INFLUENCE OF MICRO METEOROLOGICAL FACTORS ON FLOWERING IN VANILLA
(*Vanilla planifolia* Andrews)

By

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THESIS

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DEPARTMENT OF PLANTATION CROPS AND SPICES

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2008

DECLARATION

I hereby declare that the thesis entitled "**Influence of micro meteorological factors on flowering in vanilla (*Vanilla planifolia* Andrews)**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Dedicated to My
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TABLE OF CONTENTS

CHAPTER	TITLE	PAGE NO.
1	INTRODUCTION	1-3
2	REVIEW OF LITERATURE	4-27
3	MATERIALS AND METHODS	28-37
4	RESULTS	38-87
5	DISCUSSION	88-101
6	SUMMARY	102-103
	REFERENCES	i-xv
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Effect of moisture stress on soil moisture content at root zone depth (0-15cm) in vanilla	38
1 (a)	Plantation Crops and Spices	38
1 (b)	Farmer’s field	39
2	Effect of moisture stress on leaf colour in vanilla (*Vanilla planifolia* Andrews)	41
3	Effect of moisture stress on wilting symptoms on leaves and vines in vanilla (*Vanilla planifolia* Andrews)	44
4	Effect of moisture stress on leaf thickness in vanilla (*Vanilla planifolia* Andrews)	45
5	Effect of moisture stress on relative leaf water content in vanilla (*Vanilla planifolia* Andrews)	46
5 (a)	Plantation Crops and Spices	46
5 (b)	Farmer’s field	46
6	Effect of moisture stress on membrane stability in vanilla (*Vanilla planifolia* Andrews)	49
6 (a)	Plantation Crops and Spices farm	49
6 (b)	Farmer’s field	49
7	Effect of moisture stress on total chlorophyll content in vanilla (*Vanilla planifolia* Andrews)	51
7 (a)	Plantation Crops and Spices farm	51
7 (b)	Farmer’s field	52
8	Effect of moisture stress on epicuticular wax content in vanilla (*Vanilla planifolia* Andrews)	54
8 (a)	Plantation Crops and Spices farm	54
8 (b)	Farmer’s field	55
9	Effect of moisture stress on soluble protein content in vanilla (*Vanilla planifolia* Andrews)	57
9 (a)	Plantation Crops and Spices farm	57
9 (b)	Farmer’s field	57

10	Effect of moisture stress on total free amino acids in vanilla (*Vanilla planifolia* Andrews)	60
10 (a)	Plantation Crops and Spices	60
10 (b)	Farmer's field	60
11	Effect of moisture stress on proline content in vanilla (*Vanilla planifolia* Andrews)	62
10 (a)	Plantation Crops and Spices	62
10 (b)	Farmer's field	63
12	Effect of moisture stress on peroxidase activity in vanilla (*Vanilla planifolia* Andrews)	65
12 (a)	Plantation Crops and Spices	65
12 (b	Farmer's field	65
13	Effect of moisture stress on total sugar content in vanilla (*Vanilla planifolia* Andrews)	67
13 (a)	Plantation Crops and Spices	67
13 (b	Farmer's field	69
14	Effect of moisture stress on potassium concentration in leaf tissues in vanilla (*Vanilla planifolia* Andrews)	70
14 (a)	Plantation Crops and Spices	
14 (b	Farmer's field	
15	Effect of moisture stress on flowering in vanilla (*Vanilla planifolia* Andrews)	72
16	Temperature and relative humidity in selected locations	77
17	Mean light intensity (lux) in vanilla gardens (July 2006 to July 2007)	84
18	Flower bud initiation and flowering in vanilla	84
19	Correlation coefficients of flowering in vanilla with temperature and relative humidity	86

LIST OF FIGURES

Figure No.	Title	Page No.
1	Total soil moisture content as influenced by soil moisture stress	40
2a	Relative leaf water content as influenced by soil moisture stress	47
2b	Membrane stability as influenced by soil moisture stress	50
3a	Total chlorophyll content as influenced by soil moisture stress	53
3b	Epicuticular wax content as influenced by soil moisture stress	56
3c	Soluble protein content as influenced by soil moisture stress	58
3d	Total free amino acids as influenced by soil moisture stress	61
3e	Proline content as influenced by soil moisture stress	64
3f	Peroxidase activity as influenced by soil moisture stress	66
3g	Total sugar content as influenced by soil moisture stress	68
3h	Potassium concentration in leaf tissues as influenced by soil moisture stress	71
4a	Trends in maximum temperature in vanilla garden at two locations	78
4b	Trends in minimum temperature in vanilla garden at two locations	78
4c	Trends in mean temperature in vanilla garden at two locations	79

4d	Trends in relative humidity in vanilla garden at two locations	79
4e	Diurnal variation in temperature at farmer’s field	80
4f	Diurnal variation in temperature at PC & S farm	80
5a	Light intensity from different directions in farmer’s field	82
5b	Light intensity from different directions in PC & S farm	82
5c	Light infiltration percentage in farmer’s field	83
5d	Light infiltration percentage in PC & S farm	83
6	Pattern of flowering in vanilla (*Vanilla planifolia* Andrews)	85
7	Flowering vs. temperature and relative humidity in farmer’s field	87
8	Light intensity vs. flowering in farmer’s field	87

LIST OF PLATES

Plate No.	Title	Page No.
1	Experimental set up Selected plots for investigations Plantation Crops and Spices farm Farmer’s field Stevenson screens installed at experimental fields Plantation Crops and Spices farm Farmer’s field Taking soil samples for moisture evaluation Recording observations on light intensity	29
2	Wilting symptoms on leaves 1 month stress vs. control 1½ month stress vs. control 2 months stress vs. control 2½ months stress vs. control	42
3	Wilting symptoms on leaves and vines 1 month stress vs. control 1½ month stress vs. control 2 months stress vs. control 2½ months stress vs. control	43
4	Various stages of flowering in vanilla Vegetative bud Floral bud Flower bud emergence Inflorescence emergence Flowering	74

Introduction

1. INTRODUCTION

Vanilla (*Vanilla planifolia* Andrews), the tropical orchid spice is cultivated for its pleasant flavour. Originated in the humid tropical rain forest in Mexico and parts of Central America, the crop occupies a major share of cropped area in Madagascar, Indonesia, Mexico, Comoros and Reunion. Nearly 90 per cent of world production comes from Madagascar, Indonesia and Mexico. Globally, the crop occupies an area of 76,998 ha with a production of 5738 t.

Vanilla was introduced to India by the East India Company during eighteenth century but commercial cultivation of the crop started only from 1990 onwards by the initiatives of Spices Board, Government of India. Preliminary research on the crop was undertaken at Kallar Burliar Fruit Research Station, Nilgiris and Horticultural Research Station, Ambalavayal under Kerala Agricultural University in early 1960's. The earlier research efforts in the crop helped in standardization of the techniques of propagation, hand pollination, crop management and processing aspects.

The crop is now commercially cultivated in Karnataka, Kerala and Tamil Nadu, occupying an area of 6936 ha with a production of 259 t of cured beans (DASD, 2008). India exported 200 t of vanilla during 2007-08 with an export earning of Rs.1, 775 lakhs. The export of organic vanilla has helped India to earn better prices in major markets of Indian vanilla i.e., USA, Germany and France.

Vanilla grows better in areas with an annual rainfall of 150-300 cm and a temperature range of 25-32°C. Areas which receive rain for 8-9 months and which have a dry climate in the remaining 3-4 months are ideal for growing vanilla. It requires warm humid conditions for proper growth and productivity. Vanilla could be cultivated from sea level up to an altitude of 1500 meters above MSL.

Vanilla vines being very tender, could not withstand heavy winds. Hence areas, where heavy winds are anticipated, suitable wind breaks should be grown around the garden. Vanilla does not grow well under intense light or heavy shade. It grows well under trees which provide filtered light of about 25 to 50 per cent. In southern parts of peninsular India, places like Wayanad and Idukki districts of Kerala, Coorg, North and South Kanara districts of Karnataka are best suited for cultivation of vanilla (Spices Board, 2003).

Due to high price prevailed for the crop during 2000 to 2003, large scale expansion of the crop took place in almost all the districts of Kerala. Extensive cultivation and adoption of many non-scientific practices to get an early crop led to many production constraints in the crop. In new areas, where the cultivation of the crop is expanded, the ideal climatic requirements for proper growth and production of the crop are not often met with. Hence studies on microclimate and its influence on growth and yield of the crop and manipulation of microclimate to achieve high and sustained production with quality beans are of great significance in vanilla in the changed environmental conditions. The fleshy herbaceous perennial vine usually starts flowering third year after planting. There is only one flowering season in vanilla. Longer cuttings (>1m) as planting materials and better management practices adopted could bring about early flowering in vanilla. Flowering is a complex phenomenon in vanilla, the physiology and biochemistry of which need in depth investigations. Rest of the events viz. fruit set and yield could be manipulated by adjusting the hand pollination and regulating the number of beans per inflorescence, there by size of beans.

Flowering in vanilla is influenced by many factors like physiological maturity of the plant, the extent of moisture stress in the garden, microclimate of the garden, management practices adopted in the garden, accumulation of nutrients and flowering substances in plants etc. Lack of flowering in shoots prepared for flowering was reported as a problem by many farmers. Systematic research efforts on the influence of different parameters on flowering in vanilla are lacking. The moisture stress which is essential for inducing flowering in vanilla is practiced by growers at different intensities. As a result, many plants could not recover the severe stress effects and they eventually dry even after commencement of irrigation or predisposed to various diseases.

Hence present investigations were taken up at College of Horticulture, Kerala Agricultural University, Thrissur to study the influence of soil moisture stress and micro-meteorological factors on flowering in vanilla. The studies were conducted in Plantation Crops and Spices farm of College of Horticulture and in a selected farmer's field at Thrissur district, a newly introduced area for the expansion of the crop.

Review of literature

2. REVIEW OF LITERATURE

Stress is an essential pre-requisite for flowering in many crop plants. The stress can be either biotic or abiotic. Biotic stress is due to attack of insects or disease incidence. The abiotic stress may be due to temperature, water scarcity, salt, anoxia and geotropism. Different operations like pinching apical bud, decapitation, ringing, girdling, pruning etc. are found to induce flowering in many crop plants. Similarly micro-meteorological factors like temperature, light, relative humidity etc. also influence flowering in many plants.

2.1 INFLUENCE OF SOIL MOISTURE STRESS ON FLOWERING IN CROP PLANTS

Soil moisture stress induces flowering in fruit plants like mango, carambola, rambutan, mangosteen, durian and in plantation crops like black pepper, cashew, cacao and coffee. Soil moisture stress is essential in many plants for floral differentiation to occur but further growth of flower buds initiated and flowering occurs after getting water stimulus either by irrigation or through rainfall. Moisture stress has got profound influence on flowering in the following crops.

2.1.1 Fruit crops

2.1.1.1 Litchi

The effect of soil moisture tension on growth and floral development of field grown *Litchi chinensis* was investigated by Nakata and Sueihisa (1969). A high soil moisture tension for four to six months promoted floral initiation and fruit set in litchi but inhibited the growth of trunk and emergence of flushes. Low moisture tension throughout the experimental period was inhibitory to floral bud initiation. They also studied the biochemical response of litchi due to moisture stress. Nitrogen and potassium levels in leaf samples were unaffected by water stress. But the phosphorus levels in stem and leaves were lowered by high soil moisture tension.

The effect of temperature and leaf water stress on growth and flowering of litchi was studied by Menzel *et al*. (1989). They found that day shoot and root temperature interacted to control flowering in litchi. Flowering was very weak when day shoot and root temperatures exceeded 20°C. A combination of warm shoots (30°C day / 25°C night) and cool roots (constant 12.5°C) induced lower leaf water potential (-1.0 to -2.0 MPa) in litchi and totally suppressed the growth.

Another study was conducted by Jerway *et al*. (1997) to examine the effects of temperature and water stress on two year old air layers of litchi. Severe water stress given to plants reduced flushing and terminal buds formed were unable to produce panicles even after re-watering. But a moderate stress given to plants induced flowering in more than 80 percent of terminal buds after rewatering.

2.1.1.2 Mango

Biotic and abiotic stresses induced flowering in mango. Rameshwar (1988) reported that commercial production of mango was generally limited to areas where there was no rainfall during the pre- flowering period of mango. In areas with heavy rainfall during the pre- flowering period, mango trees grew vegetatively with less flowering due to the absence of a well defined drought spell. In areas close to equator, where the temperature fluctuations were least and minimum temperature was high, water stress played a major role in mango flowering. He reported that the metabolism of hormones and aminoacids were affected by stress factors. During pre-flowering period, auxins, ethylene and abscisic acid accumulate due to stress, while gibberellins and cytokinins decrease. He reported the involvement of more than one factor for mango flowering. Stress induced flowering in mango when the involvement of other factors were satisfied.

Bally *et al*. (2000) conducted a study in 15 year old ‘Kensington’ mango tree in the dry tropic of Australia. The treatments given were viz. irrigation throughout the year, withholding of irrigation from maturation of the first vegetative flush following harvest until 90 per cent of buds were anatomically floral and withholding irrigation from maturation of the first vegetative flush following harvest until 70 per cent of inflorescence had emerged. It was observed that withholding irrigation significantly increased the number of terminals that flowered and also yield in mango.

2.1.1.3 Citrus

Nir *et al.* (1972) conducted studies on the effect of water stress on 'Eureka' lemon trees. The anatomical studies revealed that flower differentiation in 'Eureka' lemon trees occurred during stress period. The generative bud formed did not undergo further development until water was supplied. Gibberellic acid applied at the normal irrigation condition, inhibited summer flower formation and stimulated vegetative growth. In contrast, CCC induced flower formation when applied by the end of August. Southwick and Davenport (1986) opined that the influence of moderate water stress in the flowering response of citrus was similar to that of low temperature stress induced flowering in 'Tahiti' lime. Severe water stress resulted in much greater flushing and flowering and increased the percentage of flowering shoots.

Moisture stress pattern in citrus orchard before monsoon flowering was studied by Ghadekar *et al.* (1993) in Nagpur orange (*Citrus reticulata*) over a period of 9 years. Forzatura technique was adopted to quantify the moisture stress. Moisture stress was found to vary between 134.25 to 239.98 mm having the corresponding CPE values 505.4 to 854 mm. The moisture stress was found to induce monsoon blossom in Nagpur orange. The study indicated the possibility to induce and retain flowering in Nagpur orange by suitable climatic modifications at micro level. The cumulative pan evaporation values could be taken as a guide to predict and regulate the start and break of water stress for assured flowering in monsoon season. Krajewski and Rabe (1995) reviewed critically the various aspects of citrus flowering including the water deficit stress.

The effect of water stress on flower bud formation and plant hormone content of Satsuma mandarin trees was investigated by Koshita and Takahara (2004). Severe and moderate water stresses were imposed by irrigating the plants once in every 7-10 days and once in every three days respectively. The severely water stressed trees produced fewer flower buds than the moderatively water stressed ones. Also, the ratio of the leafy to leafless inflorescence was 3 fold higher in severely stressed trees. All the nodes of the moderatively water stressed trees produced flowers and the ratio of the leafless to leafy inflorescence was 1.7 fold higher. They observed that the levels of $GA_{1/3}$ were enhanced by severe water stress.

2.1.1.4 Guava

Singh *et al.* (1997) conducted studies in 12 year old guava varieties 'Allahabad safeda' and 'Sardar' in Lucknow under field conditions to know the effect of stress on flowering in guava. Stress was exerted on trees by withholding water from January to June and the non stressed control trees were watered at an interval of 10-15 days. Just after termination of stress in the third week of June, vegetative flushes were induced in which flower bud formation was observed in rainy season. Longer periods of stress along with high temperature (36.17°C -39.80°C) and low relative humidity (22.7% to 23.33%) caused delayed flowering. 'Allahabad safeda' responded more to moisture stress for regulation of flowering than 'Sardar'. In 'Sardar', in addition to soil moisture stress a chemical that can inhibit the growth may also be essential to regulate flowering.

2.2.1.5 Mangosteen

Salakpetch and Poonnachit (2006) conducted studies on 23 year old mangosteen trees at Thailand to find out the effect of soil moisture stress on flowering. Irrigation with either 1.85 times the total daily evaporation every 3rd day or an initial application of 35-40 mm of water per tree plus half the initial rate applied at 7 day intervals induced production of largest amount of flowers and fruits. It was demonstrated that irrigation strategies after attaining a threshold water stress condition could be used as an agro-management practice to stimulate flowering in mangosteen.

2.1.1.6 Durian

Chandraparnik *et al.* (1992) reported that a continuous dry period of 10-14 days was found to induce flower initiation in durian. But irrigation was required to promote growth and development of the flower buds to anthesis. However, rainfall greater than 10 mm / day for about three to five continuous days suppressed the development of flower buds during the first stage of emergence.

2.1.2 Plantation Crops

2.1.2.1 Coffee

Alvim (1977) reported that coffee flowering was controlled by day length and rain fall distribution. Bud differentiation was induced by short days (photoperiodism), where as bud anthesis was associated with rainfall following a dry period or a sequence from dry to wet period. Moisture stress decreased ABA translocation into flower buds thus rendering the buds to grow. Subsequent release of moisture stress increased GA concentration thus inducing bud growth and anthesis.

2.1.2.2 Cashew

Alvim (1960a) demonstrated that moisture stress was a pre - requisite for bud break in cashew and dormancy could be prolonged indefinitely, by irrigating the plants frequently. A high plant water potential was necessary for flower opening but it had no effect without a moisture stress period. A suitable temperature range, low relative humidity, and bright sun shine hastened the process of flowering in cashew.

2.2.2.3 Cacao

The importance of moisture stress in breaking the dormancy in cocoa was reported by Alvim (1960b). However, anthesis occurred only after rain. Sale (1970) and Boyer (1973) reported that vigorous flushing was observed in watered plants of cacao after the stress period. Less flowering was observed in frequently irrigated plants of about two years, where as plants of the same age but previously submitted to soil moisture stress flowered vigorously.

2.2 PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN CROP PLANTS DUE TO MOISTURE STRESS

2.2.1 Physiological

2.3.1.1 Leaf thickness

Herrera *et al.* (2000) conducted studies in Peperomia plant (*Peperomia carnevalii)* to find out the effect of drought on CAM and water relations. Drought caused reduction in leaf thickness due to shrinkage of the hydrenchyma, but not of the mesophyll. Reduction in leaf thickness was associated with the occurrence of a gradient of osmotic potential between these two tissues.

Influence of different soil moisture on anatomy of maize was studied by Stoyanova *et al.* (2002). The structure of the 7th, 8th and 12th leaf of maize plants grown under conditions of 80, 60 and 40 per cent of full moisture content was studied. The data from anatomical analysis showed that gradual depletion of soil moisture did not provoke substantial histological changes.

Leaf anatomical adaptation of *Cenchrus ciliaris* against drought stress was investigated by Nawazish *et al.* (2006). Leaf thickness in the ecotype obtained from Faisalabad, Pakistan decreased with increasing drought stress level from 310.45 µm at 100 per cent field capacity to 195.23 µm at 50 per cent field capacity. An opposite response to drought stress was recorded in the ecotype from another location with salinity, where leaf thickness was increased from 214.56 µm at 100 per cent to 293.45 µm at 50 per cent field capacity.

2.2.1.2 Relative leaf water content

Relative leaf water content (RLWC), which is directly related to leaf water potential is an alternative measure of plant water status (Roberts and Knoerr, 1977; Sinclair and Ludlow, 1985).

Stephenson *et al.* (1989) conducted studies to find out the plant water relations of stressed young potted macadamia trees under glass house conditions. Critical levels of water status beyond which irreversible damage occurred were determined. Relative leaf water content, leaf and xylem water potentials declined exponentially during the first two weeks. Soft young leaves wilted earlier than mature hardened leaves. A decrease in RLWC, photosynthesis, respiration and growth characters were observed by Singh *et al.* (1997), when they studied the response of applied potassium on plant growth and CO_2 exchange in chickpea under normal soil moisture and water stressed conditions.

The influence of water deficit on vegetative growth, physiology, fruit yield and quality in brinjal was investigated by Kirnak *et al.* (2001). The water stress resulted in significant reduction in RLWC. Severe water stress (40 per cent of pot capacity) resulted in RLWC of 66 per cent, where as control plants recorded 96 per cent RLWC. Charugupta (2006) observed the relationship between plant growth, biomass and seed yield under moisture stressed and non stressed conditions in mustard. Soil moisture stress was induced at different magnitudes viz. mild (50 per cent of control), moderate (35 per cent of control), severe (20 per cent of control) at the time of 100 per cent flowering. In severely water stressed plants, RLWC significantly reduced (59.12 per cent) while it was 83.40 per cent in control plants.

Relative leaf water content was used as a parameter to select genotypes tolerant to drought in several crop plants. Physiological and biochemical changes in cluster bean genotypes under water stress were studied by Kuhad and Sheoran (1986). Water stress was created by withholding irrigation at flower initiation stage. Water stress resulted in reduction in RLWC in all plants. The decrease was more in the genotype Durgajay (55 per cent) and HG 75 (53 per cent) than other genotypes over control.

Responses of tall, dwarf and hybrids of coconut to moisture stress (stimulated with an osmoticum of polyethylene glycol and air desiccation) were investigated by Voleti *et al.* (1990). The two methods of stimulation had differential effect on the genotype. In general, RLWC of all genotypes was reduced more significantly due to air desiccation than osmotic stress. In coconut, the stress tolerant hybrids like LO × GB; LO × COD and WCT maintained a high RLWC under moisture stress conditions. The difference in RLWC between control and stressed condition was only 6.4 per cent in LO × GB and 8.9 per cent in both LO × GB and WCT.

The effect of soil moisture stress and water relation on cropping behavior in guava varieties was investigated by Singh *et al.* (1997). Even at low RLWC 'Sardar' variety maintained normal metabolic activities than 'Allahabad safeda'.

Decrease in relative leaf water content with increase in water stress was reported in black pepper by Krishnamurthy *et al.* (1998). RLWC ranged in black pepper from 82 to 95 per cent in accession ACC 1622 and 64 to 91 per cent in accession ACC 891, after 8 days of water stress.

Stress physiology of cocoa was reviewed by Balasimha (1999). He reported that RLWC of leaves of rainfed cocoa plants were lower than those of irrigated plants.

Efficacy of RLWC as an index for water stress tolerance in rice was reported by Tyagi *et al.* (1999). Drought tolerant genotype CR 143-2-2 Salam Pikit and JD8 showed lower reduction in RWC under water stress condition as compared to susceptible genotype PR 110 and P169.

Yadav and Chanrabhushan (2001) studied the effect of moisture stress on growth and yield in rice genotypes. Water stress lowered RLWC at both tillering and flowering stages. The genotypes IET 11677, IET 11675, IET 11672 and Annada maintained higher RLWC. The genotype IET 11677 was more tolerant to moisture stress as compared to other genotypes. The impact of moisture stress and ameliorants on growth and yield of sunflower variety CO 4 was studied by Velu and Palanisami (2002). One irrigation was withheld at vegetative, flowering and post flowering stages. Water stress reduced the RLWC content in sunflower.

The influence of water stress on physiological responses of three sorghum genotypes was investigated by Alhamadani and Barger (2003). Three cycles of drought induced lower RLWC. The bloom cultivar ROKY 62 maintained a higher RLWC than bloomless cultivar ROKY 62 and sparse bloom cultivar ROKY 62.

According to Upreti and Murthi (2004) water stress treatments led to decline in RLWC in onion variety PBR and Arka Pragathi. Arka Kalyan exhibited stability in RLWC under stress conditions at 30 and 45 days after transplanting.

2.2.1.3 Membrane stability

The cell membrane damage and leakage of electrolytes under stress are measures of cell membrane stability (Blum and Ebercon, 1981). Water stress treatment resulted in significant increase in electrolytic leakage in brinjal as compared to control treatment (Kirnak *et al*., 2001). Electrolytic leakage was slightly higher in mature leaves than in developing ones.

Stress tolerant genotypes were selected in several crops based on electrolytic leakage. Electrolytic leakage in water stressed coconut hybrids was less as compared to dwarfs (Kasturibai *et al.,* 1988).

Efficacy of membrane stability as an index for selection of rice genotypes against water stress was investigated by Tyagi *et al.* (1999).

He observed that drought tolerant genotypes CR-143-2-2, Salam Pikit, JD8 and N22 showed higher membrane stability compared to susceptible genotypes PR110 and P169. The possible mechanism of water stress tolerance in wheat genotypes was studied by Sairam and Saxena (2000). Water stress imposed at different stages after anthesis resulted in a decrease in membrane stability and the genotype PBW 175 had the highest membrane stability, where as WH 542 exhibited the lowest membrane stability. Genotype HD 2402 was intermediate in membrane stability.

Membrane stability decreased significantly in black pepper when subjected to water stress for two days, the highest stability was observed in Taliparamba I and lowest in Kottanadan. Only six varieties survived water stress for four days and the membrane leakage was maximum in Uthirankotta 2 and least in Poonjarmunda (Thankamany, 2000).

Tea clones were screened for drought tolerance by Thomas *et al.* (2004). It was observed that China cultivars recorded significantly lower values of relative membrane injury (70.2) followed by Assam cultivars (63.6) and Cambodia cultivars (60.4).

Changes in electrolytic leakage in onion at 30 and 45 days after transplanting in response to water stress were studied by Upreti and Murti (2004). There was an increase in electrolytic leakage at both growth stages, the increase being more marked at 30 days than at 45 days. The variety Arka Kalyan exhibited higher stability and variety Arka Pragathi lower.

2.2.2 Biochemical parameters

2.2.2.1 Total chlorophyll

Water stress in general, decreases the total chlorophyll content in leaves (Hsiao, 1973). Prakash and Ramachandran (2000) conducted studies to find out the effects of moisture stress and antitranspirants on leaf chlorophyll content in brinjal. Moisture stress imposed at different growth stages decreased the chlorophyll content. Similar observations were made by Kirnak *et al.* (2001) in brinjal. In severely moistured stressed plants (40 per cent of pot capacity), the total chlorophyll content was reduced by 55 per cent as compared to control.

Water stress imposed at different stages after anthesis resulted in decrease in chlorophyll in wheat (Sairam and Saxena, 2000). Total chlorophyll content was used as a measure of tolerance or susceptibility reaction of the genotypes. Genotype PBW 175 had the highest content of chlorophyll and WH 542 being the susceptible genotype exhibited the lowest content of chlorophyll.

