowering prior to the outbreak of monsoon may result in wastage of the potentiality of the plants and cause reduction in yield, as pollination andberry set in pepper, which depend on rain would be affected. In the correlation analysis in Panniyur-1, the rainfall during the first and second fortnights prior to differentiation was significantly correlated with the process. In Karimunda, the rainfall during the first fortnight prior to differentiation only recorded significant correlation. This shows that after the receipt of sufficient quantity of rainfall, less time is taken by Karimunda compared to Panniyur-1 to reach the peak differentiation activity. The earliness, and the protracted nature in the flowering habit of

Karimunda further reinforces this point.

5.2.2. Histology of flower bud differentiation

Normally each node of a pepper vine has a leaf an axillary bud and four to six adventitious roots. At flowering the spikes are produced opposited to the leaves.

5.2.2.1. The vegetative meristem

The vegetative apex was found to be more or less conical and surrounded by leaf primordia that had initiated from it (Plates I to IV). No fundamental differences were observed in respect of the shape or the tissue organization, between the two varieties studied. The conical nature of the vegetative primordium has been reported in pepper (Nalini, 1983) as well as in other crop plants like mango (Singh, 1960), jaman (Mishra and Bajpai, 1973), grapes (Chadha and Cheema, 1971) and strawberry (Pathak and Singh, 1977)

The size and shape of the apex varied considerably during a single plastochrone of leaf initiation, from broadly conical just prior to initiation (Plate I and II) to sharply conical immediately afterwards (Plate III and IV). It can be presumed that leaf initiation would continue from the apex, as long as it remained in vegetative stage. The elongation of the axis reported by Nalini (1983) was not observed in the present studies. The non-median or

oblique nature of the sections might have led Nalini (1983) to postulate that the axis of the vegetative buds elongated after the initiation and to denote that as the second stage in the development of vegetative buds. In mango, Singh (1960) could not observe any distinct stage in the development of vegetative buds. In cauliflower, the vegetative apex remained conical and continued to differentiate leaves as long as it was not cold treated (Sadik, 1962).

The vegetative apex in pepper followed the tunica-corpus concept of tissue arrangement. The tunica was found to be bi-layered in pepper shoot apex (Plate II). Schimidt (1924) who postulated the tunica-corpus theory divided the apex into two regions. According to him, the outer tunica layer, in which the plane of division was principally anticlinal, covers the inner corpus in which there was no distinct plane of division.

5.2.2.2. The transition

In plates V and VI, the vegetative apex appears flattened and transformed into a convex hemispherical structure with a mantle-core configuration. Broader expanse of the meristematic tissue together with flattening of the apex have been reported as the histological features of the apical meristem on transition (Janik, 1972). Broadening and flattening of the apical meristem on transition have been

observed in citrus (Abbot, 1935; Randhawa and Disna, 1947; Mishra and Yamdagni, 1968); karaunda (Mishra, et al. 1968), grapes (Chadha and Cheema, 1971), jaman (Mishra and Bajpai, 1973), coffee (Alvim, 1973) and litchi (Shukla and Bajpai 1974). The outer layers 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. This indicates that the mantle region was in actively dividing stage (Plate V, VI). Sometimes a few periclinal divisions occur in tunica, which is a contradiction of the original definition (Schimidt, 1924). Later Popham and Chan (1950) introduced the term mantle for all the outer layers of the apex which can be distinguished histologically from the inner cell mass, core without taking into account the plane of divisions in these layers. The tunica layer which is distinct during the vegetative stage is not distinguishable at the transition stage due to the active division of cells. On transition, the actively dividing cells were deeply stained.