Water stress treatments led to decline in chlorophyll content in onion (Upreti and Murthi, 2004). Maximum reduction in chlorophyll content was observed in PBR followed by Arka Pragathi. Arka Kalyan exhibited stability in chlorophyll content under stress conditions at 30 and 45 days after planting. Total chlorophyll content was not significantly influenced by water stress in black pepper (Thankamany, 2000). The total chlorophyll content in mustard decreased significantly under mild, moderate and severe water stress conditions (Charugupta, 2006). Moisture stress was induced by withholding water for six and twelve days at 20, 40 and 60 days after sowing. Moisture stress significantly reduced the chlorophyll content in plants subjected to 12 days period of stress in maize. (Bedse *et al*., 2007).

2.2.2.2 Epicuticular wax content

Layer of epicuticular wax on the surface of higher plants reduces cellular permeability and thereby helps to protect the plants from excess water loss (Schonherr, 1976).

Water stress enhanced the epicuticular wax content in sorghum leaves (Ebercon *et al.* (1977).

Kurup *et al.* (1993) found that in coconut cultivar WCT and crosses involving WCT, the wax content of sixth leaf exhibited a sharp increase during the stress period. The hybrid WCT × COD had higher increase in wax content than WCT and COD × WCT. The feasibility of physiological parameters as a selection criterion for drought tolerance in hybrid coconut accessions was investigated by Vincent *et al.* (2005). Fifteen genotypes of coconut comprising five each in talls, dwarfs and hybrids were screened for drought tolerance. Among all the genotypes, dwarfs had low epicuticular wax content than either talls or the hybrids. The tall genotypes ECT and AO had higher epicuticular wax content when compared to other genotypes. In hybrids, VHC-2 (ECT × MYD) recorded higher epicuticular wax content during the stress period.

Higher deposition of epicuticular wax in drought tolerant varieties of cacao was observed by Balasimha (1999). Samdur *et al.* (2003) also observed a substantial increase in wax content in peanut genotypes under moisture stress condition.

Thankamany (2000) observed that the epicuticular wax content was relatively high in water stress tolerant black pepper varieties like Panniyur 5 and Padarpan followed by Kalluvally 4, Kumbakodi and Poonjarmunda.

2.2.2.3 Soluble protein

The quality and quantity of protein were altered by water stress in many plants. The normal protein metabolism was adversely affected and special type of proteins called stress proteins were accumulated during stress period.

A decrease in total soluble protein was observed in 'Valencia' orange under water stress deficit as reported by Joseph and Yelenosky (1988). The reduction in soluble protein was recoverable after five days of re-watering.

Physiological response of rice varieties to different levels of moisture stress was reviewed by Jha and Singh (1997).The protein content decreased with increase in moisture stress. The tolerant genotypes viz. Janki, BR-8, SBR-33-41-41 and Rasi recorded higher values of protein content than susceptible ones under moisture stress conditions. The maximum decrease in soluble protein was observed in tolerant genotype IR-36 and minimum in susceptible genotype Janki.

In cocoa accessions, total soluble protein remained unchanged under drought. (Balasimha, 1999). In brinjal soluble protein decreased in all stages of evaluation viz. vegetative, flowering and fruiting stages (Prakash and Ramachandran, 2000).

2.2.2.4 Total free amino acid

Accumulation of free amino acid was observed under stress condition in all crop plants and free amino acid has a possible role in osmotic adjustment.

The amino acid metabolism in bermuda grass during water stress was studied by Barnett and Naylor (1966). Amino acids were continually synthesized during water stress treatments in two varieties of bermuda grass.

The total free amino acid content was higher in moisture stress tolerant genotypes of rice like Janki, BR-8, SBR-313-41-41 and Rasi (Jha and Singh, 1997). The amino acid content showed an increasing trend due to severe water stress in black pepper varieties. Poonjarmuna, Panniyur-1, Panniyur-5 and Padarpan and a decreasing trend in varieties Kumbakodi, Uthirankotta-2 and Kalluvally-4 (Thankamany, 2000). Drought stress caused an increase in the total free amino acid content in bhindi as reported by Sankar *et al*. (2007). The variety JK Haritha recorded maximum accumulation and minimum by Mahyco variety.

2.2.2.5 Proline

Proline increased proportionately faster than other amino acids in plants under water stress, has been suggested as an evaluating parameter for irrigation scheduling and for selecting drought resistant varieties (Bates, 1973).

Proline accumulation in response to drought stress was reported in a two year old olive plant grown in a controlled water deficit for 20 days. Leaf and root samples were analyzed before and during the experimental periods. The levels of proline increased in parallel with the severity of drought stress in both leaves and roots of the plant (Sofo *et al.*, 2004). Increased proline synthesis due to soil moisture stress was reported in brinjal by Sarker *et al.* (2005), in mustard by Charugupta (2006), in guava by Singh *et al.* (1997) and in cocoa by Balasimha (1999).

Moisture stress was induced in coconut genotypes by air desiccation and polyethylene glycol. The accumulation of proline did not differ much between the two types of stress stimulation, except in dwarfs which exhibited higher proline content in polyethylene glycol stressed leaves than in air desiccated ones (Voleti *et al.*, 1990).

In another study conducted by Kasturibai and Rajagopal (2004), coconut hybrid seedling combinations comprised of three dwarfs (COD, CGD and MYD) each crossed with three talls (WCT, FMS and Fiji) was evaluated under moisture stress situations. Stress was induced by air desiccation and recovery potential of seedlings from moisture stress condition was observed. Proline concentrations showed significant differences between the hybrid seedling combinations during non stress, stress and after stress evaluations. Proline accumulation capacity did not show any relationship with the recovery potential of the seedlings.

Vincent *et al.* (2005) reported proline content as an indicator for drought tolerance in coconut. They reported that the tall genotypes showed higher proline content than hybrids and dwarfs. Among the tall genotypes, ECT and AO recorded higher proline. Among the hybrids, VHC-2 (ECT × MYD) recorded higher proline content.

Nahar and Gretzmacher (2002) reported that in tomato varieties, significant increase in leaf proline content was observed during water stress. There was more than 100 per cent increase in proline content at 40 per cent field capacity as compared to control. The highest accumulation of proline was found in BR-2 (4.17 per cent), followed by BR-1 (3.46 per cent), BR-5 (3.40 per cent) and BR-4 (3.23 per cent).

Jeyamraja *et al.* (2004) observed accumulation of proline in tea clones during dry season resulted in increased osmotic potential. Proline was found higher in Upasi-2, followed by Upasi-1 during drought season. The Cambodian clones recorded a relatively higher proline accumulation than Assam and China clones (Thomas *et al*., 2004).

In bhindi varieties, drought induced bio chemical modifications and proline accumulation (Sankar *et al*., 2007). The variety JK Haritha recorded higher proline content while the variety Mahyco the lower. Bedse *et al.* (2007) observed that proline content in maize genotypes increased due to moisture stress at all the growth stages viz. 20, 40 and 60 days after sowing as compared to control plants. The highest increase in proline content was noted in the stressed plants at 40 days after sowing. Among the varieties, higher proline content was recorded in PKVM Shatak than Ganga 11 and YMH 9805.

2.2.2.6 Activity of peroxidase enzyme

At cellular level, moisture affects the enzyme activities as water forms the site of activity for most of enzymes. In general, water stress increased the enzyme activities in most of the crops.

Thakur *et al.* (1981) scanned maize cultivars for peroxidase isozyme changes under varying levels of water stress. A highly significant negative correlation was found between enzyme activity and water deficit. Enhanced enzyme activity on a fresh weight basis was recorded in the tolerant maize genotype Agathi-76 with increasing stress. According to Duan (1992), peroxidase activity increased at the beginning of water stress in tea seedlings but decreased when water stress was prolonged.

Higher peroxidase activity under moisture stress condition was reported in tolerant rice genotypes viz. Janki, BR-8, SBR-313-41-41 and Rasi as reported by Jha and

Singh (1997). Sairam and Saxena (2000) observed that tolerant wheat genotype PBW 175 recorded higher peroxidase activity, while WH 542, being the susceptible genotype exhibited the lowest enzyme activity. Genotype HD 2402 was intermediate in enzyme activity.

The peroxidase activity was reported as an efficient indicator of water stress in sweet pepper (Jadoski *et al.*, 2000).

2.2.2.7 Total sugar

The total sugar content was influenced by water stress in many plants. Woodhams and Kozlowski (1954) conducted studies in tomato and beans and observed that severe drought brought about serious depletion of starch and sugars. Cortes and Sinclair (1987) observed an increased accumulation of soluble sugars due to water stress in sorghum. Yasuyoshi *et al.* (1998) reported that in cherry tomato, the total sugar content of the fruit increased as moisture stress increased but that the content of sucrose was lower than that of glucose and fructose in different stress periods studied.

Influence of moisture stress on solute accumulation in field grown tomato cultivars was studied by Rao *et al*. (1999). Maximum solute accumulation was responsible for better maintenance of turgor and osmotic adjustment during water stress and the total sugars were found higher in the variety Arka Meghali. Similar increase in the content of soluble sugars was observed in drought tolerant accessions of cocoa (Balasimha, 1999) and in senna (Agarwal and Pandey, 2002).

2.2.2.8 Potassium concentration in tissues

Potassium plays a major role in survival of crop plants under environmental stress condition. It is essential for many physiological processes such as photosynthesis, translocation of photosynthates into sink organs and activation of enzymes (Marschner, 1995; Mengel and Kirkby, 2001).

The effect of potassium on water relations, CO_2 exchange and plant growth under water stress in chickpea revealed that potassium mitigated the adverse effect of water stress and facilitated increase in dry weight of leaves, steam and root (Singh *et al*., 1997). Effect of potassium levels on growth and yield of maize under moisture stress at different phenophases was investigated by More *et al.* (2004). During moisture stress, drought tolerance reaction of maize was increased by the application of potassium. Thankamani and Ashokan (2004) reported that the concentration of potassium in the leaves of black pepper varieties varied significantly due to water stress. The potassium content of all the varieties increased at severe moisture stress and the maximum increase in potassium content was observed in Poonjarmunda, Kumbakodi and Panniyur-5.

2.3 INFLUENCE OF MICRO METEOROLOGICAL FACTORS ON FLOWERING IN CROP PLANTS

2.3.1 Temperature

2.3.1.1 Fruit crops

2.3.1.1.1 Mango

Rameshwar (1988) reviewed the various stress factors influencing flowering in mango. A late cold spell (10°C) during the month of January – February induced a second flush of flowering at Sangareddy region of Andrapradesh. Flowering in north Indian cultivars was adversely affected by a mean minimum temperature of 17°C. Shinde *et al.* (2005) reported that minimum temperature and relative humidity was significantly correlated with percentage of flowering in mango variety Alphonso in Vengurla district of Maharashtra.

2.3.1.1.2 Citrus

Southwick and Davenport (1986), Krajewski and Rabe (1995), Okuda *et al.* (2004) studied the influence of various factors on citrus flowering. Southwick and Davenport (1986) found that a day temperature of 18°C and night temperature of 10°C induced a time dependant flowering in ‘Tahiti’ lime. Krajewski and Rabe (1995) reviewed elaborately flowering in citrus with respect to events in buds leading to flowering, flowering behaviour, phenology of flowering and various factors influencing flowering in citrus like photoperiod, temperature, water deficit stress, nitrogen metabolism etc. The relationship between floral evocation and bud dormancy in Satsuma mandarin was studied by Okuda *et al.* (2004). The buds formed after floral evocation developed floral bud within a few days at 32°C and 80 per cent relative humidity.

2.3.1.1.3 Litchi

Menzel *et al.* (1989) reported that day shoot temperatures and root temperatures interacted to control the level of flowering in litchi. Flowering was very weak when day shoot temperatures and root temperatures exceeded 20°C.

2.3.1.1.4 Pine apple

The effect of night temperature on flowering and fruit size in smooth ceynne pine apple was reported by Freend (1981). Flowering was most rapid at night temperature of 20°C and slowest at night temperature of 15°C.

2.3.1.1.5 Surinam cherry

Preez *et al.* (1994) observed that flowering in Surinam cherry was largely dependent on rainfall and temperature and fruit set was dependent on high relative humidity. Flowering was found best after the rains following a dry period.

2.3.1.1.6 Apple

Flower buds were formed more readily during warm weather than during cold weather (Gribanvonovskji, 1969). 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 initiation of bud break in apple. Kamata (1992) investigated the effect of temperature on the number of days required from release of imposed dormancy to flowering in apple cultivars viz. Amrican summer, Pearmain, Jonathan, Ralls, Vanet and an indicator cherry tree. Mean air temperatures were negatively correlated with days to flowering for all three apple cultivars but less strongly for indicator cherry. Accumulated air temperatures and soil temperatures were negatively correlated with days to flowering for all plants studied.

2.3.1.1.7 White sapote

The effects of temperature on flower induction in white sapote (*Casimiroa edulis* cv. Florida) were investigated by Yonemoto *et al.* (2005) under the field condition in Japan during the year 2002-03. Floral induction was found at a mean temperature of less than 20°C, which prevailed from November to December in the field condition. The effective day- night temperature regime in the green house was 15°C -24°C for flowering and day temperature of 30°C inhibited floral induction. It was concluded that a day-night temperature of 15°C -26°C promoted flower induction in the plant.

2.3.1.1.8 Ber

The flowering habit of ber cultivars viz. Gola, Kaithali, Banarsi, Kadaka, Umran, Mundia and Seb at six locations in India were studied by Vishal *et al.* (2000). Flowering was generally dependent on temperature particularly on the variation between maximum and minimum temperature. A maximum temperature of 32.4°C-36.9°C and a minimum temperature of 20.7°C-25.3°C, along with a temperature difference of 8.6°C-13.3°C, prevailing continuously for minimum of one month induced flowering in all cultivars across the arid region.

2.3.1.2 Vegetable crops

2.3.1.2.1 Pigeon pea

Maximum bud blooming and pod setting were observed in pigeon pea when the minimum and maximum day temperature ranged between 13°C and 25°C respectively.

Maximum floral bud drop was recorded at a minimum and maximum day temperature of 4°C -6°C and 12°C -14°C (Pandey, 2004).

2.3.1.2.2 Allium

The effect of low temperature on growth and flowering ability of allium was studied by Esnault *et al.* (2005). The best flowering rates were observed when the plants were grown for three months in a phytotron under a 12 h photo period with day and night temperature of 10°C and 6°C respectively, then transplanting them into the field in March.

2.3.1.3 Flower crops

2.3.1.3.1 Tuberose

The flowering pattern of tuberose as main and ratoon crops were studied during different months of a year (Ghosh and Pal, 2004). The correlation between temperature and flowering attributes was determined. In the main crop, all flowering characters were negatively and significantly correlated with mean temperature.

2.3.1.3.2 Texas firebush (*Hamelia patens*)

The influence of climatic factors on flowering of Hamelia was studied by Armitage (1995). A green house environment with moderately high temperature (25°C) induced flowering in the plant.

2.3.1.3.3 Pyrethrum

Brown and Menary (1994) observed that night temperatures of 6°C and 12°C for two and three weeks promoted rapid inflorescence initiation and development in pyrethrum. High day temperatures (25°C) combined with low photon flux prevented flowering in pyrethrum.

2.3.1.3.4 Miltonia

Matsui and Yoneda (1999) observed that plants failed to flower when maximum temperature exceeded 32°C, but flowered early when temperature was maintained below 32°C.

2.3.1.3.5 Antirrhinum

The effect of different environmental conditions on flowering time was investigated by Cremer *et al.* (1998). Temperature was inversely correlated with flowering time and leaf number. Lowering of temperature from 15°C to 12°C resulted in reduction of flowering time.

2.3.1.3.6 Acacia

Acacia required temperatures at or above a mean maximum of 18°C and a minimum of 13°C for bud formation and at or below a mean maximum of 16°C and a minimum of 9°C for flowering (Morgan and Sedgley, 2002).

2.3.1.4 Spices

2.3.1.4.1 Pepper

Rajan (1985) reported that the maximum and minimum temperature in the preceding summer and the subsequent monsoon showers played an important role in triggering the flower bud differentiation in black pepper.

2.3.1.4.2 Saffron

The effect of temperature on the flower formation in saffron was studied by Molina *et al.* (2005). The optimum temperature for flower formation was in the range of 23°C to 27°C. Flower emergence required a markedly lower temperature of 17°C.

2.3.2 Relative humidity

2.3.2.1 Mango

Shinde *et al*. (2005) conducted studies in mango variety Alphonso to find out the effects of weather parameters on floral expression in Vengurla district of Maharashtra during 2000 to 2002. A relative humidity of 64.99% to 69.61% was attributed to low percentage of hermaphrodite flowers during the second phase of flowering. The percentage of hermaphrodite flowers reached 13-17 per cent at a relative humidity of 68-85% during the 1st and 3rd phases of flowering. Thus significant correlation with relative humidity and percentage of hermaphrodite flowers was observed in mango.

2.3.2.2 Ber

Relative humidity of 70.6 to 82.5 per cent, prevailing continuously for a minimum of one month induced profuse flowering in ber cultivars (Vishal *et al*., 2000).

2.3.2.3 Tuberose

The flowering pattern of tuberose as main and ratoon crops were studied during different months of a year by Ghosh and Pal (2004). All flowering characters were negatively and significantly correlated with average relative humidity in the main crop. The ratoon crop exhibited positive and significant correlation between average relative humidity and floral characters, except floret diameter.

2.3.2.4 'Satsuma' mandarin

The relationship between floral evocation and bud dormancy in 'satsuma' mandarin was observed by (Okuda *et al.* (2004). The buds formed after floral evocation developed floral buds within a few days at a relative humidity of 80 per cent.

2.3.3 Light intensity

2.3.3.1 Flower crops

2.3.3.1.1 Orchid

Effect of light intensity on flowering in *Oncidium* spp was studied by Song *et al.* (1998). Three treatments were imposed such as no shading, one 50 per cent shade net or two 50 per cent shade nets. Oncidium cultivar showed the best quality inflorescence under 50 per cent shading net, which gave a light intensity of 230-420 µmol m^{-2} s^{-1} at midday. The flowering percentage at this degree of shading was 83.3 and 100 per cent in cut and potted varieties of *Oncidium* spp.

2.3.3.1.2 Miltonia

Low light intensity (87.5 per cent shading) caused irregular flowering in miltonia and the number of flower stalks per plant was small. Intermediate and high light intensity increased the number and diameter of flower stalks (Matsui and Yoneda, 1999).

2.3.3.1.3 Acacia

Morgan and Sedgley (2002) conducted a study in acacia to know the environmental control of bud formation and flowering of clonal acacia tree. Light intensity affected flowering, with less than half the number of plants flowering in shade house compared with outside.

2.3.4 Rainfall

2.3.4.1 Mango

Chacko and Randhava (1971) reported that high rainfall had a negative effect on flower bud differentiation in mango. Heavy rains during the critical period of flower bud initiation stimulated vegetative growth at the expense of fruit production. Sergent *et al.* (1993) reported that quantity of rainfall during the months of October, November and January at the rate of 40.10, 15.50 and 3.25 mm, respectively produced highest yields in mango.

2.3.4.2 Coffee

The flowering behaviour of coffee as related to climatic factors was examined in detail by Alvim (1973). He observed that rainfall was the main external factor controlling flowering in coffee. Anthesis of flower bud in coffee was seen associated with rain following a dry period or a transition from dryness to wetness (hydroperodism). Alvim (1977) observed that rain or irrigation after a period of moisture stress could break the flower bud dormancy in coffee.

2.3.4.3 Pepper

Nalini (1983) observed that flower bud initiation in black pepper was triggered by the receipt of pre monsoon showers after a spell of dry weather. Rajan (1985) reported that maximum and minimum temperature in the preceding summer and the subsequent monsoon showers played an important role in triggering flower bud differentiation in black pepper.

2.3.4.4 Cashew

The importance of rainfall on the productivity of cashew was evident from the investigation of Veeraraghavan and Vasavan (1997). A well distributed rainfall during October and November was required for optimum flowering in cashew.

2.3.4.5 Cocoa

Smith (1964) found that irrigation increased the growth rate and flower production in cocoa but did not affect the percentage of setting. It was found that irrigated trees flowered earlier and produced greater number of flowers than the unirrigated trees. Hutcheon *et al.* (1973) observed that irrigation increased the rate of flower production in both unshaded and shaded cocoa. Madhu (1984) found that the mean monthly rainfall one month prior to flowering along with temperature and sunshine determined flower production in cocoa.

Materials and Methods

3. MATERIALS AND METHODS

The present investigations on “Influence of micro-meteorological factors on flowering in vanilla” (*Vanilla planifolia* Andrews) were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala and in a selected farmer’s field in Thrissur district of Kerala during October, 2005 to August, 2007. The objective of the study was to find out the influence of soil moisture stress and micro-meteorological factors on flowering in vanilla. The physiological and biochemical responses of vanilla plants due to moisture stress were also studied in detail. The studies were done in five year old vanilla plants maintained in the Plantation Crops and Spices farm (PC & S farm) of the Department of Plantation Crops and Spices and in a selected farmer’s field at Karoor, Thrissur district. (Plate 1). The soil type in selected plots was loamy and the plants were given uniform management practices as per Package of Practices Recommendations of Kerala Agricultural University (KAU, 2007).

The investigations were undertaken as two separate experiments as detailed hereunder.

EXPERIMENT-I

3.1 INFLUENCE OF SOIL MOISTURE STRESS ON FLOWERING IN VANILLA

For studying the influence of soil moisture stress on flowering in vanilla, moisture stress was induced in vanilla gardens at four levels viz., one, 1½, two, and 2½ months along with a control, wherein no moisture stress was given and plants were irrigated as normal. The details of treatments are as shown below:

T_1 – Control – Irrigation weekly twice from December 1st to February 15
T_2 – Withholding of irrigation for 1 month (December 1st to December 31st)
T_3 – Withholding of irrigation for 1½ month (December 1st to January 15th)

Plantation Crops and Spices farm

Farmer’s field

Selected plots for investigations

Taking soil samples for moisture estimation

Recording observations on light intensity

Plantation Crops and Spices farm

Farmer’s field

Stevenson screens installed at experimental fields

Plate 1. Experimental set up

T_4 – Withholding of irrigation for 2 months (December 1st to January 31st)
T_5 – Withholding of irrigation for 2½ months (December 1st to February 15th)

The various treatments were imposed in different blocks of the garden in farmer's field, consisting of 25 plants / block, barring the outer rows of plants in the block. In Plantation Crops and Spices farm, each treatment consists of three plants per treatment and treatments were replicated twice.

In the present investigations, moisture stress could be induced only from December onwards as there was rainfall (0.2-39.8mm) during November, 2006. Plants with uniform growth in which flowering was stabilized were tagged in both the locations, tip of the hanging shoots prepared for flowering was nipped off during November and various treatments were imposed.

Total soil moisture content was determined in the root zone depth (0-15 cm) at two locations viz., PC & S farm of College of Horticulture and selected farmer's field in Thrissur. (Plate 1) The soil was taken using soil auger at the beginning and end of stress period and total moisture content in soil was estimated gravimetrically.

The physiological and biochemical responses of vanilla plants due to different periods of moisture stress were also studied in the present investigations. Fresh samples were used for the estimation of various physiological and biochemical parameters except for total sugar and K concentration in tissues wherein dry samples were used. For drying leaf samples, they were first wiped with tissue paper and dried in a hot air oven at 60 ± 5°C for a period of 10 days. The dried leaves were finely powdered to pass through a sieve of mesh size 72. The various physiological and biochemical parameters were analyzed before and after induction of stress from the same plant. The various parameters under stress in different treatments were compared with that of control at the elapse of different stress periods. The following observations were recorded.

3.1.1 Physiological and biochemical responses of vanilla plants due to moisture stress

3.1.1.1 Physiological parameters

3.1.1.1.1 Leaf colour

Colour of leaf was noted at the beginning and end of stress period in plants of various treatments and was graded as dark green, green and light green.

3.1.1.1.2 Wilting symptoms on leaves and vines

Wilting symptoms on leaves and vines were noted at the beginning and end of stress period and the symptoms were graded based on the development of lines on leaves and vines.

3.1.1.1.3 Leaf thickness

Thickness of leaves at various periods of stress was recorded using Vernier Calipers and compared with that of control plants.

3.1.1.1.4 Relative leaf water content

Relative water content (RWC) expresses water content in sample as percentage of water in fully hydrated (turgid) tissue. The relative water content in leaf samples at various stress periods was measured as per Hsiao (1973) and was expressed as percentage of water in the fully hydrated (turgid) tissue. It is calculated using the following formula

$$\text{RWC} = \frac{(W_f - W_d)}{(W_t - W_d)} \times 100$$

where, W_f – Fresh weight of leaf sample
W_t – Turgid weight of leaf sample
W_d – Oven dry weight of leaf sample

3.1.1.1.5 Membrane stability

The cell membrane stability was studied by observing the leakage of the membrane under water stress as reported by Lutts *et al*. (1995). For this, ten leaf discs (0.1 g) were dipped in 15 ml distilled water for 3 h. The leaf discs were removed and the electrical conductivity ($E.C_1$) of the solution was measured. After the initial measurements, leaf discs were returned to original solution and boiled in distilled water for ten minutes. Leaf discs were removed and the solution was cooled. The electrical conductivity of the solution was determined again ($E.C_2$). The membrane stability was calculated as $E.C_1$ / $E.C_2$.

3.1.1.2 Biochemical parameters

3.1.1.2.1 Total chlorophyll

Chlorophyll content of leaves was estimated following the method of Starner and Hardley (1967). Chlorophyll was extracted in 80 per cent acetone and the absorption was read at 645, 663 and 652 nanometer using a spectrophometer against 80 per cent acetone as blank. Chlorophyll a, chlorophyll b and total chlorophyll of each sample were calculated using the following formulae

$$\text{Chlorophyll a (mg g}^{-1}\text{ of tissue)} = \frac{12.7\ (\text{OD at 633 nm})\ 2.69\ (\text{OD at 645 nm}) \times V}{1000 \times w}$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ of tissue)} = \frac{22.9\ (\text{OD at 645 nm})\ 4.68\ (\text{OD at 663 nm}) \times V}{1000 \times w}$$

$$\text{Total Chlorophyll (mg g}^{-1}\text{ of tissue)} = \frac{20.2\ (\text{OD at 645 nm})\ 8.02\ (\text{OD at 663 nm}) \times V}{1000 \times w}$$

Where, OD = Optical density

V = Final volume of 100 per cent acetone extract

w = Fresh weight of tissue in gram

3.1.1.2.2 Epicuticular wax content

The epicuticular wax content in leaf sample was determined following the method of Ebercon *et al*. (1977). Fresh leaf sample (1g) was immersed in redistilled chloroform for two min. The extract was then filtered and evaporated in a boiling water bath. After adding 5 ml of $K_2Cr_2O_7$, the sample was placed in boiling water bath for five min. The The optical density was read at 590 nanometer using carbo wax 3000 as standard. The epicuticular wax content was expressed in mg g^{-1} of tissue.

3.1.1.2.3 Soluble protein

Soluble protein was estimated using Lowry's method (Lowry *et al*., 1951). The fresh leaf sample was ground with phosphate buffer and to supernatant solution, alkaline copper solution and folin's reagent were added and kept under dark for 30 min. The blue colour developed was estimated spectrophotometrically. The protein content was determined from the standard graph and expressed as mg g^{-1} of fresh leaf sample.

3.1.1.2.4 Total free amino acids

Total free amino acids of samples were estimated spectrophotometrically as reported by Sadasivam and Manickam (1992) using 80 per cent ethanol for extraction. The amino acids were decarboxylated by ninhydrin and the intensity of colour developed was read at 570 nanometer using spectrophometer.

3.1.1.2.5 Proline

The content of proline in leaves was estimated following the method reported by Bates (1973). The extracted proline in aqueous sulphosalicylic acid was made to react with ninhydrin in acidic pH and the absorbance was read at 520 nanometer using a spectrophometer. The proline content was determined from a standard curve of pure proline and was expressed as µg g^{-1} of fresh weight.

3.1.1.2.6 Activity of peroxidase enzyme

The peroxidase activity was determined as reported by Sadasivam and Manickam (1992). The enzyme extract was prepared by grinding with phosphate buffer. One unit of enzyme activity was defined as the amount of enzyme required to increase the absorbance by 0.05 / min. The enzyme activity per litre of the extract is calculated using the following formula.

$$\text{Enzyme activity units / litre} = \frac{3.18 \times 0.1 \times 1000}{6.39 \times 1 \times \Delta t \times 0.1} = \frac{500}{\Delta t}$$

3.1.1.2.7 Total sugar

Total sugars of dried leaf samples were estimated by the method of Dubois *et al*. (1951). Powdered sample (100 mg) was hydrolyzed with 2.5 N HCl, followed by neutralisation with solid Na_2CO_3. The extract was pipetted and 1 ml phenol solution and 5 ml 95% H_2SO_4 were added and kept in a water bath at 25-30°C for 20 min. Total sugar content was measured using spectrophotometer.

3.1.1.2.8 Potassium concentration in tissues

Potassium concentration in tissues was determined as per the procedure reported by Jackson (1973). The dried powdered leaf sample (0.2g) was digested using the dried mixture of perchloric acid and nitric acid in the proportion 4:9 and the digested sample was read against standard KCl using flame photometer.

3.1.2 Flowering and floral characters in vanilla as influenced by moisture stress

The following observations on flowering were recorded at both the locations.

3.1.2.1 Percentage flowering

Number of plants flowered in each treatment was counted and percentage of flowering was worked out.

3.1.2.2 Number of inflorescence emerged per plant

Number of inflorescence emerged per plant was recorded in different treatments.

3.1.2.3 Number of buds per inflorescence

Number of buds emerged per inflorescence in plants of various treatments was counted and recorded.

3.1.2.4 Duration of flowering

The period from 1st flower opening to last flower opening in plants of various treatments was observed and recorded.

Experiment-II

3.2 INFLUENCE OF MICRO METEOROLOGICAL FACTORS ON FLOWERING IN VANILLA

The micro meteorological parameters in the PC & S farm and in selected farmer's field were recorded from October, 2006 to May, 2007. Single Stevenson screens were installed at two locations to record the surface air temperature and diurnal variations at two locations. (Plate 1). The Stevenson's readings were recorded daily at 7.25 am and 2.25 pm. Digital light meter was used to record the light intensity in both the locations. (Plate 1). The micro meteorological situations at both the locations were compared and correlations were worked out to know the influence of micrometeorological factors on flowering in vanilla. The following parameters were recorded in the selected locations.

3.2.1 Temperature (Maximum and Minimum)

Maximum thermometer was placed on the upper platform of single Stevenson screen horizontally at an angle of 2° with bulb to the lower side. The mercury level inside the thermometer provided the maximum temperature in ^{0}C recorded during last 24 h. After taking reading, the thermometer was shaken gently till it reaches the reading level of dry bulb thermometer by positioning it vertically in the right hand.

The minimum thermometer was placed on the lower platform of Stevenson screen below the maximum thermometer at an angle of 2° with the bulb to the lower side as in the case of maximum thermometer. The right side position of the glass index provided the minimum temperature in ^{0}C recorded during last 24 h. (Rao, 2003). After taking reading, minimum thermometer was tilted towards right side such that glass pin touched the surface of alcohol column.

3.2.2 Relative humidity

The dry bulb thermometer was placed vertically in the Stevenson screen towards left side to record air temperature in °C at a given point of time. The wet bulb thermometer was placed on the right side of the box, the bulb of which was wrapped with muslin cloth and tied with cotton thread dipped in distilled water to keep the bulb wet through capillary action of water.

The relative humidity was worked out from dry bulb and the wet bulb readings using hygrometric table and was expressed in percentage.

3.2.3 Light intensity

Light intensity in the garden was measured using Digital Light meter from 12.00-2.00 pm, weekly twice and light intensity was expressed in lux. The percentage light infiltration was worked out based on the light intensity in open. Light intensity readings were recorded from July 2006 to July 2007.

3.2.4 Bright sunshine hours and wind speed

The data on bright sunshine hours, wind speed and number of rainy days were collected from meteorological observatory of College of Horticulture, Kerala Agricultural University, Vellanikkara.

3.3 STATISTICAL ANALYSIS

Statistical analysis of data recorded was carried out as per the techniques described by Panse and Sukatme (1985). The biochemical and physiological parameters before and after moisture stress and stress vs. control were compared using t test. Pearson correlations were used to work out the correlations of micro meteorological parameters with flowering in vanilla.

Results

4. RESULTS

The results pertaining to the study entitled "Influence of micro meteorological factors on flowering in vanilla (*Vanilla planifolia* Andrews)" are presented in this chapter.

4.1 INFLUENCE OF SOIL MOISTURE STRESS ON FLOWERING IN VANILLA

Moisture stress was induced in vanilla garden at four different levels viz., one, 1½, two and 2½ months to induce flowering in vanilla.

4.1.1 Total soil moisture content

At the two locations studied, soil moisture content decreased significantly as the stress period advanced. The maximum reduction in soil moisture content was observed in plants stressed for longer periods (Table 1 and Fig.1).

Table 1. Effect of moisture stress on soil moisture content at root zone depth (0-15cm) in vanilla (*Vanilla planifolia* Andrews)

a) Plantation Crops and Spices farm

Period of moisture stress	Soil moisture content (%)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	13.11	10.18	9.74	2.00	10.18	10.45	-5.94	57.4
1 ½ month	12.20	7.79	18.48	0.01	7.79	10.85	-1.69	14.1
2 months	12.38	6.52	7.00	6.00	6.52	11.08	-1.68	14.4
2 ½ months	13.56	6.93	14.41	1.00	6.93	12.05	-1.66	14.7
Mean	12.81	7.86	12.41	2.25	7.86	11.11	-2.74	25.15

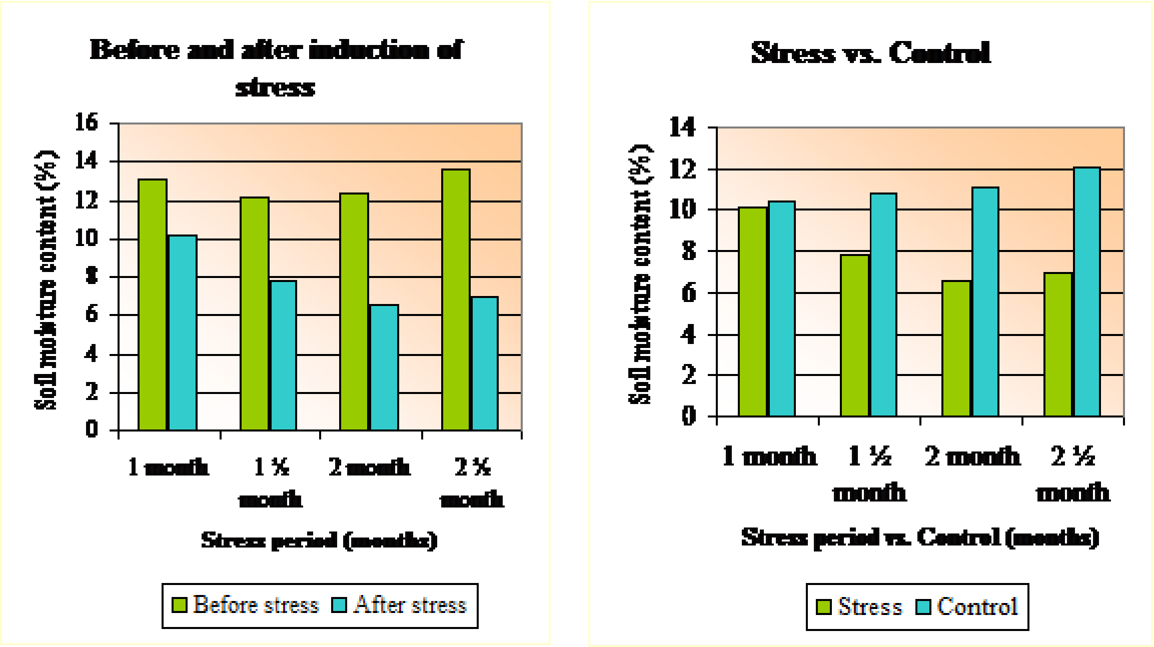
b) Farmer's field

Period of moisture stress	Soil moisture content (%)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	7.65	6.47	25.81	0.01	6.47	8.75	-1.27	25.0
1 ½ months	7.82	5.86	19.51	0.01	5.86	9.36	-1.34	22.9
2 months	7.82	5.53	15.86	0.01	5.53	10.10	-1.06	33.0
2 ½ months	8.30	5.22	26.06	0.01	5.22	10.10	-1.09	0.01
Mean	7.90	5.77	21.81	0.01	5.77	9.58	-1.19	20.22

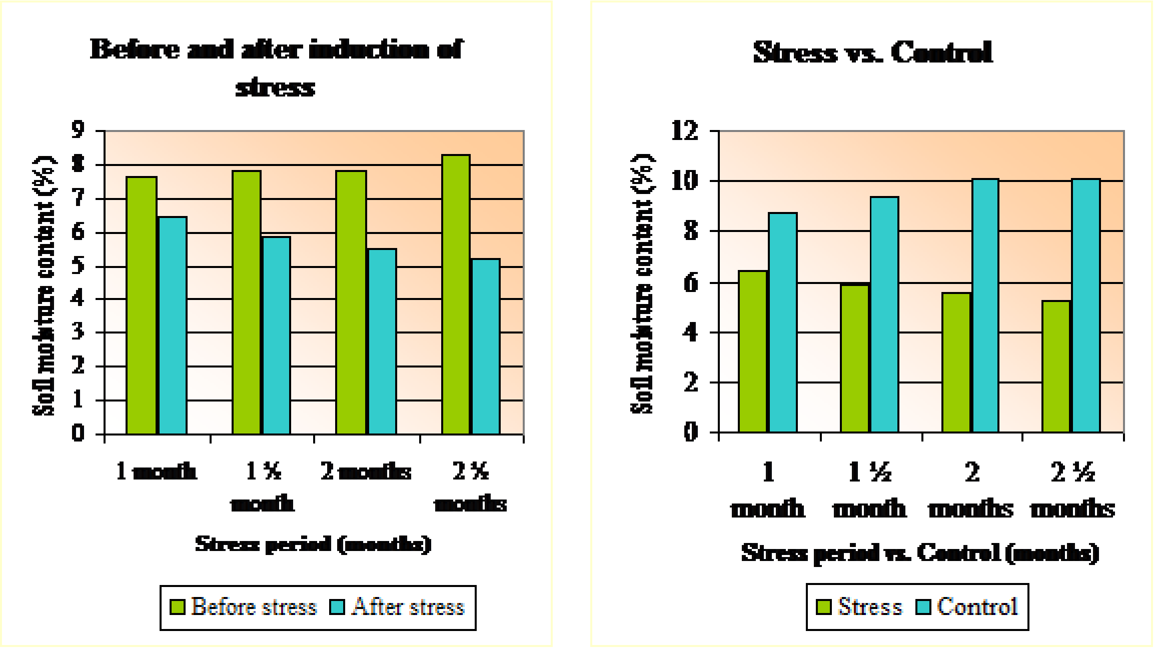
In PC & S farm, a mean decrease of 38.6 per cent in soil moisture content was observed in samples analyzed after the termination of stress period. The percentage decrease in soil moisture content varied from 22.3 per cent in one month stress period and 48.9 per cent in 2½ months stress period. The control plants however registered 41.3 per cent higher content in soil moisture at the root zone depth as compared to stressed plants.

The plants sampled from farmer's field registered a mean decrease of 27 per cent in the soil moisture content wherein, the soil moisture reduction observed was in the range of 15.4 to 37.1 per cent during various stress periods. The control plant registered higher soil moisture content (66%) at the root zone depth as compared to stressed plants. The moisture stress experienced during various stress periods viz., one month, 1½ month, two months and 2½ months were 25, 40, 45 and 50 per cent of control treatments respectively.

Stress induced physiological and biochemical response of vanilla plant and flowering and inflorescence characters are presented hereunder:



Plantation Crops and Spices farm



Farmer's field

Fig. 1. Total soil moisture content as influenced by soil moisture stress

4.1.2 Physiological and biochemical response of vanilla plants to moisture stress

4.1.2.1 Physiological response

4.1.2.1.1 Leaf colour

Leaf colour was noted at the beginning and end of stress period in plants of various treatments and was graded visually as dark green, green and light green (Table 2).

Table 2. Effect of moisture stress on leaf colour in vanilla (*Vanilla planifolia* Andrews)

Treatments	Period of moisture stress	Leaf colour at the beginning of stress period	Leaf colour at the end of stress period
T_1	No stress (control)	Dark green	Dark green
T_2	1 month	Dark green	Green
T_3	1½ month	Dark green	Green
T_4	2 months	Dark green	Light green
T_5	2½ months	Dark green	Light green

At the beginning of the stress period, leaf colour in plants of various treatments was dark green. As the duration of stress period advanced, the colour of the leaf became faded and turned to light green. The fading was more in two and 2½ months stress periods.

4.1.2.1.2 Wilting symptoms on leaves and vines

Wilting symptoms appeared as paling of leaves and vines, development of lines on leaves and vines and reduction in succulent nature of leaves and girth of vines. The wilting symptoms were visually graded based on the above parameters (Table 3), (Plate 2, 3).

1 month stress **1½ month stress**

2 months stress **2½ months stress**

Plate 2. Wilting symptoms on leaves

1 month stress

Control

1½ month stress

Control

2 months stress

Control

2½ months stress

Control

Plate 3. Wilting symptoms on leaves and vines

Table 3. Effect of moisture stress on wilting symptoms on leaves and vines in vanilla (*Vanilla planifolia* Andrews)

Period of moisture stress	Wilting symptoms	Development of lines on leaves and vines
No stress (Control)	Leaves and vines were succulent, dark green fleshy and thick, vines round	Nil
1 month	Paling of dark green colour of leaves and vines	Nil
1½ month	Paling of leaves and vines. Presence of lines due to moisture stress begins to appear along the length of leaves and vines. Reduction in the succulent nature of leaves. Reduction in girth of vines	Low
2 months	Lines became prominent due to moisture stress. Paling of leaves and vines. Succulent nature of leaves was lost. Reduction in girth of vines.	Medium
2½ months	Lines on leaves and vines became very prominent. Paling of leaves and vines. Succulent nature of leaves was lost and leaves became thin and reduction in girth of vine	High

The leaves and vines were dark green, succulent, fleshy, thick and vines round in plants of the control treatment, wherein no moisture stress was given. The wilting symptoms appeared as paling of leaves and vines in one month stressed plants. The leaves and vines became paler and lines began to appear when the stress period advanced to 1½ month. Also, there was reduction in the succulent nature of leaf, vine and girth of vine. The lines became still prominent, leaves and vines became paler in plants of two month stress period. In plants subjected to 2½ months stress period, lines on leaves and vines became very prominent, leaves became paler and thin. Paling of dark green colour was more pronounced in leaves as compared to vines.

4.1.2.1.3 Thickness of leaves

Thickness of leaves was measured at various periods of moisture stress using Vernier Calipers and it was compared with that of control, sampled at the termination of each stress period (Table 4).

The thickness of leaves decreased when plants were starved for moisture. Reduction in leaf thickness was more when period of stress was more and maximum decrease in leaf thickness was observed in 2½ months stress period. The same trend was observed at both the locations. The reduction in leaf thickness was statistically significant over control in leaf samples of two and 2½ months stress periods in farmer's field. The percentage reduction in leaf thickness was more in farmer's field as compared to leaf samples from Plantation Crops and Spices farm (PC & S farm). The mean percentage decrease in leaf thickness was 13 percent in farmer's field, while it was only five percent in PC & S farm as compared to control.

Table 4. Effect of moisture stress on leaf thickness in vanilla (*Vanilla planifolia* Andrews)

Leaf thickness (mm)								
Plantation Crops and Spices Farm					Farmer's field			
Period of stress	Stress	Control	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	0.595	0.616	1.009	0.337	0.653	0.658	-0.094	0.927
1 ½ month	0.592	0.617	-0.227	0.825	0.635	0.673	-0.871	0.404
2 months	0.583	0.622	-0.534	0.605	0.615	0.725	-2.697	0.022
2½ months	0.510	0.630	-1.083	0.304	0.613	0.827	-4.064	0.002
Mean	0.590	0.621	-0.835	0.518	0.629	0.721	-1.931	0.338

4.1.2.1.4 Relative leaf water content

Relative water content which is the relative measure of water content in sample as compared to fully hydrated (turgid) tissue decreased in plants stressed for various periods (Table 5 and Fig.2a).

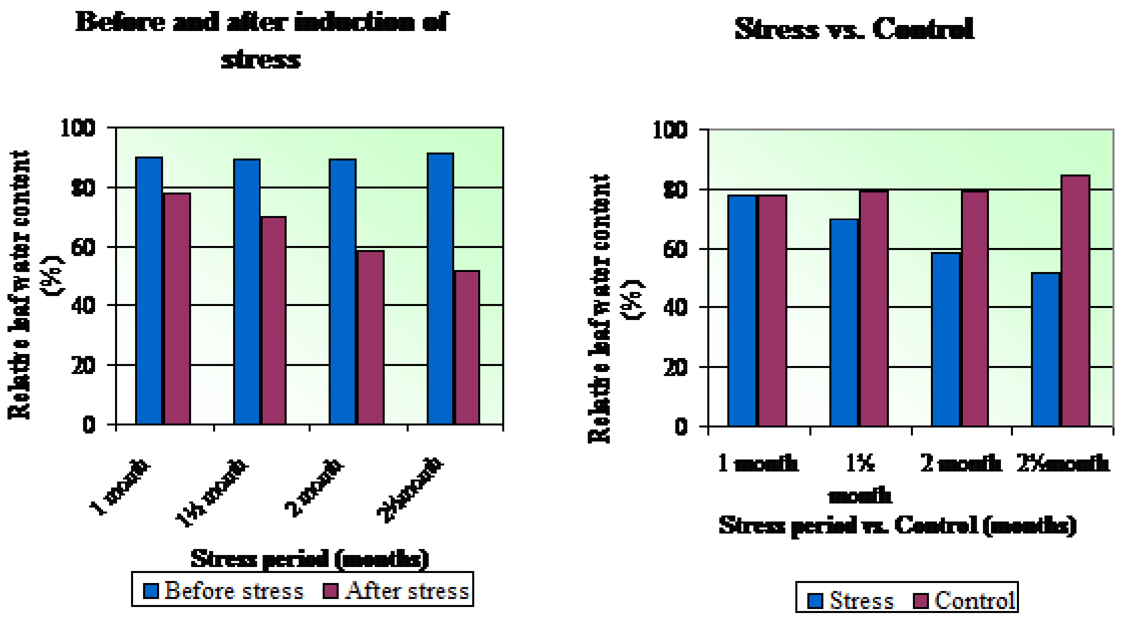
Table 5. Effect of moisture stress on relative leaf water content in vanilla (*Vanilla planifolia* Andrews)

a) Plantation Crops and Spices farm

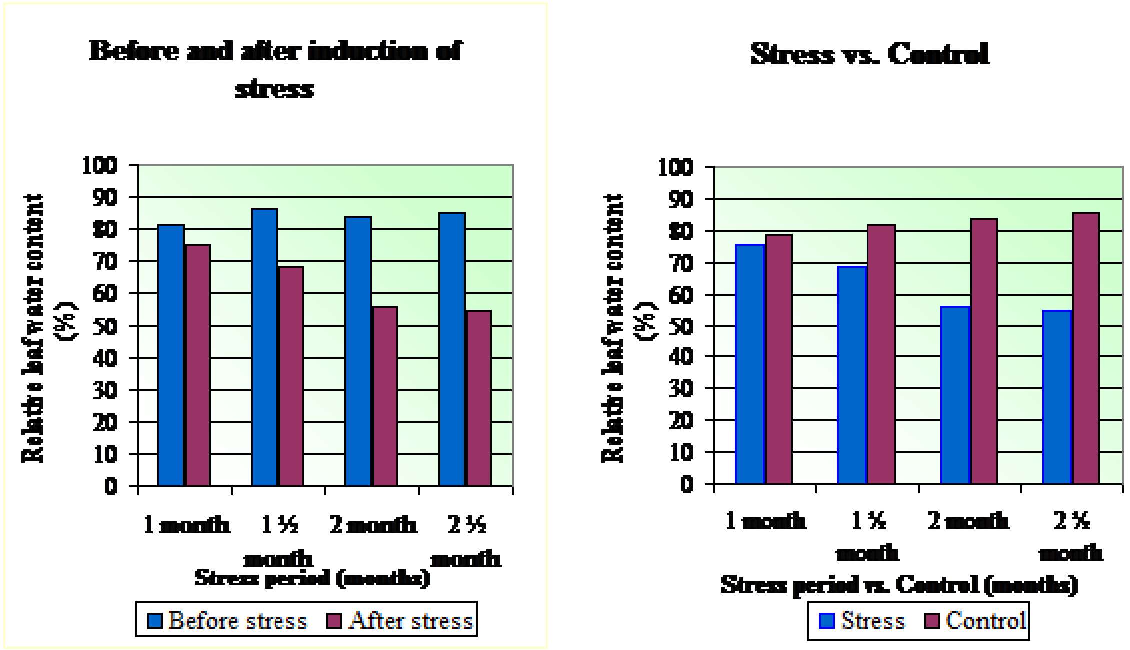
Period of moisture stress	Relative water content of leaf (%)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	90.18	77.92	23.95	0.01	77.92	78.05	3.90	0.30
1 ½ month	89.15	69.70	25.89	0.01	69.70	78.93	-3.11	1.10
2 months	89.22	58.44	169.11	0.01	58.44	79.39	-36.43	0.01
2 ½ months	90.95	51.40	43.37	0.01	51.40	84.76	-13.78	0.01
Mean	89.88	64.37	27.33	0.01	64.37	80.28	-12.36	0.36

b) Farmer's field

Period of moisture stress	Relative water content of leaf (%)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	81.19	75.25	20.15	0.01	75.25	78.43	-5.19	0.01
1 ½ months	86.60	68.29	61.18	0.01	68.29	81.56	-19.06	0.01
2 months	83.77	56.21	74.00	0.01	56.21	83.74	-63.15	0.01
2 ½ months	85.20	54.56	102.22	0.01	54.56	85.76	-65.15	0.01
Mean	84.19	63.58	64.39	0.01	63.58	82.37	38.14	0.01



Plantation Crops and Spices farm



Farmer's field

Fig. 2a. Relative leaf water content as influenced by soil moisture stress

The mean relative leaf water content (RLWC) in selected plants of PC & S farm before induction of stress was 89.88 per cent which decreased to 64.37 percent after stress periods, registering a decrease of 28.4 per cent in leaf water content. The decrease in leaf water content was more in plants stressed for longer periods. The percentage decrease in RLWC was 13.6 per cent in one month stressed plants, 21.82 per cent in 1½ month stressed plants, 34.50 per cent in two months stressed plants and 43.5 per cent in 2½ months stressed plants in PC & S farm.

Relative leaf water content in plants of the control treatment was assessed immediately after the elapse of different stress periods and was compared with that in stressed plants. The control plant samples registered 24.7 per cent higher RLWC than stressed plants. However the mean RLWC was lower in control plants as compared to water content in plants of various treatments before the induction of stress.

In farmer's field also, RLWC decreased in stressed plants the mean decrease being 24.5 per cent as compared to the water content in samples before induction of stress. Similarly, the decrease in leaf water content was more as stress period advanced. The control plants also registered higher leaf water content as compared to stressed plants. The decrease in water content before and after induction of stress and stress vs. control were statistically significant at both the locations.

4.1.2.1.5 Membrane stability

The cell membrane stability which was assessed from the initial and final leakage values decreased in plants stressed for various periods (Table 6 and Fig.2 b).