5.2.2.3. The flower bud meristem

Spike initiation

The meristem which was conspicuously hemispherical at the transition stage (Plates V and VI) further developed

as a dome shaped structure (Plate VII). The spike primordium is seen initiated. The primordia of the leaf adjacent to the spike, the bract at the base of the spike and the older leaves can also be seen in Plate VII. Nalini (1983) reported appearance of two conical primordia surrounded by a leaf sheath as the indication of the commencement of flower bud initiation. According to her, one of the primordia broadened, elongated and developed into the spike primordium while the other remained as the axillary vegetative bud primordium. But the present studies revealed that two undifferentiated conical primordia may not be visible in all the cases where differentiation had taken place. An advancement in the differentiation of the spike primordium is likely to leave the axillary bud primordium as rudimentary. A difference in the cutting plane of the sections can be another reason for not being able to observe two primordia at the commencement of the differentiation.

Spike development

The pattern of further development of the differentiated floral primordium takes different courses, determined mainly by the type and nature of the inflorescence produced. In plants producing branched inflorescence,

the primordia after differentiation produce numerous growing points which later develop into inflorescence branches, as in the case of grapes (Chadha and Cheema, 1971), coffee (Alvim, 1973) and mango (Ravisankar et al., 1979). However, in pepper, branching of the primordium was not observed. The dome shaped structure developed into a cylindrical, well developed spike primordium (Plates VIII to X).

Bract and flower initiation

During the next stage, primordial bracts and flowers are seen initiated on the sides of the cylindrical spike primordium in acropetal succession (Plate XII-XIV). In the periphery of the developing spike, meristematic zones with deeper stainability can be observed in acropetal spiral order. These loci emerge out as protuberances, denoting the sectors of bract initiation. In each sector, the tunica divided by anticlinal divisions keeping pace with the multiplication of corpus cells. From the axis of bract primordia, floral bud meristems differentiate. These findings are in agreement with those of Nalini (1983).

Flower development

In the floral primordia that were to develop as female/hermaphrodite flowers, the ovary wall developed

from the peripheral area. Inside this, differentiation of the two integuments enclosing the nucellus takes place (Plate XV-XVI). Some space seen left out in between the ovary wall and the integuments is the ovarian locule. Simultaneous to ovary wall differentiation, ovule differentiation also takes place. Joshi (1944) described the ovary of Piper longum as having a single orthotropus ovule with two integuments.

In male/hermaphrodite flowers, the flower primordial development led to two to four short stamens. All the stamens are not visible in the median longitudinal sections (Plates XVIII and XIX). Pollen grains are visible inside the pollen sac in Plate XIX. Mathew (1958) found that microsporogenesis in Piper nigrum took place when spikes were about three to five centimetres long. He also observed a four layered anther wall inside which the microsporangia divided to produce the microspores.

The above observations indicate that it is the terminal vegetative apex that gets differentiated into the inflorescence in pepper. An axillary vegetative bud continues the growth symbodially. The axillary bud was visible clearly during the later stage of the development (Plate XII).

With regard to the histological features and the stages of development, no differences were observed between the two varieties of pepper, studied Panniyur-1 and Karimunda.

5.2.3. Time taken for flower bud differentiation

The data presented in Table 7 indicate that the total time taken for the differentiation of the flower buds in pepper is about 25 days. This duration is reckoned from the commencement of spike initiation to the complete development of the gametophytes. The appearance of hemispherical pre-differentiated apex (transition stage) was not considered as the commencement of the differentiation process, as the degree of differentiation was difficult to ascertain at this stage. Nalini (1983) observed that the process of flower bud differentiation was completed within about 20 days of its commencement. The appearance of two undifferentiated conical primordia was reckoned by Nalini as the first stage. It is clearly seen in the present studies that this is a little advanced stage. Further, Nalini considered the appearance of the differentiated floral buds with embryo sac and stamens as the termination of the differentiation process. The present studies indicated that the differentiation of the integuments, ovules, pollen grains, etc. follow the differentiation of the embryosac and

stamens. Thus the difference of five days observed between the two studies does not seem at discrepancy.