Table 6. Effect of moisture stress on membrane stability in vanilla (*Vanilla planifolia* Andrews)

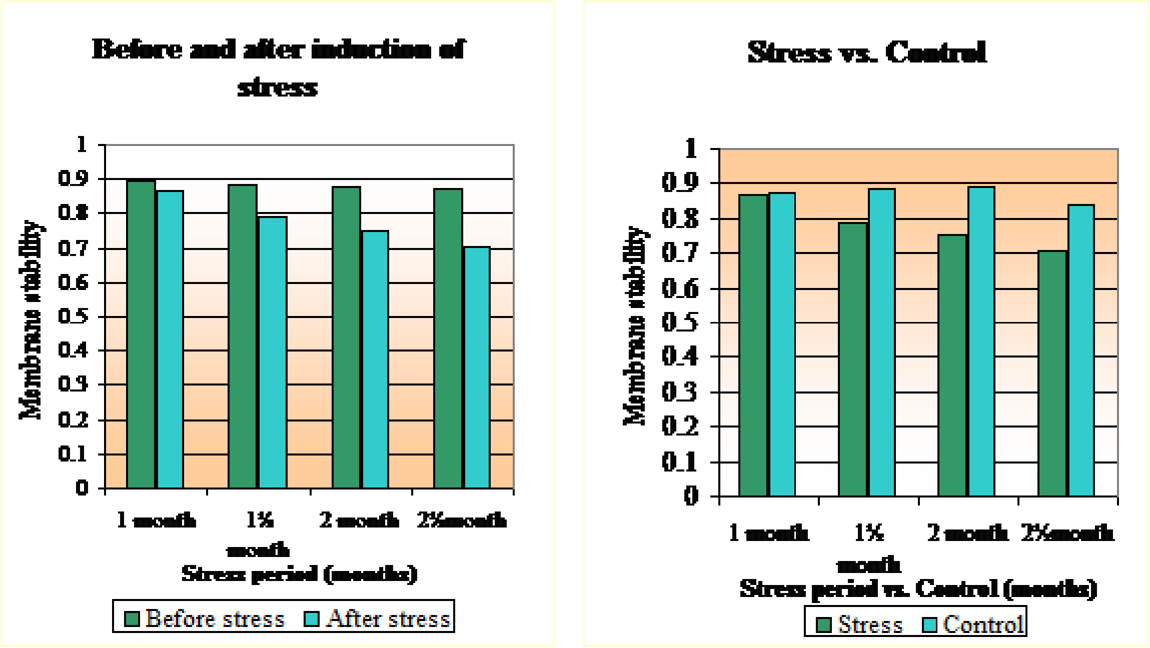
a) Plantation Crops and Spices farm

Period of moisture stress	Membrane stability							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	0.896	0.865	4.185	9.00	0.865	0.875	4.817	0.10
1 ½ month	0.886	0.789	12.710	0.01	0.789	0.887	-1.458	17.60
2 months	0.877	0.752	22.094	0.01	0.752	0.890	-16.212	0.01
2 ½ months	0.874	0.705	55.891	0.01	0.705	0.840	-27.036	0.01
Mean	0.883	0.778	23.720	2.258	0.778	0.873	-9.972	4.43

b) Farmer's field

Period of moisture stress	Membrane stability							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability	Stress	Control	t value	Probability
1 months	0.959	0.840	27.494	0.01	0.840	0.844	2.121	6.00
1½ month	0.959	0.805	29.728	0.01	0.805	0.853	0.460	65.60
2 months	0.936	0.756	21.930	0.01	0.756	0.860	-5.457	0.01
2½ months	0.953	0.713	50.638	0.01	0.713	0.878	-20.942	0.01
Mean	0.952	0.779	32.448	0.01	0.779	0.858	-5.955	18.155

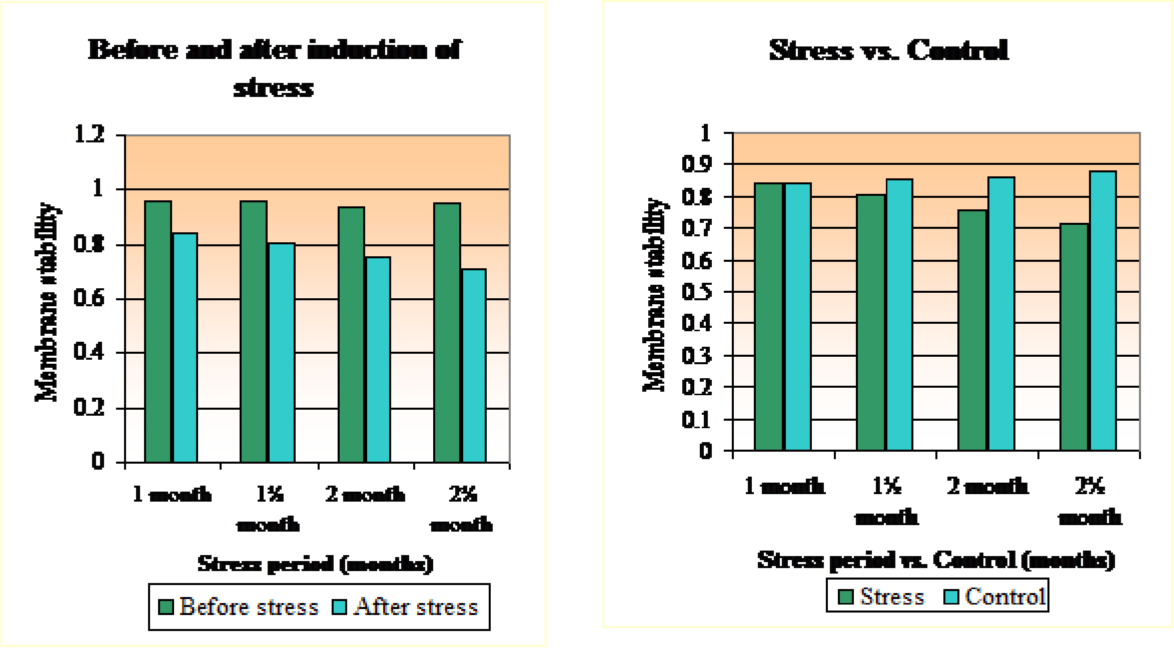
A mean decrease of 11.9 per cent in membrane stability was noted at the end of stress period in PC & S farm. The decrease in membrane stability was more pronounced and significant as intensity of stress advanced. The percentage decrease in membrane stability during various stress periods viz. one month, 1½ month, two months and 2½ months were 3.46, 10.95, 14.25 and 19.34 per cent respectively. In contrast, the control plants registered 12.21 per cent higher mean membrane stability than stressed plants at the termination of different stress periods.



Before and after induction of stress

Stress vs. Control

Plantation Crops and Spices farm



Before and after induction of stress

Stress vs. Control

Farmer's field

Fig. 2b. Membrane stability as influenced by soil moisture stress

A significant decrease in membrane stability was observed in stressed plants of farmer's field also, the mean decrease being 18.2 per cent. The percentage decrease in membrane stability was in the range of 12.41 to 25.18 in plants subjected to various stress periods in farmer's field. The control plants registered 10.10 per cent higher membrane stability than stressed plants.

4.1.2.2 Biochemical response

4.1.2.2.1 Total chlorophyll

The total chlorophyll content was assessed in samples taken from same plant before and after induction of stress and in control plants at the termination of various stress periods. The total chlorophyll content decreased in plants stressed for various periods (Table 7 and Fig. 3a).

Table 7. Effect of moisture stress on total chlorophyll content in vanilla (*Vanilla planifolia* Andrews)

a) Plantation Crops and Spices farm

Period of moisture stress	Total chlorophyll content (mg g^{-1} of tissue)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	0.254	0.244	2.677	4.4	0.244	0.260	-6.072	0.01
1 ½ month	0.249	0.230	5.273	3.0	0.230	0.273	-7.372	0.01
2 months	0.247	0.209	6.430	1.0	0.209	0.277	-20.107	0.01
2 ½ months	0.256	0.202	27.937	0.01	0.202	0.322	-14.807	0.01
Mean	0.252	0.221	10.579	2.103	0.221	0.283	-8.043	0.01

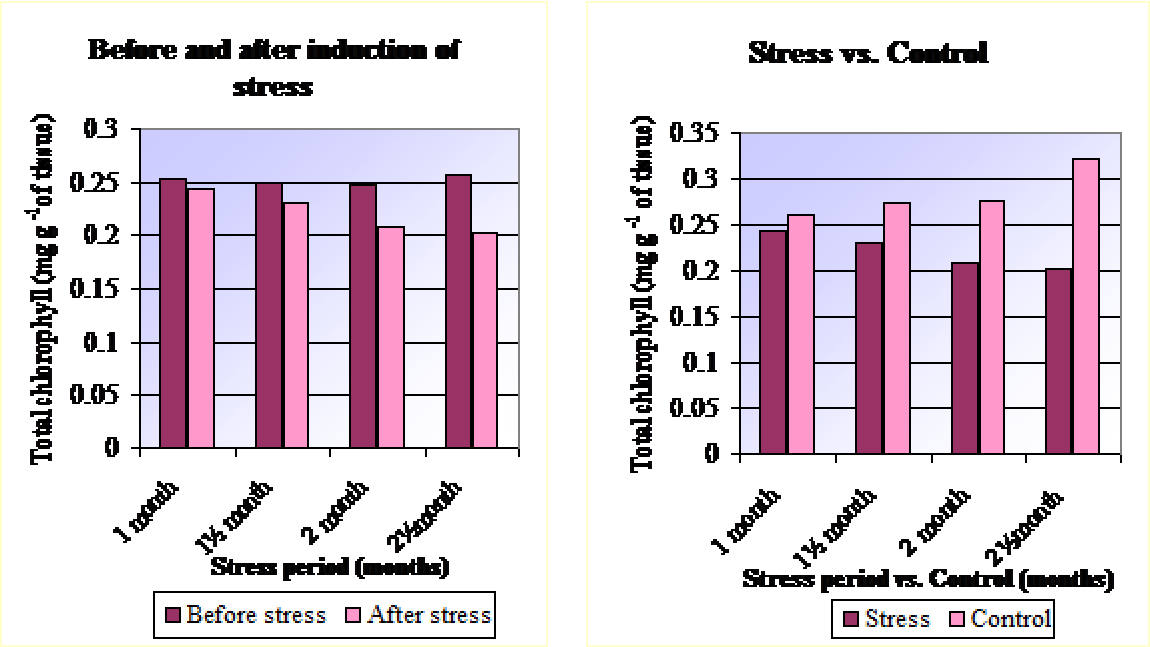
a) Farmer's field

Period of moisture stress	Total chlorophyll content (mg g^{-1} of tissue)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	0.334	0.324	15.789	0.01	0.324	0.326	10.068	0.01
1 ½ months	0.337	0.319	2.718	4.20	0.319	0.327	6.278	0.01
2 months	0.360	0.278	18.913	0.01	0.278	0.299	-3.386	0.70
2 ½ months	0.369	0.258	18.845	0.01	0.258	0.320	-17.112	0.01
Mean	0.350	0.295	14.066	1.056	0.295	0.318	-4.152	0.183

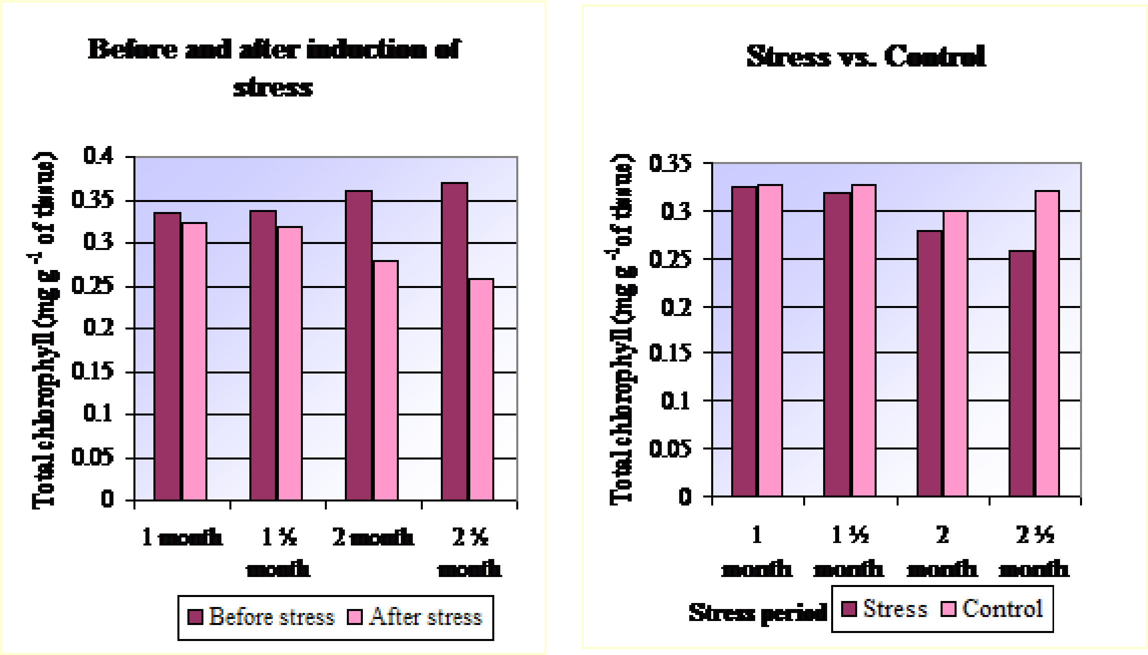
The moisture stress significantly reduced the chlorophyll content in vanilla at both the locations. Before induction of stress, the mean total chlorophyll content was 0.252 mg g^{-1} in plants sampled from PC & S farm and it decreased to 0.221 mg g^{-1} at the end of stress period, registering a decrease of 12.3 per cent in the content of total chlorophyll. The decrease in total chlorophyll content was more in plants stressed for longer periods. The percentage decreases in total chlorophyll content during various stress periods viz. one month, 1½ month, two months and 2½ months were 3.93, 7.63, 15.38 and 21.09 respectively.

The total chlorophyll content in control plants sampled at the termination of various stress periods were compared with the content in stressed plants. The control plant samples registered 28 per cent higher chlorophyll content than stressed plants.

In farmer's field also, the same pattern in the content of total chlorophyll was observed. A decrease of 15.7 per cent in mean content of total chlorophyll was observed after the termination of stress period. Similarly, the reduction in total chlorophyll content was more pronounced as the stress period advanced. In the given stress periods of one month, 1½ month, two months and 2½ months, the corresponding decreases in percentage of total chlorophyll were 2.99, 5.34, 22.78 and 30.08 respectively. Also, the control plants registered higher (7.80 %) chlorophyll content than stressed plants.



Plantation Crops and Spices farm



Farmer's field

Fig. 3a. Total chlorophyll content as influenced by soil moisture stress

The total chlorophyll content in general was high in farmer's field and reduction in the content of total chlorophyll due to moisture stress was also more pronounced in farmer's field. The mean percentage reduction in total chlorophyll due to moisture stress was 15.71 per cent in farmer's field, whereas it was 12.30 per cent in PC & S farm.

4.1.2.2.2 Epicuticular wax content

Significant increase in the content of epicuticular wax was noticed due to moisture stress at both the locations (Table 8 and Fig. 3b).

Table 8. Effect of moisture stress on epicuticular wax content in vanilla (*Vanilla planifolia* Andrews)

a) Plantation Crops and Spices farm

Period of moisture stress	Epicuticular wax content (mg g^{-1} of tissue)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	13.568	19.423	-13.344	0.01	19.423	13.176	26.078	0.01
1½ months	11.345	16.846	-45.890	0.01	16.846	10.196	16.622	0.01
2 months	11.319	18.226	-18.199	0.01	18.226	7.293	37.316	0.01
2½ months	12.621	20.563	-47.815	0.01	20.563	7.236	34.298	0.01
Mean	12.213	18.765	-31.312	0.01	18.765	9.475	28.579	0.01

b) Farmer's field

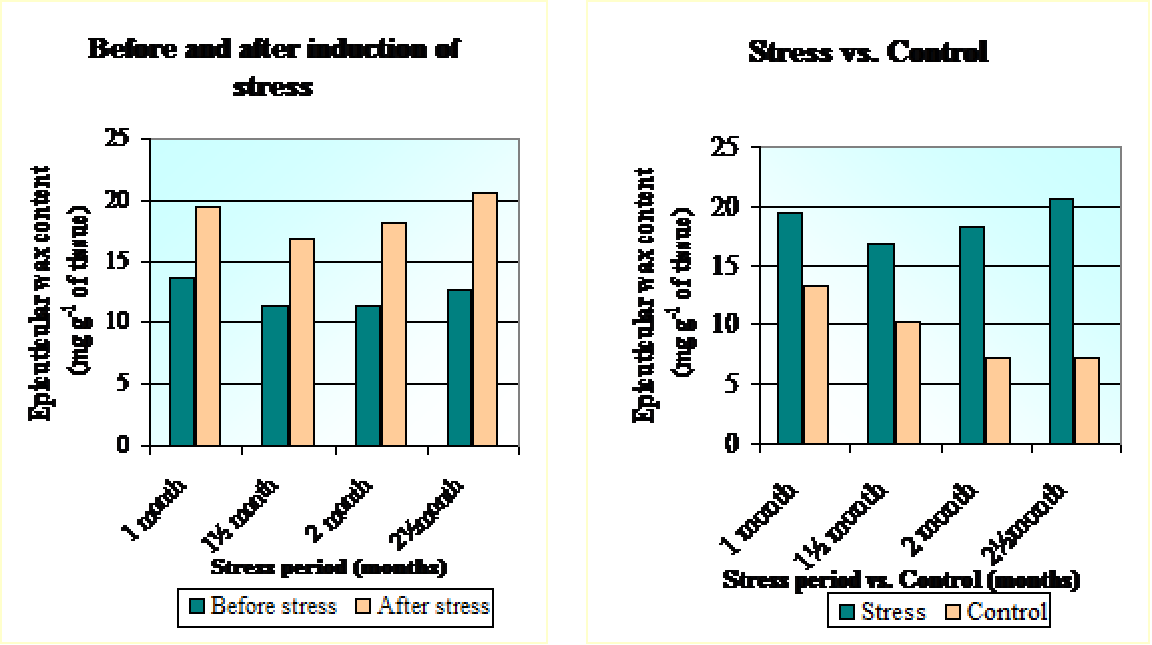
Period of moisture stress	Epicuticular wax content (mg g^{-1} of tissue)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability perecntage	Stress	Control	t value	Probability percentage
1 month	12.815	16.391	-16.287	0.01	16.391	13.479	15.903	0.01
1 ½ month	12.951	18.211	-41.887	0.01	18.211	13.337	45.559	0.01
2 month	12.794	19.372	-42.635	0.01	19.372	9.761	48.223	0.01
2 ½ month	13.160	20.412	-45.586	0.01	20.412	10.164	65.284	0.01
Mean	12.930	18.597	-36.598	0.01	18.597	11.685	43.742	0.01

In PC & S farm, the mean epicuticular wax content before the induction of stress was 12.213 mg g^{-1} and it increased to 18.765 mg g^{-1} at the end of stress period, registering an increase of 53.6 per cent. As the intensity of stress advanced, the wax content also increased. The percentage increase in epicuticular wax content during the given stress periods viz. one month, 1½ month, two months and 2½ months were 43.20, 48.50, 61.00 and 62.90 respectively.

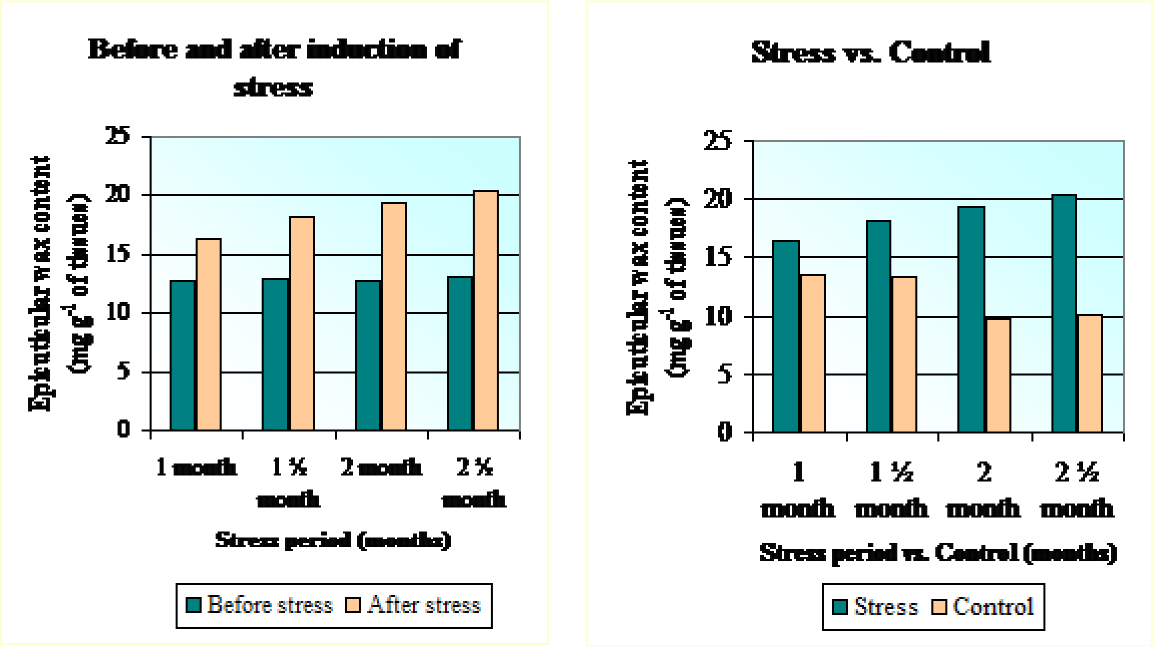
In contrast, control plants sampled after various stress periods registered 49.50 per cent lesser wax content than stressed plants.

The same trend in epicuticular wax content was noticed in farmer's field, wherein the percentage increase in wax content at the end of stress period was 43.80 per cent.

The content of epicuticular wax was comparable in samples analyzed from both the locations. However, increase in epicuticular wax content due to stress was more in samples analyzed from PC & S farm.



Plantation Crops and Spices farm



Farmer's field

Fig. 3b. Epicuticular wax content as influenced by soil moisture stress

4.1.2.2.4 Soluble protein

There was significant decrease in the content of soluble protein due to moisture stress (Table 9 and Fig. 3c).

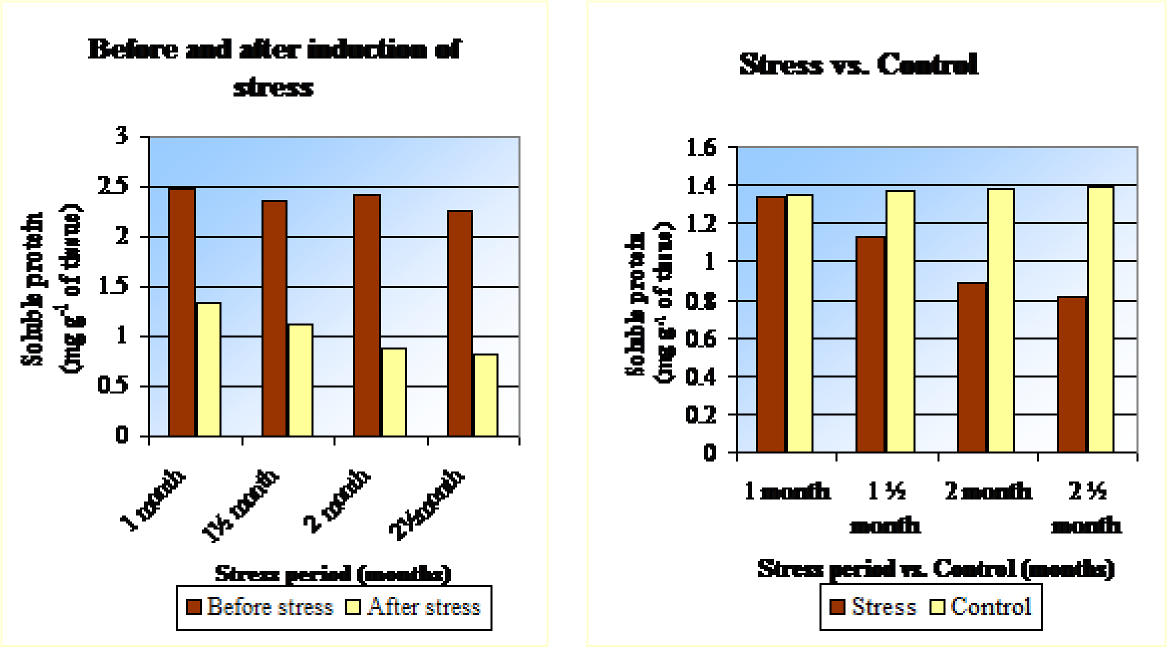
Table 9. Effect of moisture stress on soluble protein content in vanilla (*Vanilla planifolia* Andrews)

a) Plantation Crops and Spices farm

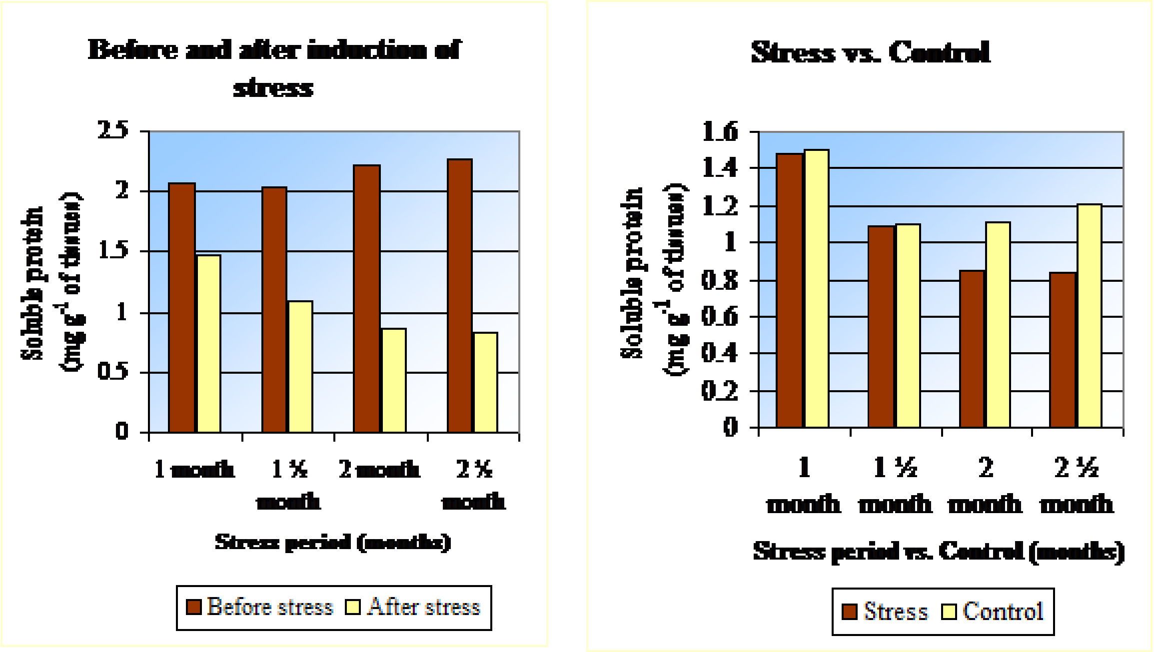
Period of moisture stress	Soluble protein content (mg g^{-1} of tissue)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	2.476	1.339	18.159	0.01	1.339	1.350	-1.340	89.60
1 ½ month	2.351	1.130	175.378	0.01	1.130	1.374	-6.120	0.01
2 months	2.416	0.887	51.057	0.01	0.887	1.382	-10.660	0.01
2 ½ months	2.257	0.815	81.652	0.01	0.815	1.393	-16.836	0.01
Mean	2.375	1.043	81.561	0.01	1.043	1.375	-8.739	22.408

b) Farmer's field

Period of moisture stress	Soluble protein content (mg g^{-1} of tissue)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	2.071	1.480	4.734	5.00	1.480	1.506	11.433	0.01
1 ½ month	2.031	1.093	19.101	0.01	1.093	1.097	2.049	6.80
2 months	2.215	0.853	33.270	0.01	0.853	1.114	-6.791	0.01
2 ½ months	2.262	0.835	23.029	0.01	0.835	1.210	-27.439	0.01
Mean	2.145	1.065	20.033	1.258	1.065	1.232	5.187	1.708



Plantation Crops and Spices farm



Farmer's field

Fig. 3c. Soluble protein content as influenced by soil moisture stress

The decrease in the content of soluble protein was more as the stress period advanced in both the locations. The soluble protein content decreased to a mean level of 56 per cent at the termination of stress period in PC & S farm. Different stress periods viz. one month, 1½ month, two months and 2½ months recorded a decrease of 45.92, 51.94, 63.29 and 63.89 per cent respectively in the content of soluble protein. The corresponding values of soluble protein recorded in control plants sampled at the termination of various stress periods registered a mean increase of 31.8 per cent over stressed plants.

In samples from farmer's field, the soluble protein content reduced to a mean level of 50.40 per cent. The percentage decrease in soluble protein content during various stress periods was 28.50, 46.20, 61.50 and 63.00 respectively. The control plants (no moisture stress) recorded 15.60 per cent higher soluble protein content than plants in the stressed condition.

The decrease in soluble protein content before stress vs. after stress and stress vs. control were statistically significant at both the fields except the soluble protein content at 1 month stress vs. control in PC & S farm.

4.1.2.2.4 Total free amino acid

The moisture stress significantly increased the content of total free amino acid in vanilla at both the locations (Table 10 and Fig.3d).

Table 10. Effect of moisture stress on total free amino acids in vanilla (*Vanilla planifolia* Andrews)

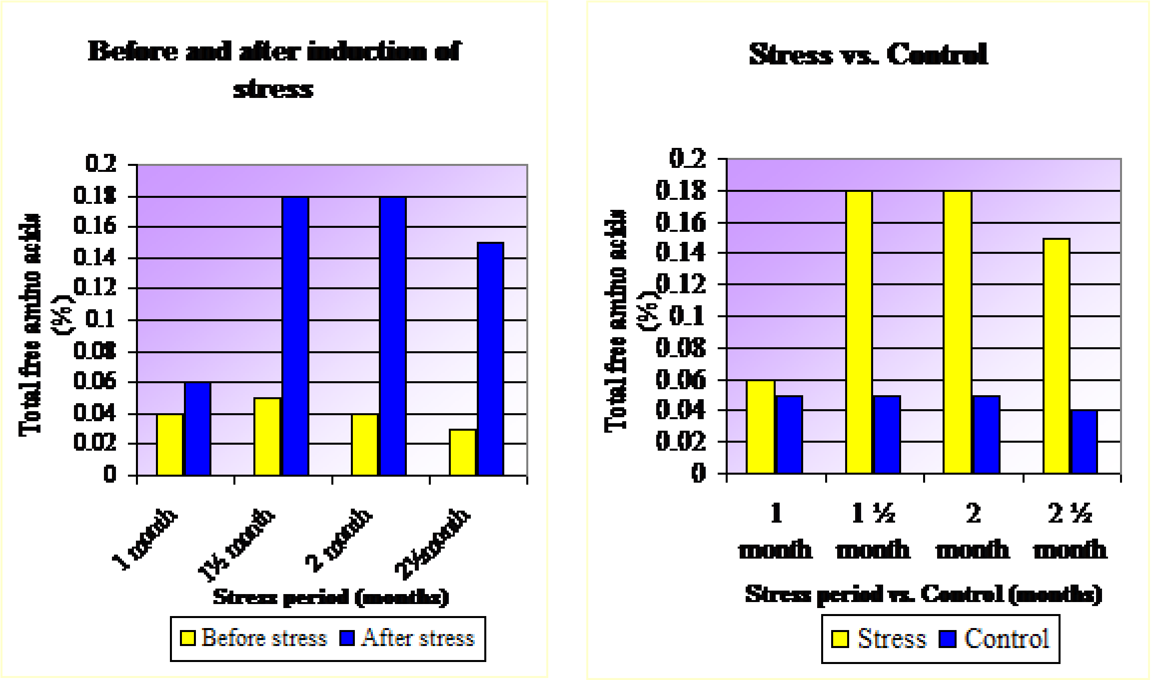
a) Plantation Crops and Spices farm

Period of moisture stress	Total free amino acid content (%)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	0.04	0.06	-2.40	6.20	0.06	0.05	8.67	0.01
1 ½ months	0.05	0.18	-20.57	0.01	0.18	0.05	8.68	0.01
2 months	0.04	0.18	-24.18	0.01	0.18	0.05	21.14	0.01
2 ½ months	0.03	0.15	-107.25	0.01	0.15	0.04	34.26	0.01
Mean	0.04	0.14	38.61	1.56	0.14	0.05	18.19	0.01

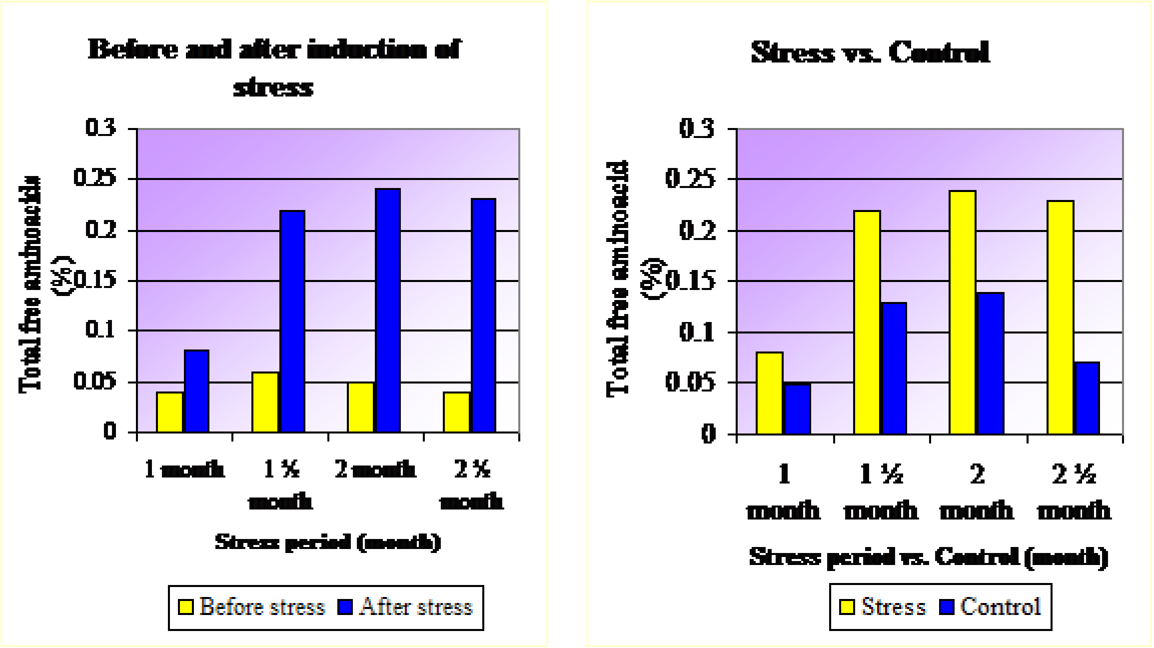
b) Farmer's field

Period of moisture stress	Total free amino acid content (%)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	0.04	0.08	-6.759	0.10	0.08	0.05	6.86	0.01
1 ½ month	0.06	0.22	-29.018	0.01	0.22	0.13	20.18	0.01
2 months	0.05	0.24	-132.109	0.01	0.24	0.14	19.42	0.01
2 ½ months	0.04	0.23	-108.800	0.01	0.23	0.07	61.69	0.01
Mean	0.05	0.19	-69.172	0.03	0.19	0.10	27.04	0.01

The increase in total free amino acid content due to moisture stress was 2.5 times higher than the content in samples before stress in PC & S farm. The increase in total free amino acid was higher in plants stressed for longer periods. Different stress periods such as one month 1½ month, two months and 2½ months recorded an increase of 0.5 fold, 2.6



Plantation Crops and Spices farm



Farmer's field

Fig. 3d. Total free aminoacids as influenced by moisture stress fold, 3.5 fold and 4 fold in the content of total free amino acid. The total free amino acid content in the control plant samples was 0.64 times lesser than the stressed plants.

The total free amino acid due to moisture stress observed in plants sampled from farmer's field was 2.8 times higher than in plants without moisture stress. The increase in total free amino acid content during various stress periods viz. one month, 1½ month, two months and 2½ months were 1 fold, 2.6 fold, 3.8 fold and 4.7 fold respectively. The control plants registered lesser total free amino acid content than the stressed plants.

The increase in the content of total free amino acid due to moisture stress was more in farmer's field.

4.1.1.2.5 Proline

Generally the proline content increased as the intensity of stress advanced in both the locations (Table 11 and Fig.3e).

Table 11. Effect of moisture stress on proline content in vanilla (*Vanilla planifolia* Andrews)

a) Plantation Crops and Spices farm

Period of moisture stress	Proline content (µg g^{-1} of tissue)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	3.641	3.804	-2.781	3.90	3.804	3.704	1.168	27.00
1 ½ months	3.519	4.134	-13.558	0.01	4.134	3.560	4.370	0.10
2 months	3.502	4.306	-14.534	0.01	4.306	3.495	3.263	0.90
2½ months	3.569	5.465	-27.709	0.01	5.465	3.100	24.551	0.01
Mean	3.558	4.427	-14.646	0.983	4.427	3.465	8.338	7.003

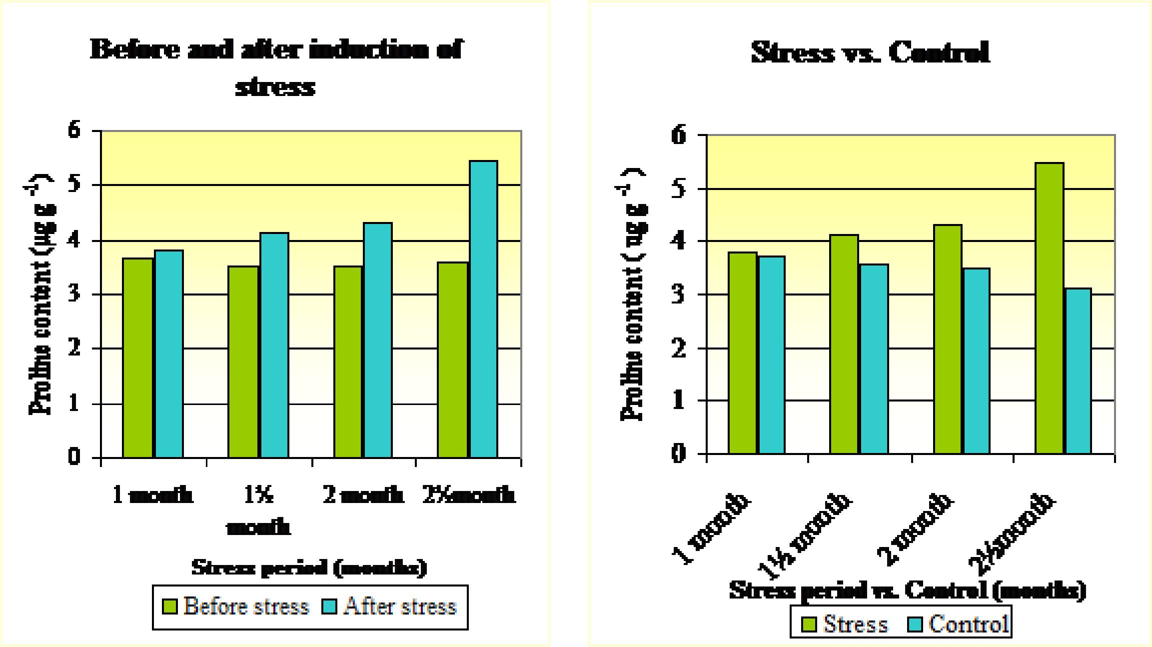
b) Farmer's field

Period of moisture stress	Proline content (µg g^{-1} of tissue)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	4.856	5.302	-14.123	0.01	5.302	3.858	19.242	0.01
1 ½ month	4.677	5.172	-4.761	0.50	5.172	3.197	15.579	0.01
2 months	4.797	5.608	7.448	0.10	5.608	3.028	19.374	0.01
2 ½ months	4.616	5.463	-4.667	0.50	5.463	2.968	29.978	0.01
Mean	4.736	5.386	-7.749	0.278	5.386	3.263	21.043	0.01

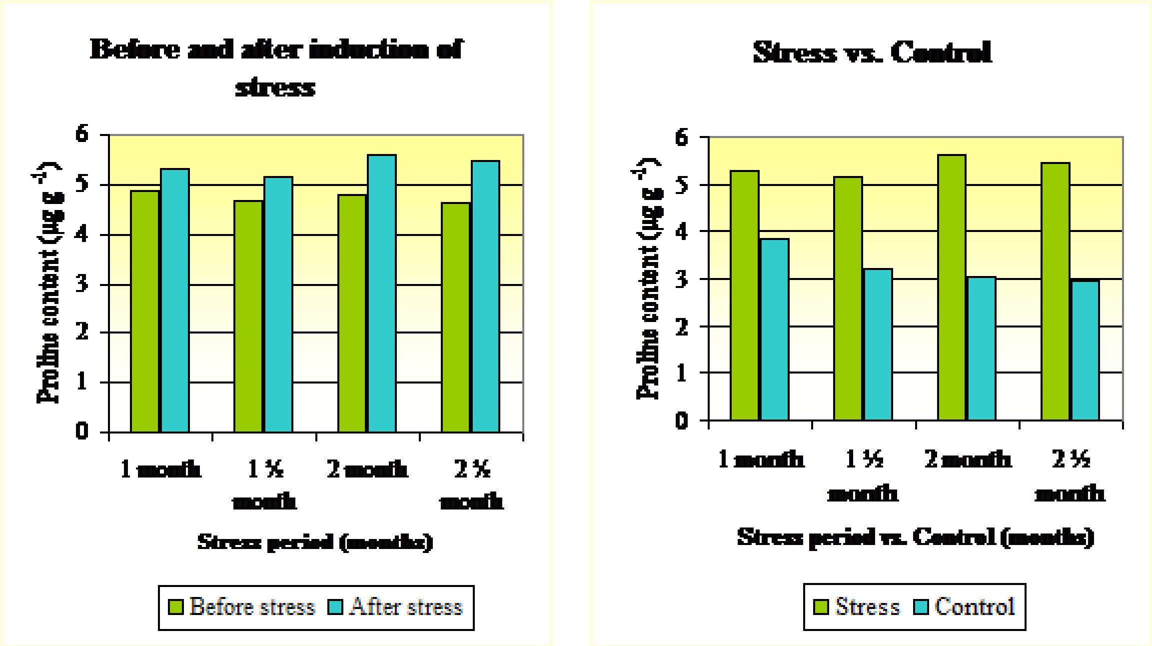
Before the induction of stress, the mean content of proline in PC & S farm samples was 3.558 µg and it increased to 4.427 µg after the cessation of stress period, registering an increase of 24.40 per cent in the content of proline. The percentage increase in proline content during various stress periods were 4.50, 17.50, 23.0 and 53.0 in one month, 1½ month, two months and 2½ months respectively. The mean proline content in control plants sampled after termination of various stress periods were compared with the proline content in stressed plants. Control plants registered 21.70 per cent lesser proline content as compared to the stressed plants.

In farmer's field, mean increase in proline content at the end of stress periods was 13.7 per cent higher than in plants without stress. The maximum percentage increase in proline content (18.4%) was observed in 2½ months stress period and the least in one month (9.2%) stress period. Control plant samples registered 39.4 per cent lesser proline content as compared to the stressed plants.

The proline content in general was high in plants sampled from farmer's field and the percentage increase in proline content was high in PC & S farm.



Plantation Crops and Spices farm



Farmer's field

Fig. 3e. Proline content as influenced by soil moisture stress

4.1.2.2.6 Activity of peroxidase enzyme

The activity of peroxidase enzyme increased due to moisture stress in vanilla samples collected from both the locations (Table 12 and Fig.3 f).

Table 12. Effect of moisture stress on peroxidase activity in vanilla (*Vanilla planifolia* Andrews)

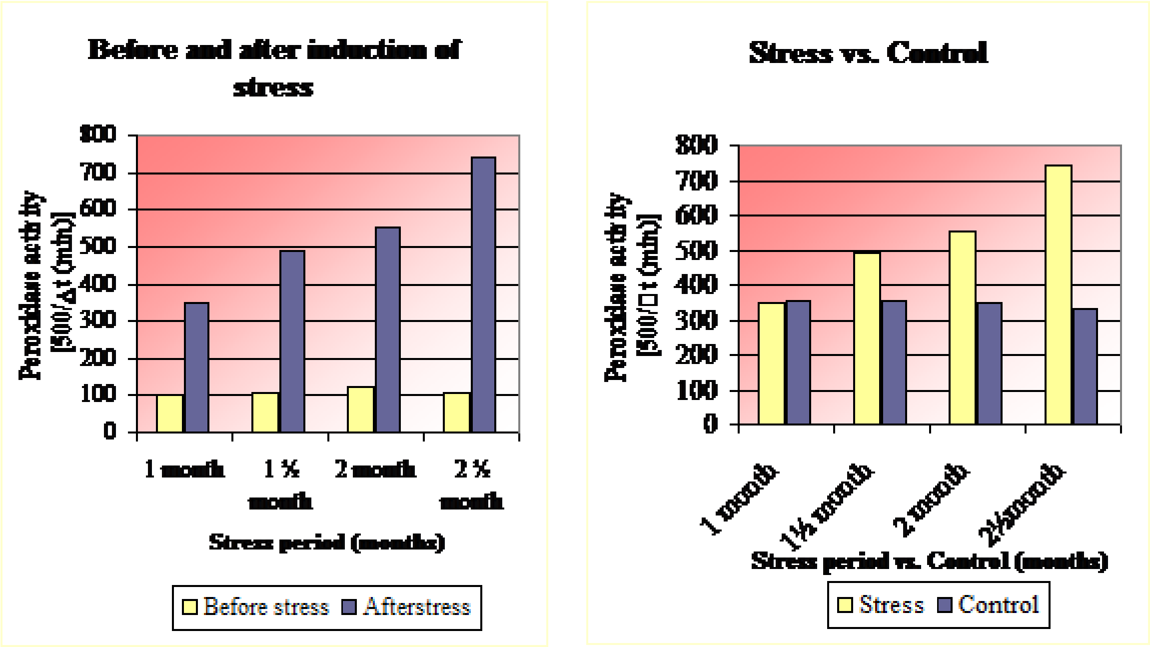
a) Plantation Crops and Spices farm

Period of moisture stress	Peroxidase activity $\left(\frac{500}{\Delta t\ (\text{min})}\right)$							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	101.722	349.892	-71.649	0.01	349.892	356.414	-1.411	18.90
1 ½ months	107.064	491.117	-84.525	0.01	491.117	356.381	63.436	0.01
2 months	122.627	555.395	-37.000	0.01	555.395	347.722	23.197	0.01
2 ½ months	106.678	742.531	-139.874	0.01	742.531	329.549	58.769	0.01
Mean	109.523	534.734	-83.262	0.01	534.734	347.517	35.998	4.733

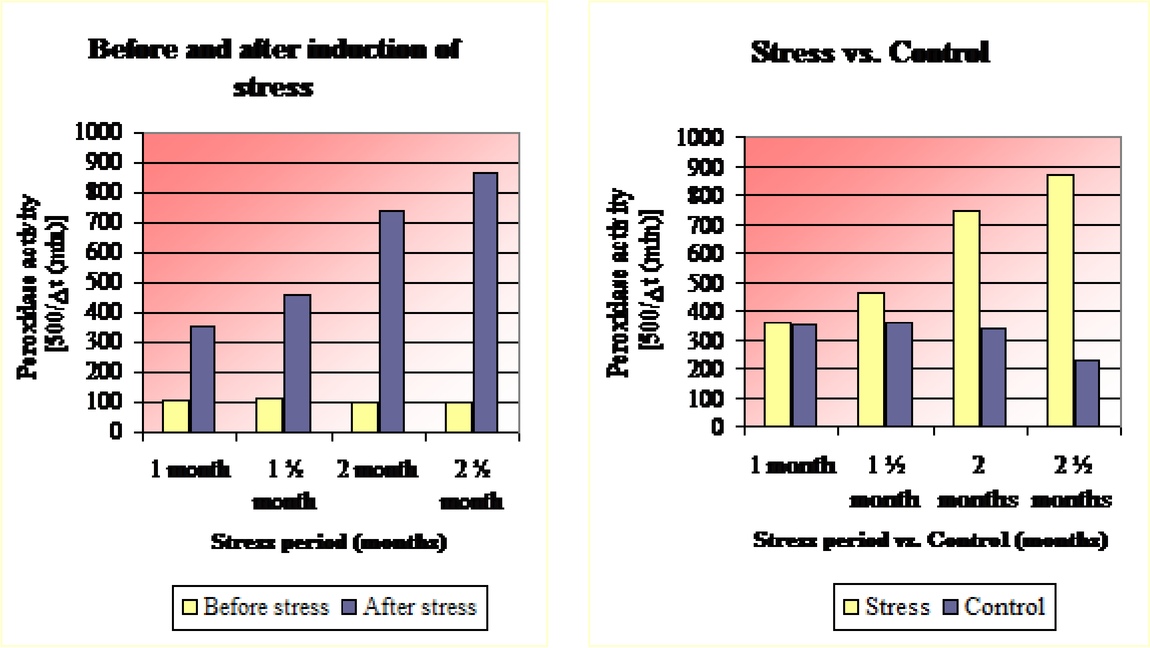
b) Farmer's field

Period of moisture stress	Peroxidase activity $\left(\frac{500}{\Delta t\ (\text{min})}\right)$							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	108.812	356.048	-126.732	0.01	356.048	354.365	-1.423	18.50
1 ½ month	110.681	461.885	-35.071	0.01	461.885	355.507	10.507	0.01
2 months	101.813	742.531	-99.723	0.01	742.531	339.280	77.134	0.01
2 ½ months	98.292	865.709	-50.830	0.01	865.710	225.152	43.923	0.01
Mean	104.900	606.543	-78.089	0.01	606.544	318.576	32.535	4.633

The enzyme activity was 3.9 times higher in samples collected from PC & S farm after the termination of stress period. The increase in peroxidase activity was more in



Plantation Crops and Spices farm



Farmer's field

Fig. 3f. Peroxidase activity as influenced by soil moisture stress plants stressed for longer periods. Among the various stress treatments, 2½ months stress period recorded the highest increase in the enzyme activity (5.9 fold) and the least in one month stress period (2.4 fold). In contrast, the control plant samples registered lesser peroxidase activity than the stressed plants.

The same pattern in enzyme activity was observed in farmer's field also. At the end of stress period, an increase of 4.8 fold higher peroxidase activity was observed. The increase in enzyme activity was in the range of 2.3 to 7.8 fold in farmer's field during various stress periods. The control plant samples registered 0.5 times lesser peroxidase activity as compared to the stressed plants.

One month stress period given to vanilla plants had not altered the peroxidase activity significantly at both the locations.

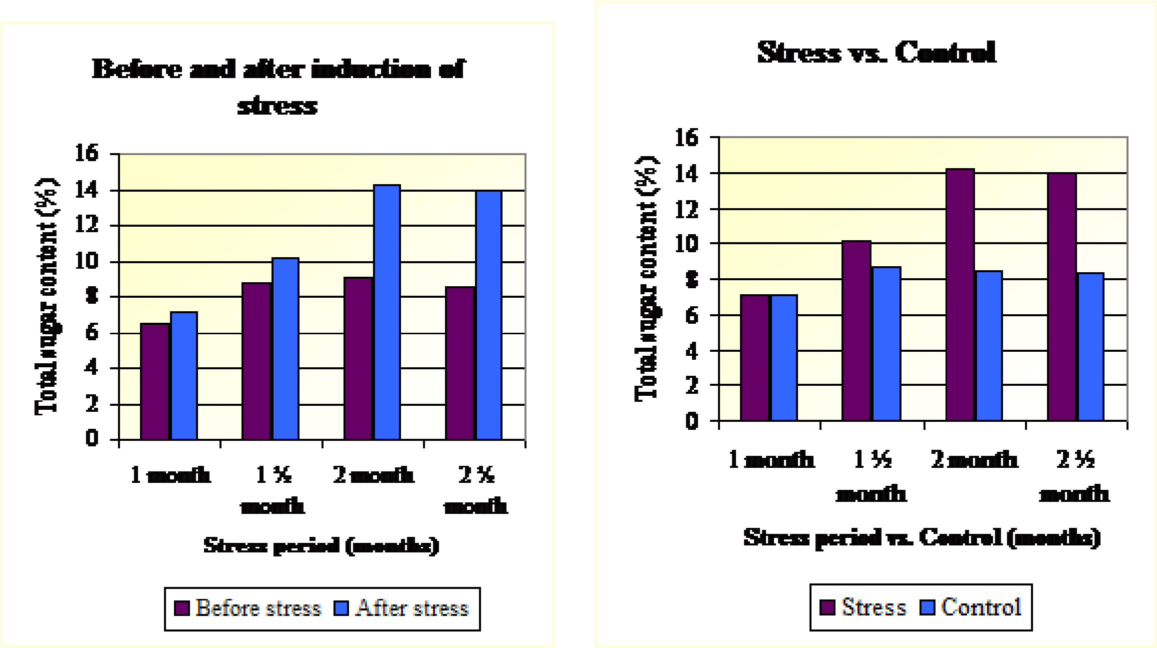
4.1.1.2.8 Total sugar

A significant increase in total sugar content was observed in plants stressed for different periods in both the locations (Table 13 and Fig. 3g).