5.2.4. Microtechnique

In the present studies, certain modifications were tried for dehydration and paraffin embedding of the specimens. Isopropyl alcohol, was found to be useful exactly in the same manner as the ethyl alcohol for dehydration purposes in the tertiary butyl alcohol (TBA) series. Sass (1951) had reported that isopropyl alcohol may be used for dehydration of certain plant tissues. He also suggested that the texture and cutting property of soft paraffin could be modified by the addition of other materials like rubber, bee's wax or ceresin. In the present studies, "paraffin with ceresin" was found to be superior to the other embedding media, in terms of cutting property and adherence between the sections to form the ribbons.

Haidenhan's Azan is a very promising triple stain for animal tissues, as reported by Mallory (1961). The stain components were, azocarmin, Orange G. and anilin blue. Reports on the use of Haidehhan's Azan in plant

tissue are not available. The present studies revealed that the stain is well adapted to plant tissues also. Contrasting colours were exhibited by the cell wall, the cytoplasm and the nucleus. Carmine gave bright red colour to the nuclei, while Orange G. acted as a cytoplasmic conter-stain.Anilin blue stained the cell walls blue.

6. SUMMARY

6.1. Studies were undertaken at the College of Horticulture, Vellanikkara during 1983-'84 in two black pepper varieties, Panniyur-1 and Karimunda, to collect information on the factors influencing flower bud differentiation/flowering and on the histological aspects of the process.

6.2. The maximum and minimum temperatures in the preceding summer and the subsequent monsoon showers, played important roles, in triggering the flower bud differentiation activity. These factors exhibited significant positive correlation with the process. Maximum and minimun humidity showed positive correlation with process at the time of differentiation, while sunshine hours recorded negative correlation.

6.3. The carbohydrate content and the C/N ratio failed to show statistically significant correlation with flower bud differentiation. However, carbohydrate accumulation and C/N ratio build up were observed prior to the peak differentiation period. Nitrogen content showed significant positive correlation with the process at the time of flower bud differentiation andalso during the preceding fortnights. Phosphorus and potassium content did not show much variation during the course of studies and their correlation with flower bud differentiation also were not significant.

6.4. Auxins were found to be not consistent at any Rf position of the chromatograms. Inhibitor content

recorded a decline during the summer months and the low level persisted throughout the period of peak differentiation activity (June-July) Inhibitors were consistent at Rf 0.5 - 0.7.

6.5. The low water potential in the plants during the summer months (caused by high atmospheric temperature, scanty rainfall, low relative humidity and high transpiration) appears to have reduced thetranslocation of inhibitors from the leaves to the buds, reducing its content in the latter. The moisture supplied by the scanty rainfall during the summer months of supplemented by irrigation was not effective in raising the water potential of the plant, under conditions favouring high transpiration.

6.6. Specimens for microtomy could be stored in FAA without damage. Haidenhan's Azan was found to give acceptable staining of the (pepper) plant specimens.

6.7. Maximum flower bud differentiation was observed from the middle of June to the end of July in Panniyur-1. The peak appeared a little advanced in Karimunda, from the beginning of June to the middle of July. Compared to Panniyur-1, Karimunda showed fair amounts of flower bud differentiation during some ofthe other months also.

6.8. Basic differences were not observed in respect of the histological features of differentiation, between the two varieties under study, Panniyur-1 and Karimunda.

6.9. The vegetative bud appeared conical and surrounded by leaf primordia. The leaves continued to be initiated from it during vegetative phase.

6.10. The change over of the shape of the meristem from conical to hemispherical indicated the transition from vegetative to floral phase. The apex at this stages exhibited high meristematic activity.

6.11. After transition the hemispherical structure enlarged into a dome shaped one, thus initiating the spike. The primordia of the bract (spike bract) and the subtending leaf also were visible at the spike initiation stage. The dome shaped structure developed into the cylindrical spike primordium. On the periphery of it, floral primordia developed as finger like projections in the axils of pointed bract primordia (flower bract). The above stages were seen completed before visual emergence of the spike. The male and female gametophytes developed after the emergenc of the spike. At maturity the ovules were seen covered by a two-layered integument and an ovary wall. In hemaphrodite flowers, stamens developed at the sides of the ovary. The process of differentiation was seen completed within about 25 days of initiation.