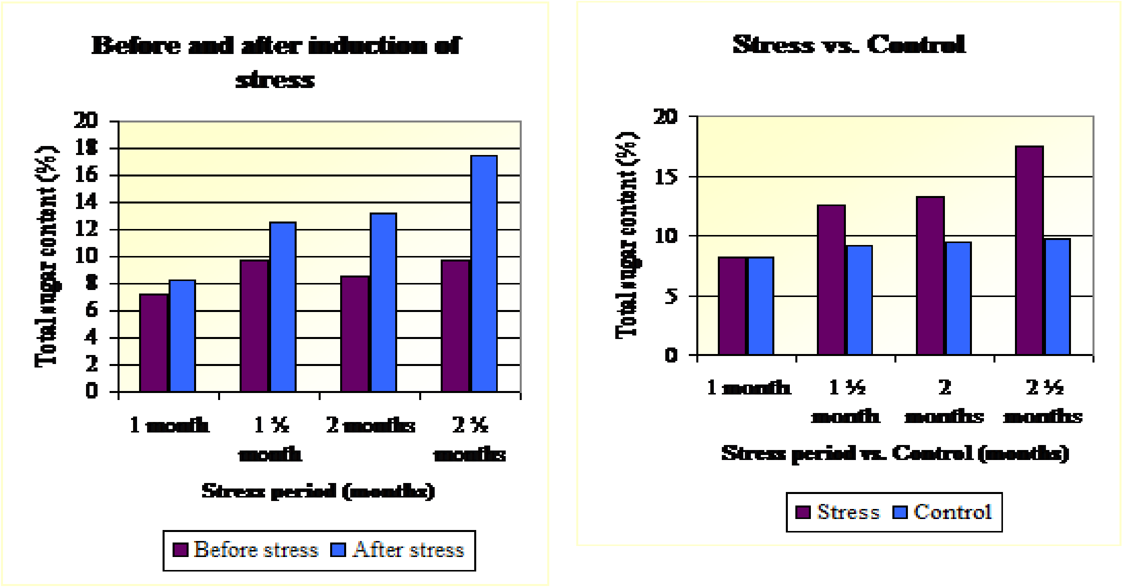
Table 13. Effect of moisture stress on total sugar content in vanilla (*Vanilla planifolia* Andrews)

a) Plantation Crops and Spices farm

Period of moisture stress	Total sugar content (%)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	6.46	7.14	-3.73	1.40	7.14	7.12	-7.16	0.01
1 ½ month	8.73	10.19	-14.95	0.01	10.19	8.73	13.81	0.01
2 months	9.04	14.23	-41.84	0.01	14.23	8.49	27.42	0.01
2 ½ months	8.58	13.99	-62.32	0.01	13.99	8.30	30.73	0.01
Mean	8.20	11.39	-30.71	0.36	11.38	8.16	64.80	0.01



Plantation Crops and Spices farm



Farmer's field

Fig. 3g. Total sugar content as influenced by soil moisture stress

b) Farmer's field

Period of moisture stress	Total sugar content (%)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	7.14	8.21	-4.97	0.40	8.21	8.20	0.35	73.60
1 ½ month	9.70	12.50	-14.39	0.01	12.50	9.15	7.64	0.01
2 months	8.50	13.20	-41.87	0.01	13.20	9.46	31.06	0.01
2 ½ months	9.80	17.46	-62.33	0.01	17.46	9.66	62.04	0.01
Mean	8.79	12.84	-30.89	0.11	12.84	9.12	25.27	18.41

The mean increase in total sugar content was 38.90 per cent at the end of stress period in plants sampled from PC & S farm. Different stress periods such as one month, 1½ month, two months and 2½ months recorded a percentage increase of 10.53, 16, 72, 57.41 and 63.05 respectively in total sugar content. When the stressed plant samples were compared with the control plants, the control plants recorded 28.29 per cent lesser sugar content.

In farmer's field, the increase in total sugar content observed in the plants stressed for various periods was 46 per cent. The percentage increase in total sugar content during various stress periods was in the range of 14.99 to 78.16. Control plants recorded 28.97 per cent lesser total sugar content as compared to stressed plants.

4.1.2.2.8 Potassium concentration in tissues

Potassium concentration increased significantly with advancement of stress periods in plants sampled from both the locations (Table 14 and Fig.3 h).

Table 14. Effect of moisture stress on potassium concentration in leaf tissues in vanilla (*Vanilla planifolia* Andrews)

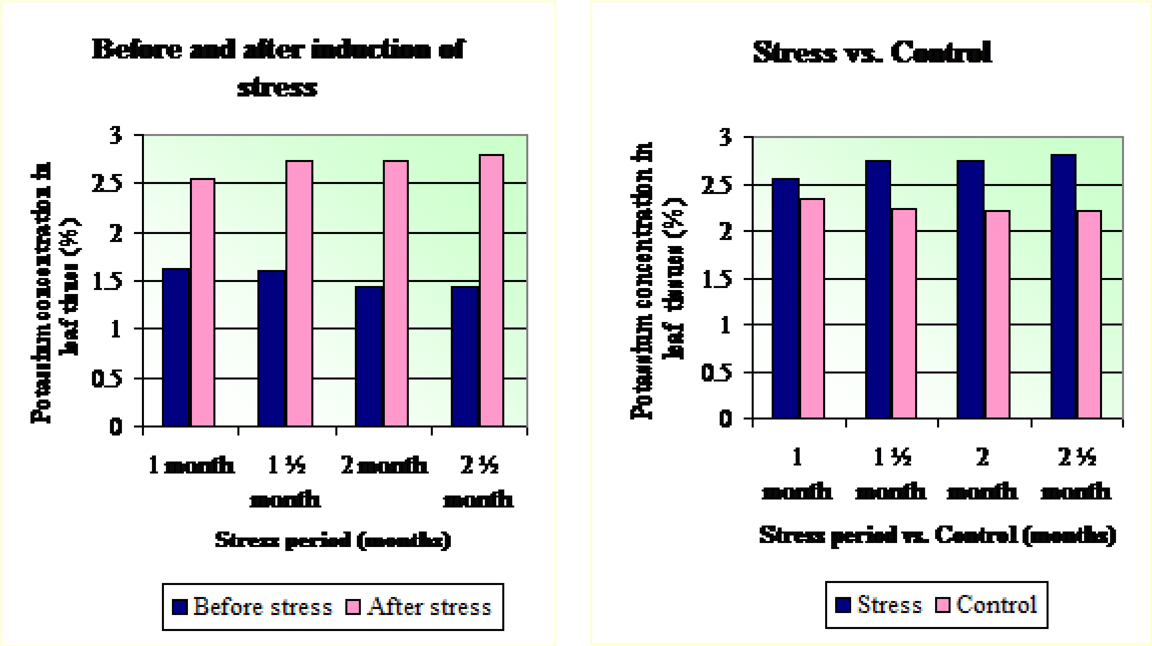
a) Plantation Crops and Spices farm

Period of moisture stress	Potassium concentration in tissues (%)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	1.614	2.546	-24.685	0.01	2.546	2.332	3.989	0.30
1 ½ month	1.609	2.739	-53.324	0.01	2.739	2.241	23.748	0.01
2 months	1.444	2.739	-63.990	0.01	2.739	2.218	30.367	0.01
2 ½ months	1.444	2.799	-18.956	0.01	2.799	2.211	9.840	0.01
Mean	1.528	2.706	-40.239	0.01	2.706	2.251	16.986	0.083

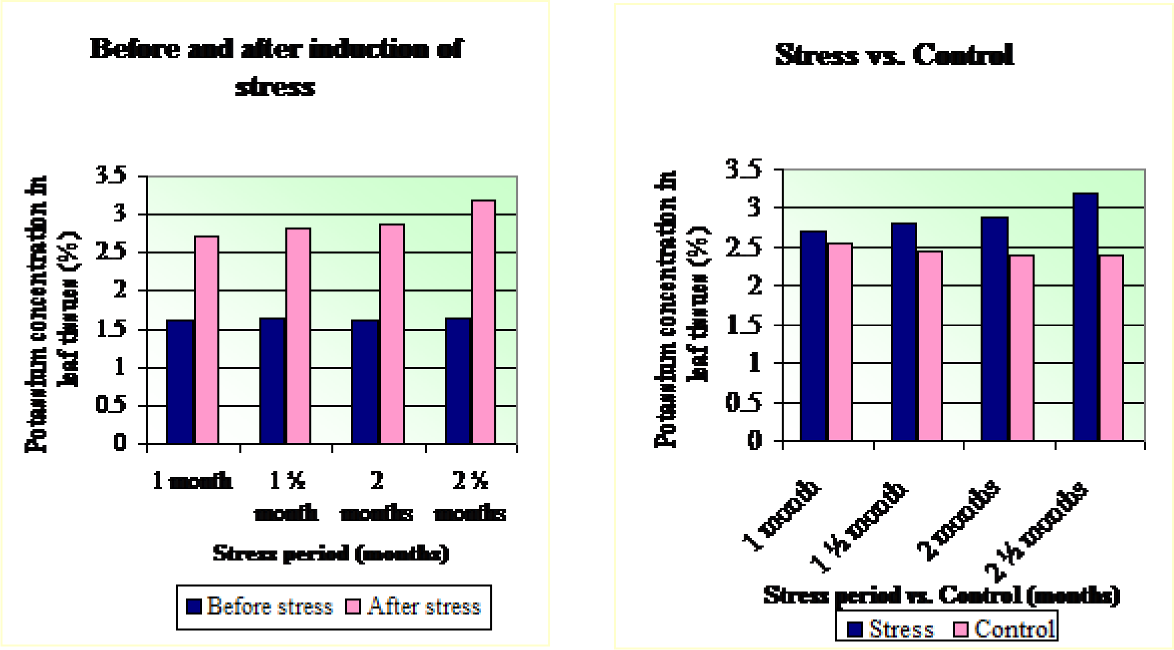
b) Farmer's field

Period of moisture stress	Potassium concentration in tissues (%)							
	Before stress vs. after stress				Stress vs. control			
	Before stress	After stress	t value	Probability percentage	Stress	Control	t value	Probability percentage
1 month	1.619	2.712	-43.657	0.01	2.712	2.535	6.414	0.01
1 ½ month	1.646	2.813	-143.866	0.01	2.813	2.448	5.417	0.01
2 months	1.612	2.884	-65.043	0.01	2.884	2.391	26.217	0.01
2 ½ months	1.635	3.188	-94.946	0.01	3.188	2.381	58.421	0.01
Mean	1.628	2.899	-86.878	0.01	2.899	2.439	24.117	0.01

Before stress, the mean potassium concentration in plants sampled was 1.528 per cent and it increased to 2.706 per cent at PC & S farm, indicating an increase of 77 per cent. The percentage increase in potassium concentration during various stress periods viz. one month, 1½ month, two months and 2½ months were 57.70, 70.20, 89.70 and 93.80 respectively. The mean potassium concentration in control plants analyzed at the elapse of different stress periods was 16.80 per cent lesser as compared to samples from stressed plants.



Plantation Crops and Spices farm



Farmer's field

Fig. 3h. Potassium concentration in leaf tissues as influenced by soil moisture stress

The same trend in potassium concentration was noticed in farmer's field also, wherein the mean potassium concentration was 1.628 per cent before the induction of stress and it increased to 2.899 per cent at the end of stress period, indicating an increase of 78.0 per cent in potassium content. The plants sampled from various stress periods recorded an increase in potassium concentration in the range of 67.51 to 95 per cent. As compared to the stressed plants, the control plants recorded 15.8 per cent lesser potassium than the stressed plants.

4.1.3 Effect of different periods of moisture stress on flowering in vanilla

For induction of flowering in vanilla, moisture stress was imposed at four levels viz., one, 1½, two and 2½ months. Effect of moisture stress on percentage flowering, number of inflorescences emerged and number of buds per inflorescence were observed. As there was only less number of plants available for experimentation in PC & S farm, data gathered on flowering from farmer's field alone were subjected to analysis and results are presented in Table 15.

Table 15. Effect of moisture stress on flowering in vanilla (*Vanilla planifolia* Andrews)

Treatment	No. of plants tagged	No. of plants flowered	Percentage flowering	Mean no. of inflorescence emerged / plant	Mean no. of buds / inflorescence	Duration of flowering
No moisture stress (control)	25	1	4	1	15.000	14
1 month	25	20	80	3.85 (2.02)	18.367 (4.292)	69
1½ month	25	15	60	4.2 (2.13)	18.844 (4.372)	69
2 months	25	13	52	3.23 (1.86)	23.138 (4.792)	71
2½ months	15	4	26.66	1.75 (1.49) CD 0.1 (0.303)	21.500 (4.635) NS	74

4.1.3.1 Percentage flowering

Different periods of moisture stress induced were found to influence flowering in vanilla. Maximum percentage flowering (80%) was observed in plants stressed for moisture for a period of one month. The percentage flowering observed in 1½ month and two months moisture stress treatments was almost uniform recording 60 and 52 per cent flowering respectively. However, the percentage flowering observed in 2½ months stress treatment was very low (26.6%). In control plants, which was irrigated normally and no moisture stress was given recorded only four percentage flowering. So a moisture stress period of one month is sufficient to induce flowering in vanilla. Various stages of flowering in vanilla are presented in Plate 4.

4.1.3.2 Number of inflorescence emerged per plant

Number of inflorescence emerged per plant during each stress treatment was calculated and analyzed statistically. Out of the five stress treatments, maximum number of inflorescence was emerged in 1½ month stress treatment (4.2). The number of inflorescence emerged in one month, 1½ month and two months stress treatments were on par recording 3.85, 4.2 and 3.23 inflorescences respectively. A stress period of 2½ months recorded minimum number of inflorescence (1.75). Since only one plant flowered in the control treatment, the data was not statistically analyzed. The number of inflorescence emerged per plant was significantly lower in 2½ months stress treatment as compared to other periods of stress.

4.1.3.3 Number of buds per inflorescence

There was no significant difference in mean number of buds per inflorescence in the various stress periods studied in the present investigations. Maximum number of buds per inflorescence emerged in two month stress period (23.138), followed by 2½ months stress period (21.500).The mean number of buds per inflorescence emerged were 18.844

Vegetative bud

Floral bud

1 month stress **1½ month stress**

Flower bud emergence

Inflorescence emergence

Flowering

Plate 4. Various stages of flowering in vanilla

and 18.367 in 1½ month and one month stress periods respectively. Control treatment was not statistically analyzed due to insufficient data.

4.1.3.4 Duration of flowering

Duration of flower opening in vanilla varied from 69 to 74 days in plants stressed for different periods of moisture stress. The duration of flowering was almost uniform in plants subjected to different stress periods. The duration of flowering was 74 days in 2½ months stress period, followed by 71 days in two months stress period. As only one inflorescence was produced in control plants, the duration of flowering was only 14 days.

4.2 INFLUENCE OF MICRO METEOROLOGICAL FACTORS ON FLOWERING IN VANILLA

The micro meteorological parameters like temperature, relative humidity, light intensity, bright sunshine hours, wind speed and number of rainy days were recorded for a period of eight months starting from October, 2006 to May 2007. The study period was divided into three viz. post monsoon (October to November) winter (December to February) and summer (March to May) periods. The micro meteorological situations at both the locations were compared during these periods. Correlations were worked out to know the influence of micro meteorological factors on flowering in vanilla.

4.2.1 Study of micro meteorological situations at two locations

The micro meteorological parameters viz. maximum, minimum and mean temperature and relative humidity were compared between two locations during October, 2006 to May, 2007.

4.2.1.1 Temperature and relative humidity

Micro meteorological parameters recorded varied in the two locations studied. The mean maximum temperature recorded at PC & S farm during the period was higher

Table 16. Temperature and relative humidity in selected locations

Sl. No.	Parameters	October-November (Post monsoon)			December-February (Winter)			March-May (Summer)			Mean		
		Locations			Locations			Locations			Locations		
		Farmer's field	PC&S farm	Variation between locations	Farmer's field	PC&S farm	Variation between locations	Farmer's field	PC&S farm	Variation between locations	Farmer's field	PC&S farm	Variation between locations
1	Maximum temperature (°C)	31.2	31.9	0.7	33.6	33.9	0.3	33.7	35.6	1.9	32.8	33.8	1.0
2	Minimum temperature (°C)	23.3	23.4	0.1	22.3	21.2	1.1	24.4	23.8	0.6	23.3	22.8	0.5
3	Mean temperature (°C)	27.3	27.7	0.4	27.9	27.5	0.5	29.1	29.7	0.6	28.1	28.3	0.2
4	Relative humidity (%)	74.0	71.0	3.0	45.0	58.0	13.0	68.0	60.0	8.0	62.3	63.0	0.7

by 1°C than that of farmer’s field. The variation was more pronounced during the period from March – May, during which period the variation observed was 1.9°C. In contrast, minimum temperature recorded during the period was lower at PC & S farm when compared to farmer’s field. The decrease was more pronounced during winter period recording a variation of 1.1°C. There was no difference observed between the locations with respect to mean temperature. (Table 16).The variation in relative humidity was more pronounced during winter period between locations. The weekly observations on micro-meteorological parameters were found to vary in the locations studied. The trends of maximum, minimum and mean temperature and relative humidity in the locations studied for a period of eight months (October, 2006 to May, 2007) are presented in Fig.4a to 4f.

The maximum temperature showed more variation in the locations studied during summer months from January to May. During the period, high temperature was recorded at PC & S farm. The maximum temperature during the period was 30.9-37.8°C which was found detrimental to the crop. The trends in maximum temperature in the selected locations during the period from October, 2006 to May, 2007 are presented in Fig. 4 a.

The minimum temperature showed variation in the locations during December to March. The minimum temperature recorded at PC & S farm during the period was lower as compared to farmer’s field. During the period, the lowest minimum temperature was recorded at PC & S farm (19.3°C). In farmer’s field, the lowest minimum temperature recorded during the period was 19.5°C (Fig. 4 b).

The mean temperature recorded was lower in farmer’s field during October-November and March-May and was higher in December-January months when compared to PC & S farm during the same field (Fig. 4c).

The relative humidity showed variation during the month of November-March, between locations and ranged from 44 to 75 per cent at PC & S farm and 32 to 88 per cent at farmer’s field. At PC & S farm, a high relative humidity was observed during the

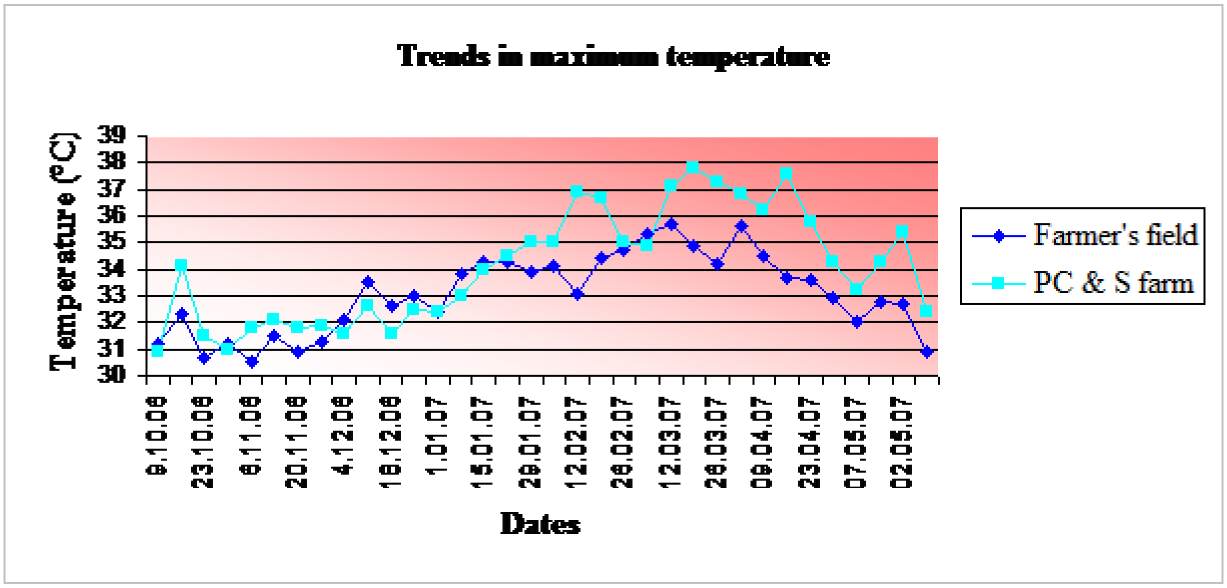


Fig.4a. Trends in maximum temperature in vanilla garden at two locations

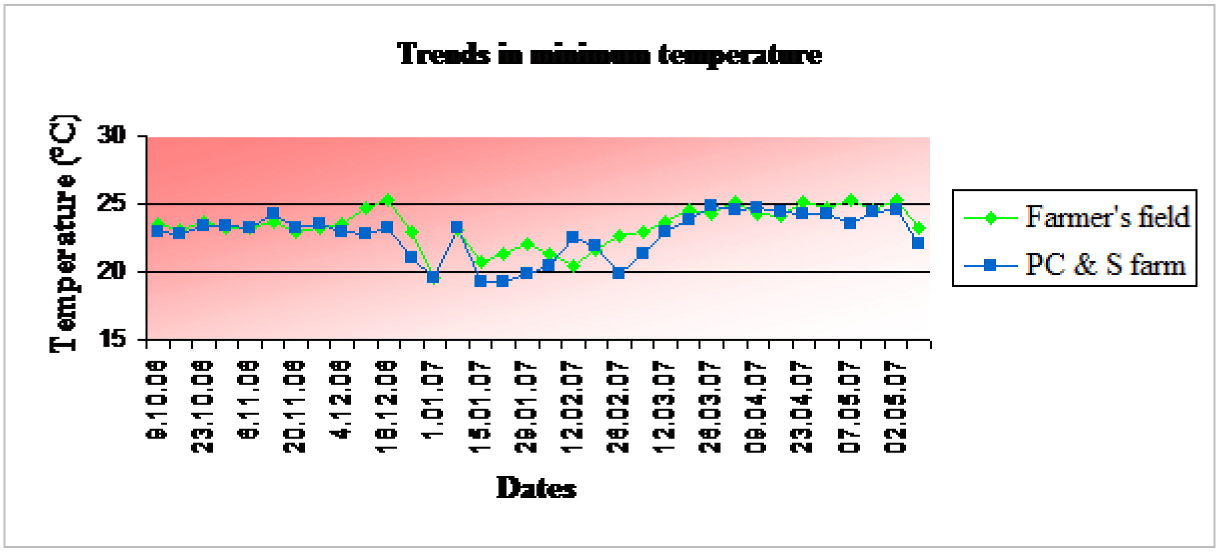


Fig.4b. Trends in minimum temperature in vanilla garden at two locations

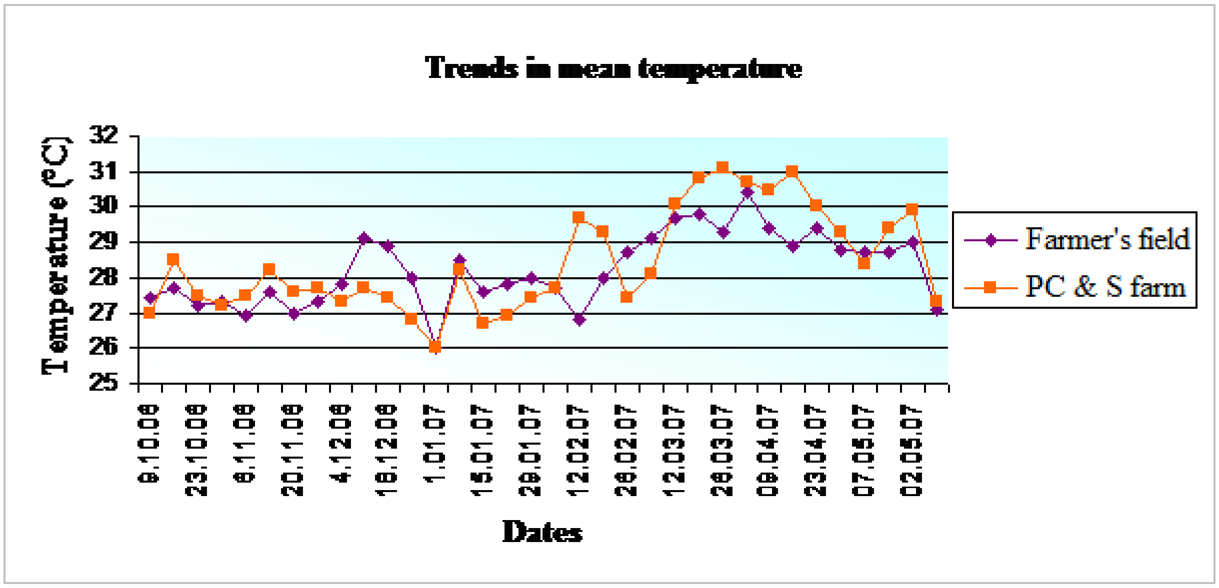


Fig.4c. Trends in mean temperature in vanilla garden at two locations

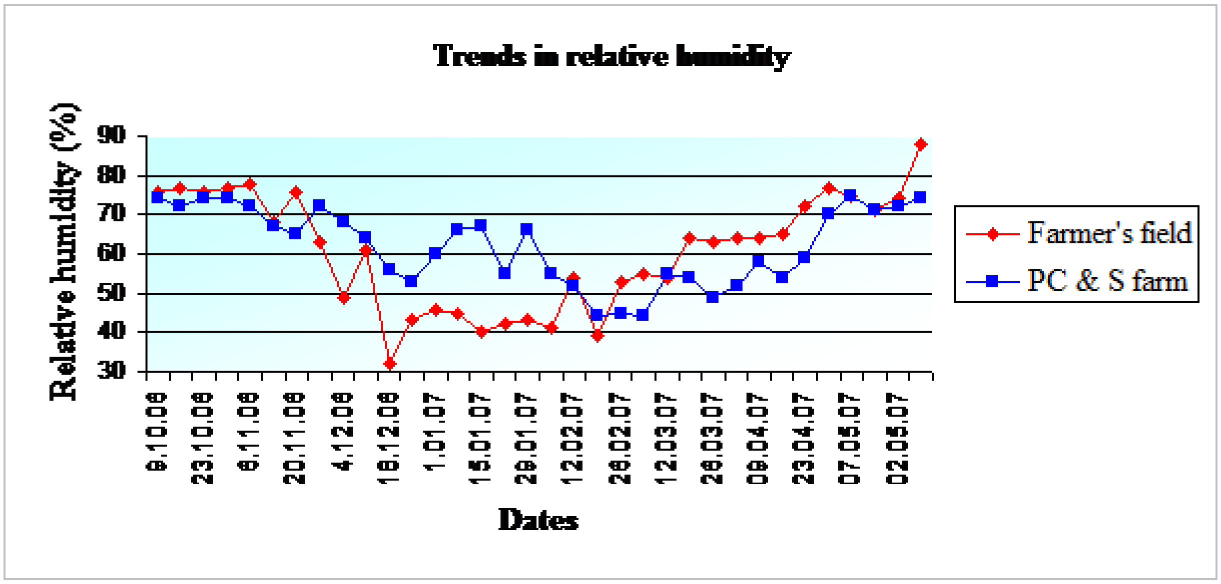


Fig.4d. Trends in relative humidity in vanilla garden at two locations

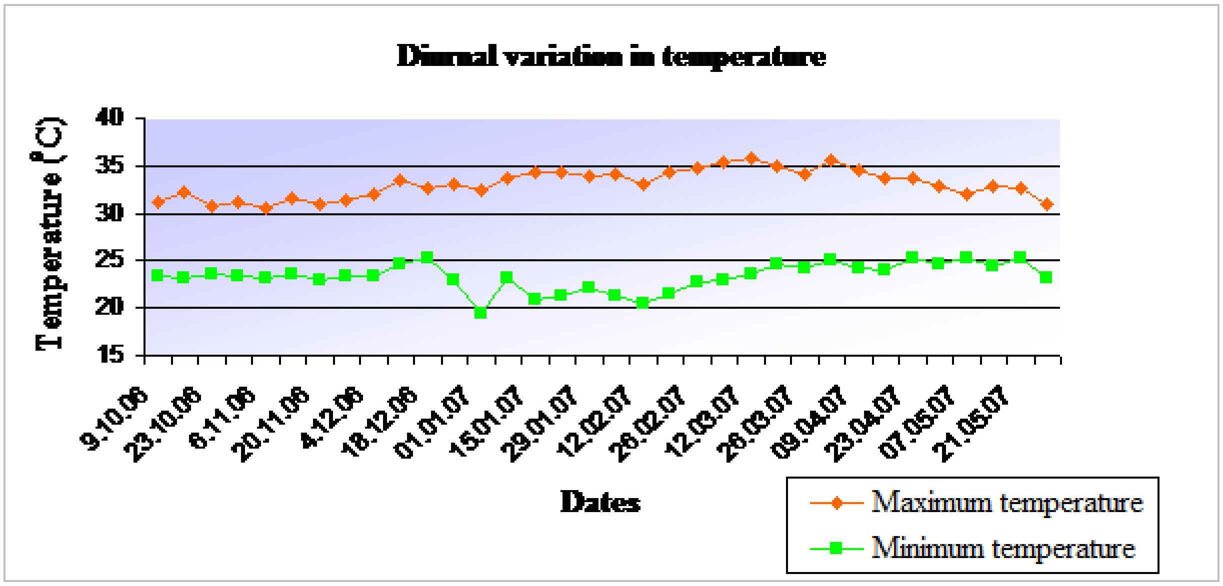


Fig 4e. Diurnal variation in temperature at farmer's field

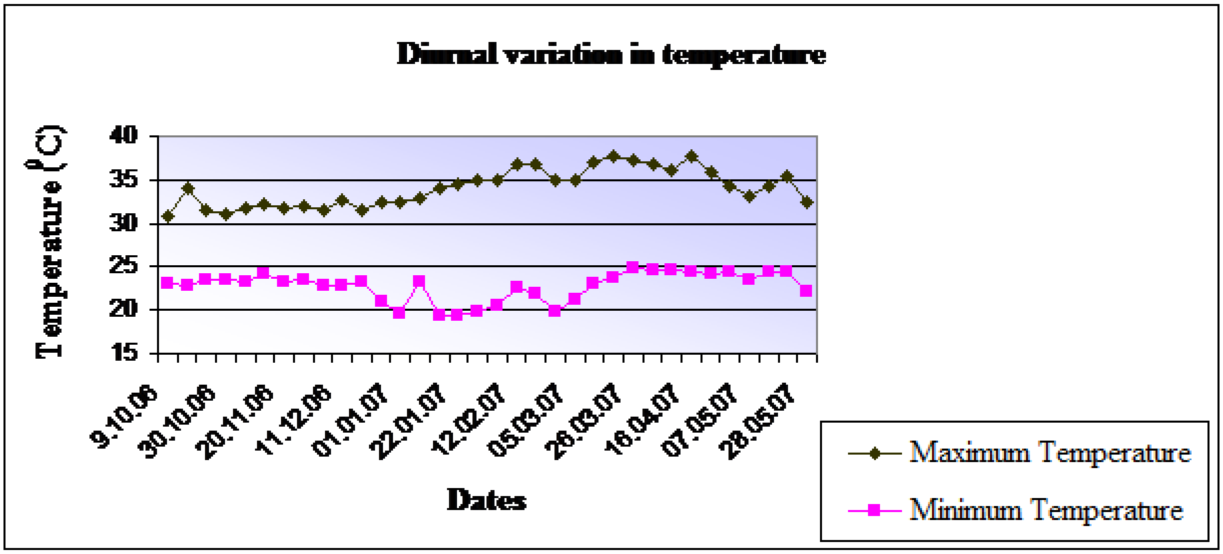


Fig. 4f. Diurnal variation in temperature at PC & S farm

month of December-February. The period of October-November and March-May recorded high relative humidity at farmer's field (Fig. 4d).

Maximum diurnal variation was observed during the month of December – March. Diurnal variation observed was less in farmer's field and a mean variation of 9.3 °C was noticed.

The diurnal variation was more at PC & S farm as compared to farmer's field. The mean diurnal variation observed in PC & S farm was 11.8^0C as against 9.3^0C at farmer's field. Maximum diurnal variation was noticed during the period from mid January to mid March at PC & S farm. The diurnal variation in temperature at both the locations is presented in Fig. 4 e and Fig. 4 f.

4.2.1.2 Light intensity and infiltration

Light intensity readings were found to vary in the two locations studied. Mean light intensity recorded at PC & S farm during the period was 232.21 lux and in farmer's field was 261.09 lux. (Table 17) and (Fig. 5a to 5d). In PC & S farm, there was uniform light intensity readings from all the four directions.

The percentage light infiltration was found to vary in the two locations selected for the study. The light infiltration was found more than 50 per cent, after lopping of standards in vanilla in May and September-October. Percentage light infiltration in farmer's field is shown in Fig.5a and that of PC & S farm in Fig.5b. The percentage light infiltration was between 25-50 for most part of the year in farmer's field while it was above 50 per cent in PC & S farm.

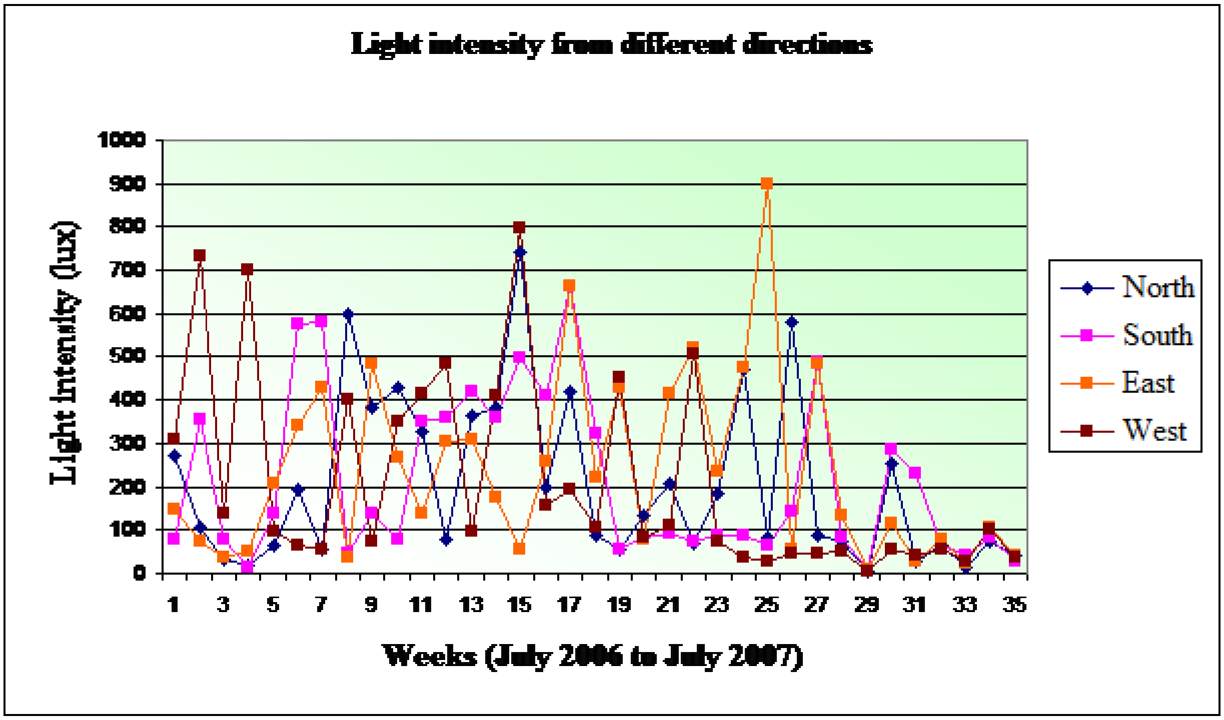


Fig. 5a. Light intensity from different directions in farmer's field

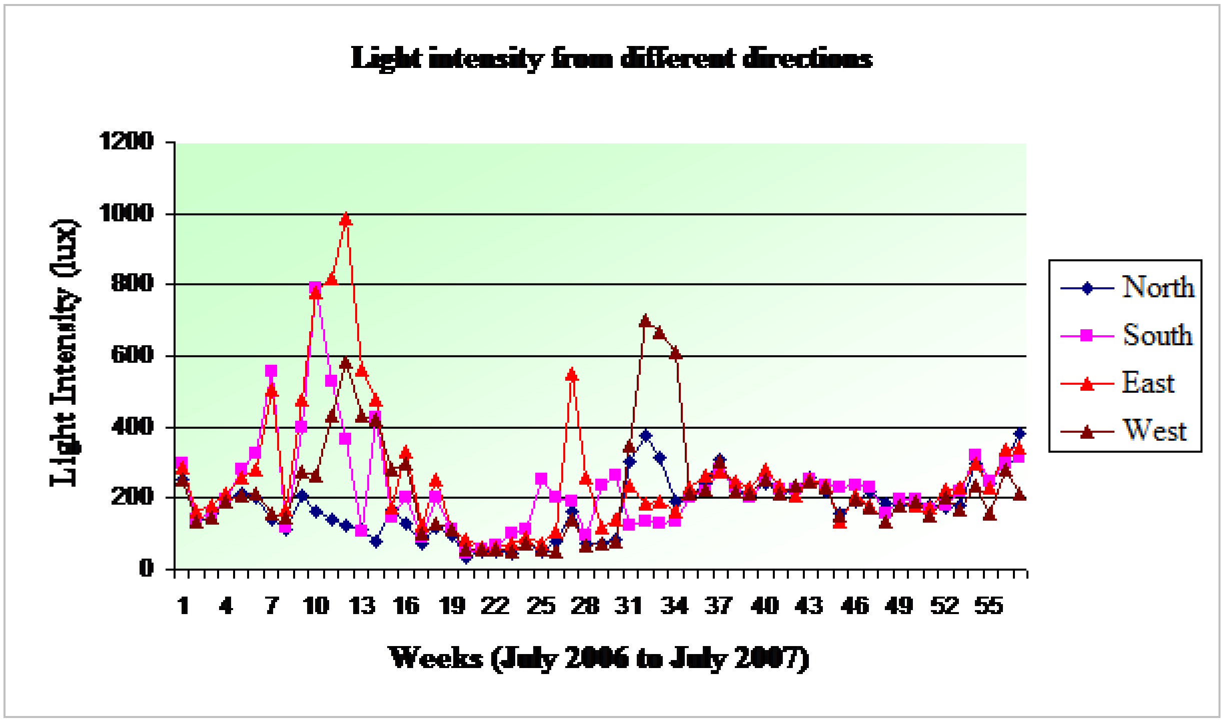


Fig. 5b. Light intensity from different directions in PC & S farm

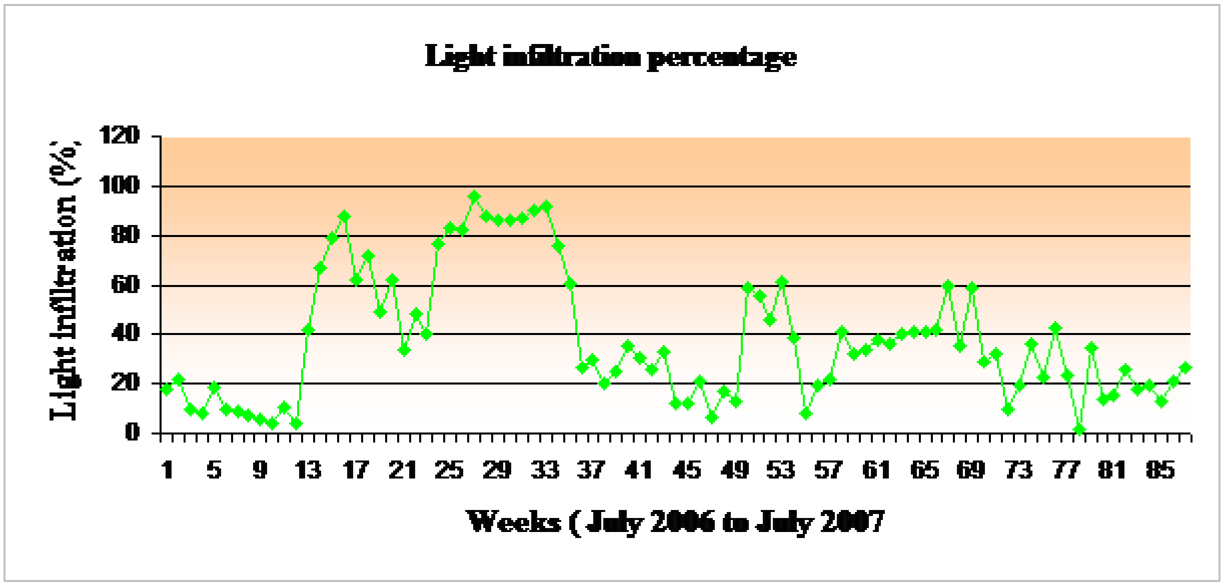


Fig. 5c. Light infiltration percentage in farmer's field

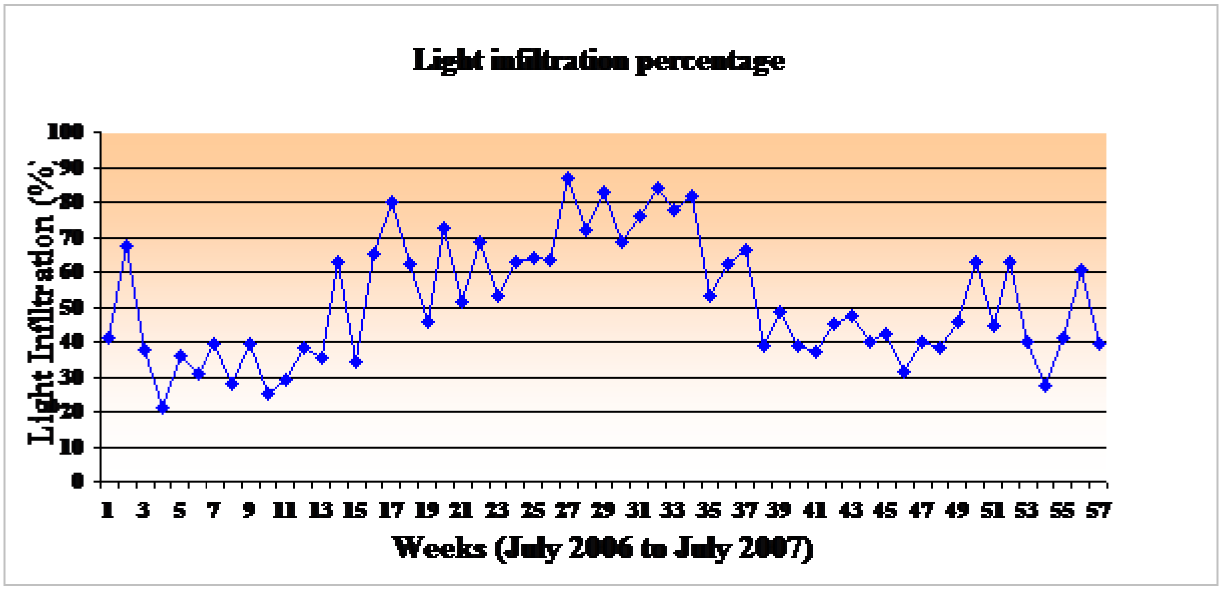


Fig. 5d. Light infiltration percentage in PC & S farm

Table 17. Mean light intensity (lux) in vanilla gardens (July 2006 to July 2007)

Location	North	South	East	West	Mean
PC & S farm	201.86	237.05	263.61	226.33	232.21
Farmer's field	247.91	261.03	271.3	264.15	261.09

4.2.2 Correlation of micro meteorological factors with flowering in vanilla

Flower initiation in vanilla was observed in farmer's field during January- February. From flower initiation to flowering, it took on an average one month.
(Table 18).

Date wise flowering observed in farmer's field is presented in Fig.6. Flowering commenced on 2nd week of February (09/02/07) and ended on last week of May. (25/05/07). During last phase of flowering (20/04/07 to 25/05/07), the number of flowers opened was negligible and ranged from zero to one. The peak flowering was observed in March and maximum flowers opened during first fortnight of March.

Table 18. Flower bud initiation and flowering in vanilla

Sl. No.	Parameters	Farmer's field
1	Date of 1st inflorescence initiation	11.1.07
2	Date of 50 per cent emergence of inflorescence	23.2.07
3	Date of 1 st flowering	9.2.07
4	Date of 50 per cent flowering	14.3.07
5	Peak flowering	14.3.07
6	Duration of flowering (Barring the last phase of flowering)	74 days

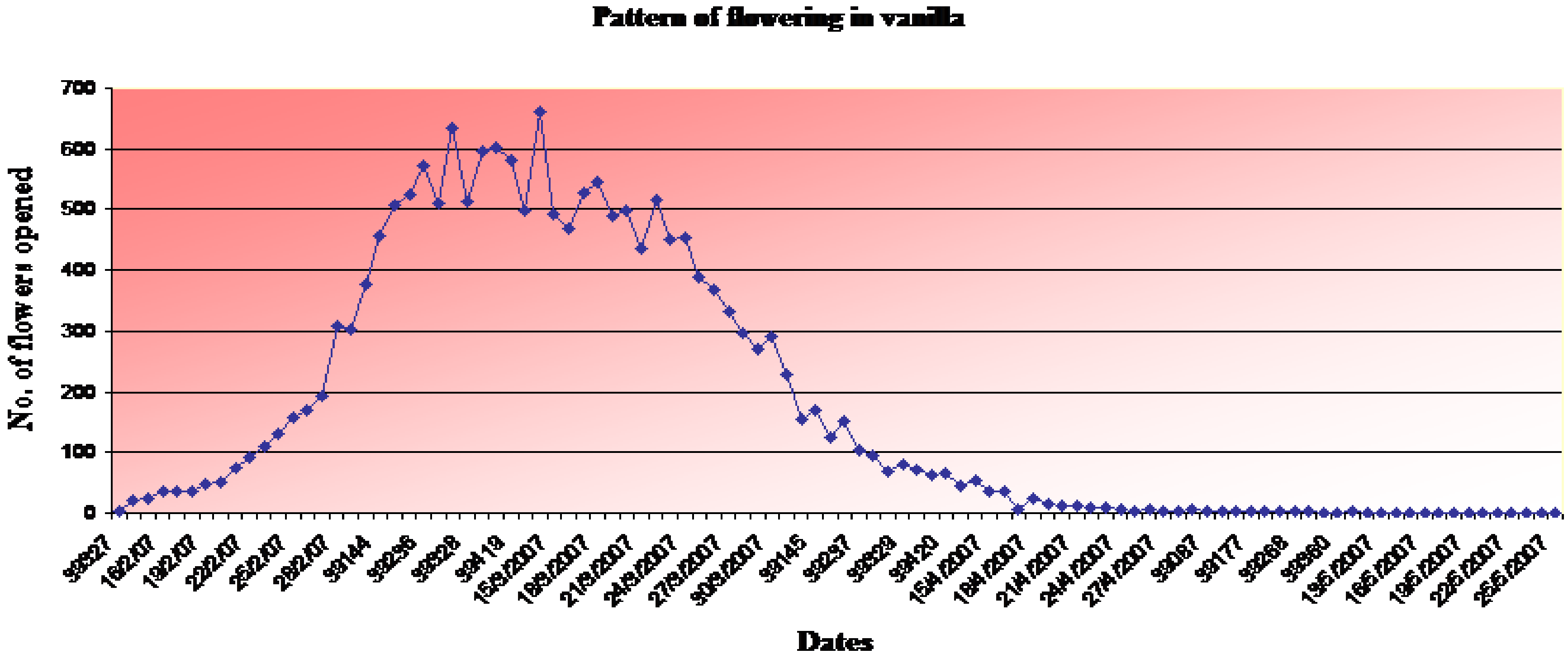


Fig. 6. Pattern of flowering in vanilla (*Vanilla planifolia* Andrews)

Flowering in vanilla was influenced by micro meteorological factors. The influence of micro meteorological factors on flowering was worked out using Pearson correlations. The correlation coefficients on flowering with micro meteorological parameters at farmer's field in Thrissur are presented in (Table 19 and Fig.7).

Table 19. Correlation coefficients of flowering in vanilla with temperature and relative humidity

Parameters	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)
Number of flowers opened during 2006 season	0.549**	-0.083	-0.365
Number of flowers opened during 2007 season	0.604**	-0.087	-0.379**

Flowering in vanilla was positively correlated with maximum temperature and negatively with relative humidity and minimum temperature during 2006 and 2007 seasons. The positive correlation of maximum temperature with flowering in vanilla is highly significant in both the years.

4.2.2.1 Correlation of light with flowering in vanilla

The number of flowers opened in farmer's field was recorded daily during 2007 season. The data on flowering were subjected to Pearson correlations with light intensity from different directions and percentage light infiltration to the field. Correlation of light with flowering in farmer's field was depicted in Fig.8. The data analyzed showed that the number of flowers opened showed highly significant positive correlations with light received from North and South directions.