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* Originals not seen.

Appendix

Appendix.I. Weather data* for the period starting from 1st August, 1983 to 31st July, 1984.

Fortnight	Maximum temperature C	Minimum temperature C	Rain fall mm	Sunshine hours hrs.	Relative maximum humidity %	Relative minimum humidity %
August I	28.17	23.46	494.0	1.14	93.5	80.9
August II	29.45	24.11	259.9	2.33	93.7	78.9
September I	30.60	23.94	154.1	3.90	93.9	74.1
September II	30.09	23.90	345.0	3.30	92.6	76.9
October I	30.76	21.54	62.7	7.74	91.0	63.7
October II	31.32	26.43	87.1	6.22	88.3	64.3
November I	32.02	21.96	43.4	7.78	84.3	58.3
November II	32.57	22.60	16.8	8.06	83.0	57.8
December I	32.19	24.10	00.0	9.10	67.8	47.9
December II	30.73	25.23	24.4	4.95	79.6	61.4
January I	31.98	23.63	00.0	8.05	70.7	49.3
January II	33.38	22.91	00.0	7.91	73.1	41.1
February I	33.94	24.42	4.8	7.28	69.1	42.2
February II	35.90	25.02	22.2	9.52	69.9	41.1
March I	33.42	24.82	00.0	4.72	82.0	54.7
March II	37.67	23.68	00.0	10.42	84.1	46.4
April I	35.21	24.29	147.6	7.30	79.7	56.4
April II	34.50	24.90	84.3	7.51	88.1	60.5
May I	34.32	26.27	29.0	9.08	85.1	53.7
May II	34.50	25.80	37.7	6.40	86.1	62.7
June I	29.62	22.98	433.0	0.95	92.7	80.1
June II	28.40	22.44	419.8	1.86	93.3	80.6
July I	28.49	22.99	417.6	3.39	91.3	79.3
July II	28.75	22.83	311.8	2.78	94.5	76.5

* Source, B class meteorological observatory, Vellanikkara.

FLOWER BUD DIFFERENTIATION IN

Piper sp.

By
RAJAN P. S.

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of
the requirement for the degree of

MASTER OF SCIENCE IN HORTICULTURE

Faculty of Agriculture
Kerala Agricultural University

Department of Horticulture (Plantation Crops & Spices)
COLLEGE OF HORTICULTURE
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1985.

ABSTRACT

Studies were undertaken at the College of Horticulture, Vellanikkara during 1983-'84 in Panniyur-1 and Karimunda varieties of pepper to collect information on the factors influencing flower bud differentiation and on the histological aspects of the process.

Among the climatic factors maximum andminimum temperature during the preceding summer as well as the monsoon showers exhibited significant positive correlation with flower bud differentiation process.

Among the nutritional factors studied, carbohydrates and C/N ratio were found to be high prior to the peak differentiation period. However, these failed to show significant statistical correlation. Significant accumulation of nitrogen was found in the shoots prior to differentiation. Phosphorus and potassium contents were not correlated with the process.

Inhibitor content of the shoots prior to differentiation was found to negatively influence the process.

Peak period of differentiation was observed from the middle of June to the end of July in Panniyur-1, while it was a little advanced in Karimunda (from the beginning of June to the middle of July). There were no fundamental differences in the histological aspects of flower bud differentiation between the two varieties.

The bud which was conical during the vegetative phase changed into a dome shaped structure during the transition. Afterwards, this grew into a cylindrical structure on the sides of which bract and flower primordia developed in acropetal succession. Differentiation and development of ovary wall, integuments, ovules, pollen sacs, pollen grains etc. followed. The whole process of differentiation was completed within about 25 days of its commencement.