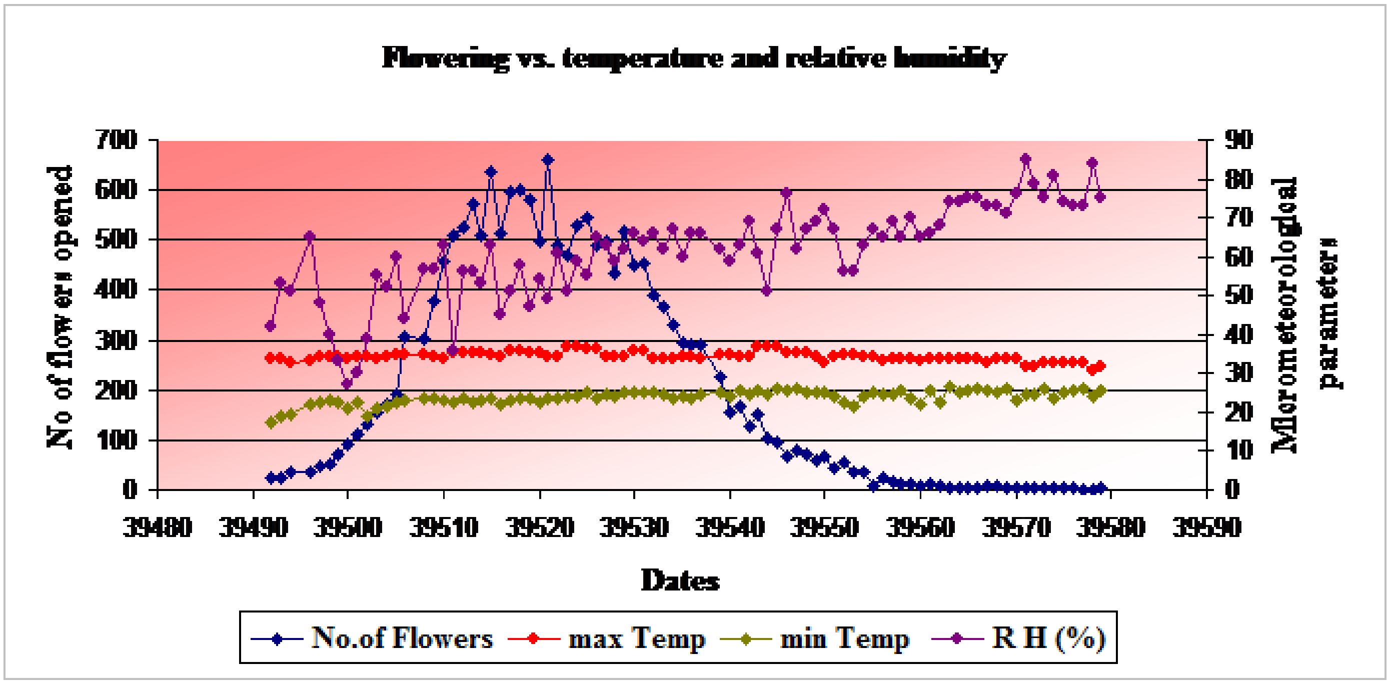


Fig. 7. Flowering vs. temperature and relative humidity in farmer's field

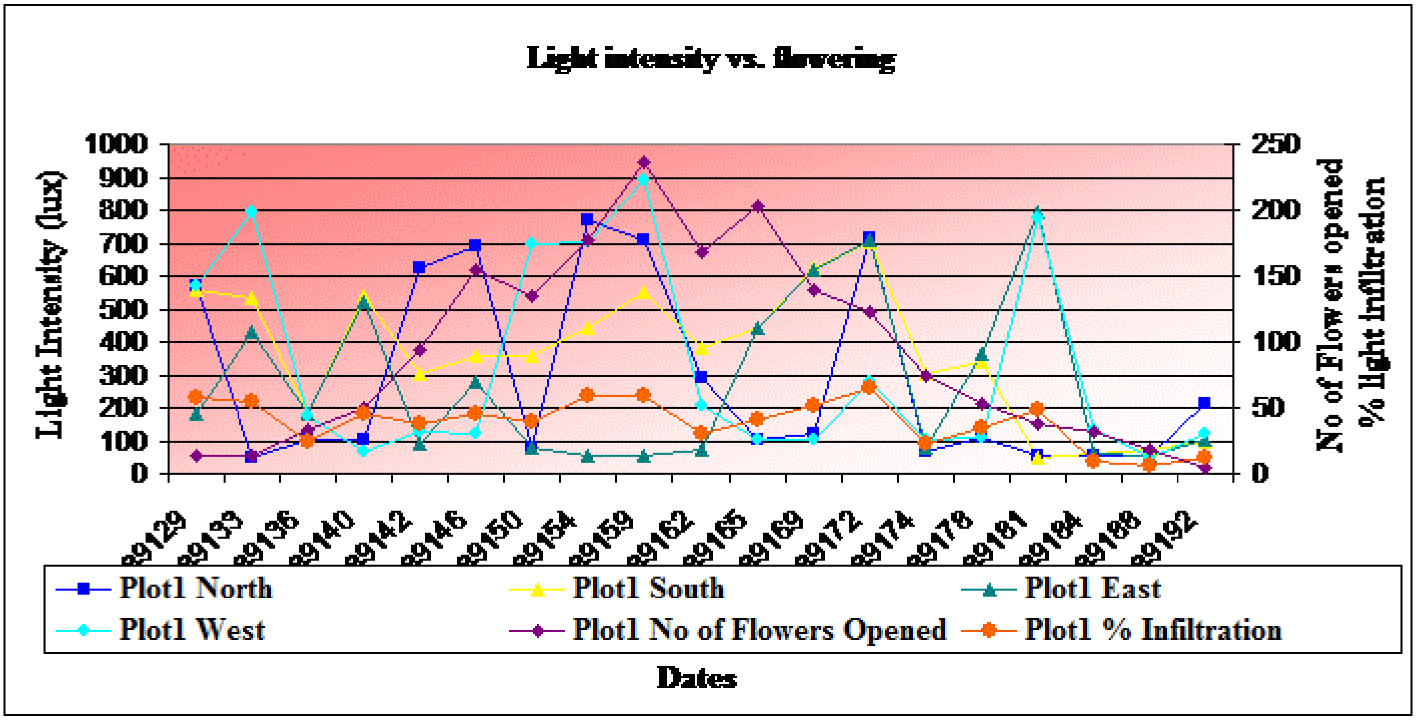


Fig. 8. Light intensity vs.flowering in farmer's field

Discussion

5. DISCUSSION

The investigations on "Influence of micro meteorological factors on flowering in vanilla" were undertaken at Department of Plantation Crops and Spices, College of Horticulture during 2005-2007. The main objective of the investigations was to study the influence of soil moisture stress and micro meteorological parameters on flowering in vanilla. The physiological and biochemical responses of vanilla plants due to moisture stress were also studied in the present investigations. The salient findings of the study are discussed in this chapter. The discussion focuses on influence of soil moistures stress on flowering in vanilla, physiological and biochemical changes in vanilla due to moisture stress and influence of micro meteorological factors on flowering in vanilla.

EXPERIMENT-I

5.1 INFLUENCE OF SOIL MOISTURE STRESS ON FLOWERING IN VANILLA

Soil moisture stress is essential in vanilla for inducing flowering. Farmers practise different periods of moisture stress to induce flowering in vanilla. Due to higher stress given to plants, many plants could not recover the stress effects and they eventually dry even after commencement of irrigation. To find out the optimum stress for induction of flowering in vanilla, four stress periods viz. one, 1½ , two and 2½ months stress periods were imposed during November to December period in 2006 season in selected vanilla gardens. The control plants in which no moisture stress was given were irrigated weekly twice.

In the present investigations, maximum flowering of 80 per cent was observed in plants stressed for moisture for a period of one month, followed by 60 per cent flowering in 1½ month stress period and 52 per cent in two months stress period. The number of inflorescence emerged per plant showed no significant difference in one, 1½ and two months stress periods. Instead, the number of inflorescence emerged was significantly lower in 2½ months stress period. There was no significant difference in the mean number of buds per inflorescence in different treatments. So a moderate stress period of 1-1½ month is required in vanilla to induce flowering and to sustain the vigour and health of vine. A stress period of beyond two months adversely affected the health of vine.

Soil moisture stress induces flowering in crop plants like mango, rambutan, citrus, coffee etc. In plants like coffee, citrus etc. soil moisture stress is essential for flower bud initiation, but further growth and their differentiation occur only after getting stimulus from water either by irrigation or through rainfall. In crops like black pepper, soil moisture stress is essential for proper flushing and then flowering in the newly formed flushes.

Stress affects the metabolism of hormones and amino acids in plants. In mango, during the pre flowering period, auxins, ethylene and absicic acid accumulate due to stress, while gibberellins decrease (Rameshwar, 1988). In Satsuma mandarin trees, Koshita and Takahara (2004) observed enhanced levels of GA_3 due to water stress. In coffee, moisture stress decreased ABA translocation to flower buds rendering the buds to grow. Subsequent release of moisture stress or a water stimulus through rainfall or irrigation increased GA concentration thus inducing bud growth and anthesis (Alvim, 1977).

As in the case of present investigations, a moderate water stress was found beneficial to induce flowering in litchi (Jerway *et al.*, 1977), satsuma mandarin (Koshita and Takahara, 2004) and guava (Singh *et al.*, 1977). Severe water stress in litchi reduced flushing and terminal buds formed were unable to produce panicles even after re-watering. The severely water stressed trees produced only fewer flower buds in satsuma mandarin and the number of leafy inflorescence produced was more in severely stressed trees.

The vanilla plants subjected to no soil moisture stress exhibited only four per cent flowering in the present study. Mango trees grew vegetatively with less flowering, when there was heavy rainfall during the pre flowering period (Rameshwar, 1988). Similar observations was made by Chandraparnik *et al.* (1992) in Durian in which rainfall greater than 10 mm / day for about three to five continuous days suppressed the development of flower buds during the first stage of emergence. In cocoa also, less flowering was observed in frequently irrigated plants as compared to plants subjected to soil moisture stress (Sale, 1970).

5.1.1 Physiological and biochemical responses of vanilla due to moisture stress

The physiological and biochemical responses of vanilla plants due to moisture stress were studied in detail in the present investigations. Physiological parameters like leaf colour, leaf thickness, relative leaf water content, membrane stability and wilting symptoms on vines and leaves were subjected to study. The biochemical parameters like total chlorophyll, epicuticular wax content, soluble protein, total free amino acid, proline, activity of peroxidase enzyme, total sugars and potassium concentration in tissues were analyzed before and after induction of stress from the same plant and also compared with the values in control plants at the elapse of each stress period. The soil moisture content in different treatments was also estimated gravimetrically before and after induction of stress.

5.1.2 Physiological response

Fading of dark green colour of leaves, reduction in thickness of leaves, relative leaf water content and membrane stability, development of lines on leaves and vines were observed as physiological changes in vanilla due to moisture stress. Plants in which no moisture stress was given, the leaves and vines were dark green, succulent, fleshy, thick and vines round. The morphological changes observed in vanilla vines due to moisture stress will be helpful to visually assess the extent of moisture stress in the garden, as the changes observed were related to the intensity of stress.

In plants, adaptation and acclimatization to environmental stresses result from integrated events occurring at all levels of organization, from morphological, anatomical, biochemical, cellular to morphological level (Taiz, and Zeiger, 2003).

Wilting symptoms appeared in vanilla as development of lines on leaves and vines (Plate 3). The wilting symptoms observed in response to moisture stress is a plant adaptation to reduce water loss from leaves and exposure to incident light there by reducing the heat stress on leaves. Cellular mechanisms in response to stress include changes in cell cycle and cell division, changes in the endomembrane system and vacuolization of cells and changes in cell wall architecture. At the biochemical level, plants alter metabolism in various ways to accommodate environmental stresses including production of osmoregulatory compounds such as proline and glycine betaine. At the molecular level, stress leads to the expression of sets of genes involved in acclimatization and adaptation to the stress. The genes mediate the cellular and whole plant responses. The sensing and activation of signal transduction cascades, mediating the changes in gene expression involve both an ABA - dependent pathway and an ABA - independent pathway. Most of these stress factors are interrelated.

The fading of green colour observed in the present study was due to decreased chlorophyll synthesis which is evident from the results presented in Table 7. At both locations, moisture stress significantly reduced the chlorophyll content and the decrease was more in plants stressed for longer periods.

The thickness of vanilla leaves decreased when plants were stressed for moisture. This may be due to the reduction in size of individual cells or shrinkage of cells, when the plants were subjected to moisture stress.

Reduction in leaf thickness observed in Peperomia plant due to moisture stress was associated with shrinkage of hydrenchyma and occurrence of a gradient of osmotic potential between hydrenchyma and mesophyll (Herrera *et al.*, 2000). Similar observations on reduction in leaf thickness due to moisture stress were reported by Stoyanova *et al.* (2002) in maize.

Relative leaf water content (RLWC), which is directly related to leaf water potential is an alternative measure of plant water status (Roberts and Knoerr, 1977; Sinclair and Ludlow, 1985). Relative leaf water content decreased in vanilla plants stressed for various periods. The reduction in RLWC was more in longer period of stress and less in short term stress periods. The reduction in RLWC under moisture stress was due to the insufficient amount of soil moisture, available to the plants. Reduction in RLWC under moisture stress was reported by Stephenson *et al.* (1989) in macadamia trees, Singh *et al.* (1997) in chickpea, Kirnak *et al.* (2001) in brinjal and Charugupta (2006) in mustard.

Cell membrane damage and leakage of electrolytes are taken as indices in studies on biotic and abiotic stresses. Moisture stress treatments resulted in damage of cell membrane and significant increase in electrolyte leakage. The stability of cell membrane decreased due to soil moisture stress in vanilla. Membrane stability was associated with lipid peroxidation (Tyagi *et al.*, 1999). The decrease in membrane stability under water stress was also partly due to the combined effects of both reduced water uptake and chlorophyll concentration (Kirnak *et al.*, 2001). The efficacy of cell membrane stability as an index of selection for water stress was reported in crops like rice by Tyagi *et al.* (1999) and in wheat by Sairam and Saxena (2000).

5.1.3 Biochemical response

5.1.3.1 Total chlorophyll

Total chlorophyll content in vanilla decreased as the intensity of stress advanced and the reduction in total chlorophyll content was more pronounced in severely stressed plants as compared to other period of stresses. The decline in chlorophyll content in stressed plants was due to the inhibition of chlorophyll synthesis or due to the destruction of chlorophyll under moisture stress as reported by Duysen and Freeman (1974) in wheat.

Reduction in chlorophyll content due to moisture stress was reported in brinjal by Prakash and Ramachandran (2000), in wheat by Sairam and Saxena (2000), in onion by Upreti and Murthi (2004) and in maize by Bedse *et al.* (2007).

5.1.3.2 Epicuticular wax content

A common developmental response to water stress is the production of a thicker cuticle that reduces water loss from the epidermis. Waxes are deposited in response to water deficit both on the surface and within the cuticle inner layer and the wax deposited in the inner layer is more important in controlling the rate of water loss.

Epicuticular wax content in vanilla increased due to moisture stress. The percentage increase in epicuticular wax content was high in 2½ months stress period. The control plants sampled after various stress periods registered lesser wax content than stressed plants. The wax layer formation on the leaf surface as an adaptive mechanism to withstand water deficit in plants under field conditions was reported by Hall and Jones (1961) and Baker (1974). Stress induced increase in epicuticular wax content was reported in sorghum by Ebercon *et al.* (1977), in coconut by Kurup *et al.* (1993) and Vincent *et al.* (2005) and in black pepper by Thankamany (2000).

5.1.3.3 Soluble protein

Soluble protein content decreased in vanilla due to moisture stress. The reduction in soluble protein was more pronounced as the intensity of stress advanced. The control plants recorded a higher soluble protein content over the stressed plants. Genkel *et al.* (1971) found that water stress caused a marked change in the protein synthesizing apparatus of plant tissue. According to Hsiao, (1970), the capacity for protein synthesis decreased considerably in response to water stress. As in the present investigations, decrease in soluble protein content was reported in ‘Valencia’ orange by Joseph and Yelenosky (1988), in rice by Jha and Singh (1997) and in brinjal by Prakash and Ramachandran (2000).

5.1.3.4 Total free amino acid

Total free amino acids increased during stress period in all the treatments. There was much variation in the total free amino acid content before and after the stress periods. The accumulation of amino acids might be due to the hydrolysis of protein and accumulation of amino acids occurred in response to changes in osmotic adjustment of their cellular contents (Greenway and Munns, 1980). The accumulation of free amino acids under stress at vegetative, anthesis and grain filling stages of sorghum indicated the possibility of their involvement in osmotic adjustment (Yadav *et al*., 2005).The results in vanilla were in conformity with the results reported in other crops, wherein accumulation of amino acids was noted in crops like rice by Jha and Singh (1997) and in bhindi varieties by Sankar *et al.* (2007).

5.1.3.5 Proline

Moisture stress enhanced proline content in vanilla in contrast to plants under no moisture stress. A major reason for increase in proline concentration due to water stress was lesser incorporation of continually synthesized proline for protein synthesis (Sharma *et al*., 1987). Proline also protects the cells from stress induced damage by acting as an osmolyte with high compatability for enzymes (Hare *et al*., 1998). The accumulation of proline during moisture stress was reported in guava by Singh *et al.* (1997) in cocoa by Balasimha (1999), in tomato by Nahar and Gretzmacher (2002), in olive by Sofo *et al*. (2004), in brinjal by Sarker *et al*. (2005) in mustard by Charugupta (2006) and in bhindi by Sankar *et al*. (2007).

5.1.3.6 Activity of peroxidase enzyme

Activity of peroxidase enzyme increased in plants stressed for various periods. Dalal and Chopra (2001) observed that enhancement in the peroxidase activity under various stress condition had been linked with protection from oxidative damage, lignifications and cross linking of cell wall to prevent the above said adverse conditions.

Nayar and Kaushal (2002) also reported that the increased activity of peroxidase enzyme along with enzyme catalase constituted potential defence mechanism against chilling induced oxidative damage in germinating wheat grains. The increase in peroxidase enzyme activity under moisture stress was reported in several crops such as tea seedlings by Duan (1992), tolerant genotypes of rice by Jha and Singh (1997) and in wheat by Sairam and Saxena (2000).

5.1.3.7 Total sugar

The intensity of moisture stress increased the total sugar content in vanilla. Longer period of stress recorded higher content of total sugar. The increase in total sugar content was probably due to mobilization of reserved polysaccharides, by decreasing the external water potential in canola as reported by Moradshahi *et al.* (2004). The current hypothesis is that sugars either act as osmotica or protect the specific macro molecules and contribute to the stabilization of membrane structures. The role of total sugar in the osmotic adjustment was emphasized by many authors in crops like cherry tomato by Yasuyoshi *et al.* (1998), in tomato by Rao *et al.* (1999), in cocoa accessions by Balasimha, (1999) and in senna by Agarwal and Pandey (2002).

5.1.3.8 Potassium concentration in tissues

Potassium is essential for many physiological processes in plants such as photosynthesis, translocation of photosynthates and activation of enzymes. Potassium concentration in tissues increased in plants stressed for various periods and the maximum percentage increase in potassium content was noted in severely stressed plants. Cakmak and Engels (1999) observed that plants suffering from environmental stresses like drought have a larger internal requirement for potassium. Environmental stress factors that enhance the requirement for potassium also cause oxidative damage to cells by inducing formation of reactive oxygen species, especially during photosynthesis (Bowler *et al.*, 1992). The reason for the enhanced need of potassium by plants suffering from environmental stress appears to be related to the fact that potassium is required for maintenance of photosynthetic CO_2 fixation.

Alleviation of detrimental effects of drought stress, especially on photosynthesis by sufficient potassium supply was reported in crops like chickpea (Singh *et al*., 1997) and in maize (More *et al*., 2004). Increased content of potassium due to moisture stress was reported in black pepper by Thankamany and Ashokan (2004).

5.2 INFLUENCE OF MICRO METEOROLOGICAL FACTORS ON FLOWERING IN VANILLA

Flowering in vanilla is a complex phenomenon which is influenced by many factors. The microclimate of the garden also plays a major role in flowering of vanilla. In the present investigations, the micro meteorological situations at both the experimental fields were compared and correlations of micro meteorological factors with flowering were worked out.

The two locations studied showed variation in maximum temperature, minimum temperature, relative humidity, light intensity and light infiltration. When both the locations were compared, the micro meteorological situations at PC & S farm were not congenial for growth and flowering of vanilla. Even though only less number of plants with uniform growth was available at PC & S farm for conducting studies, the growth and flowering of plants in general were poor in the farm when compared to farmer’s field.

Vanilla requires a warm humid climate with a temperature range of 25°C – 32°C and a relative humidity of about 80 per cent. The vine grows well under trees which provide filtered light of 25 to 50 per cent.

In both locations studied, the above requirements of the crop were not met with. The maximum temperature ranged from 30.7°C to 37.8°C, minimum temperature from 19.3°C to 25.3°C, relative humidity from 32 to 88 per cent, light intensity from 65.5 to

395 lux, light infiltration from 6.94 to 44.73 per cent. When both the locations were compared, farmer's field was found more suitable for the crop.

Flower initiation occurred in vanilla during December-February, when the minimum temperature ranged from 19.8°C to 23.5°C. From flower bud initiation to flower opening it took 30-45 days and flower opening commenced in the garden from 9th February and continued up to 25th May. Peak flowering was observed in 14th March.

The influence of low minimum temperature for flower bud initiation was reported in crops like mango, citrus, litchi and pineapple. In mango, a late cold spell of 10°C during January-February induced a second flush of flowering at Sangareddy region of Andhra Pradesh (Rameshwar, 1988). A day temperature of 18°C and night temperature of 10°C induced flowering in Tahiti lime (Southwick and Davenport, 1986). Very less flowering was reported in litchi when day shoot temperatures and root temperatures exceeded 20°C. In pineapple, flowering was most rapid at night temperature of 20°C.

In the present investigations, correlation of micro meteorological factors with flowering in vanilla was worked out. Flowering in vanilla was positively correlated with maximum temperature and negatively with relative humidity and minimum temperature. The floral primordia of vanilla initiated during December-February opened during February-May and peak flowering was observed in March. The maximum temperature recorded during the peak period of flowering in farmer's field was 35.7°C, which was found positively correlated with flowering. Requirement of a lower temperature for flower bud initiation and a higher temperature for flower opening was reported in citrus by Okuda *et al*. (2004) and in saffron by Molina *et al*. (2005).

The diurnal variation was found to vary in the two locations studied. The variation was more in PC & S farm and less in selected farmer's field. The less diurnal variation experienced in farmer's field might have favourably contributed to flowering in vanilla.

Flowering in vanilla was seen related to light intensity and light infiltration in the garden. The number of flowers opened showed a highly significant positive correlation with light received from North and South directions and also light infiltration. This showed the essential requirement of undertaking the second lopping of standards in vanilla gardens during September-October. This would help for better light intensity and infiltration in the garden which in turn helped for getting proper flowering and growth of beans. The requirement of high light intensity for proper flowering was reported in *Oncidium* spp. by Song *et al*. (1988) and in miltonia by Matsui and Yoneda (1999).

The present investigations clearly demonstrated the requirement of soil moisture stress for flowering in vanilla. A moderate stress period of 1-1½ month (November-December) was found essential for induction of flowering in vanilla. The control plants in which no moisture stress was given turned vegetative and exhibited only four per cent flowering. The moisture stress experienced during various stress periods viz., one month, 1½ month, two months and 2½ months was 25, 40, 45 and 50 per cent of control treatments respectively as revealed by the estimation of total soil moisture content at the root zone depth in the present investigations.

The moisture stress induced, altered the physiological and biochemical parameters in vanilla vines. Leaf thickness, relative leaf water content, membrane stability, soluble protein and total chlorophyll content decreased due to soil moisture stress. The content of epicuticular wax, total free amino acids, accumulation of proline, activity of peroxidase enzyme, total sugar content and potassium concentration in tissues increased due to stress. The changes in physiological and biochemical parameters were more pronounced as the intensity of stress increased.

The soil moisture stress influenced the growth and metabolism of vanilla vines. Decrease in content of soluble protein and increase in total free amino acids and total sugar observed in the present investigations might have favourably contributed to flower bud initiation and flowering in vanilla as amino acids are the precursors of hormones. However, the rate of utilization, degradation and conversion of the bio chemical substances were important for proper flowering and further recovery of stress effects in vanilla. This is evident from the present studies that plants could be recovered from stress effects of one month stress period when re-watering was done. So changes in physiological and biochemical parameters observed during one month stress period in vanilla had not adversely affected the growth and metabolic processes in vanilla. But the decrease or accumulation of biochemical substances observed in plants beyond one month stress period was found deleterious to the crop as the metabolic processes in the plant were disrupted by the increased soil moisture stress periods. The degradation and inter conversion of chlorophyll, reduction in leaf thickness and cell membrane damage observed in the present studies due to moisture stress might have influenced the metabolism of the plant. The accumulation of unwanted proteins and inhibitory substances during advanced periods of stress also could not be ruled out in this context.

The changes in physiological parameters like fading of green colour and wilting symptoms on leaves and vines could be used to visually assess the extent of soil moisture stress in the garden. The biochemical parameters recorded at ideal stress period could be used as a check to assess the extent of moisture stress in the garden more precisely and could be utilized in high-tech or precision farming systems.

The study of the microclimatic situation helps to manipulate the microclimate in the garden. The high temperature of 30.9°C-37.8°C recorded in the locations during January – May was found detrimental to the crop. This could be manipulated in the garden by giving frequent mist irrigation and by proper shade regulation. Vanilla requires high relative humidity of about 80 per cent. In the study area, the relative humidity recorded at PC & S farm varies from 44 to 75 per cent and at farmer’s field varies from 32 to 88 per cent which are not congenial for proper growth of vanilla. By proper manipulation of mist irrigation and shading, this could be manipulated to a certain extent. The stress experienced by vanilla plant is due to the combined effect of moisture stress and environmental stress factors. This is evident from the difference in time taken for soil moisture depletion in the two locations studied. The investigations also bring about the importance of physiological responses of vanilla plant due to moisture stress for visually assessing the intensity of stress.

The present investigations established the involvement of factors like soil moisture stress, temperature, relative humidity and light for flowering in vanilla. In addition, the physiological maturity and accumulation of nutrients and flowering substances in nodes of the plant also are of significance as flowering is observed in past season's shoot in vanilla and nipping of the tip of hanging shoots favours the accumulation of nutrients and flowering substances in shoots prepared for flowering. So a combination of factors determine flowering in vanilla.

The involvement of multiple factors in flowering of many crop plants was reported by several authors. Soil moisture stress induced flowering in mango, when the involvement of other factors like wounding, low temperature stress, salt stress, geotropic stress were satisfied as reported by Rameshwar (1988). Low temperature, high relative humidity and moderate periods of stress favoured flowering in guava (Singh *et al*. 1997). Coffee flowering was controlled by day length and rainfall distribution (Alvim, 1977). Short day conditions were required for flower bud differentiation in coffee whereas further growth and anthesis occurred under rainfall conditions. Temperature and leaf water stress played major role in litchi flowering. Day shoot and root temperatures below 20°C favoured flowering in litchi Menzel *et al*. (1989).

It could be concluded from the present investigations that soil moisture stress for a period of 1-1½ month during November- December is sufficient to induce flowering in vanilla. Changes in physiological parameters due to moisture stress could be used to visually assess the extent of moisture stress in the garden. The biochemical parameters recorded at ideal stress period could be used as indices to assess the extent of moisture stress in plants more precisely in high tech / precision farming systems. Manipulation of micro climate with respect to temperature, light and relative humidity is essential for getting proper flowering in vanilla.

For getting proper flowering in vanilla, the management strategies or manipulations like lopping of standards in September-October, nipping of the tip of hanging shoots prepared for flowering in November, a moderate moisture stress period of 1-1½ month in November-December and manipulation of microclimate and shade in the garden for proper light incidence from North and South directions will be of advantageous.

However, further research efforts are required to study the stress effects in vanilla including more locations differing in soil types and micro climatic conditions. Also physiology of non flowering, flowering and prolific flowering plants in vanilla and influence of micro meteorological factors on quality of cured beans of vanilla need thorough investigations.

Summary

6. SUMMARY

The investigations on “Influence of micro meteorological factors on flowering in vanilla” were conducted at Plantation Crops and Spices farm, College of Horticulture, Kerala Agricultural University and in selected farmer’s field at Thrissur district during 2005-07. The objective of the study was to find out the influence of soil moisture stress and micro meteorological factors on flowering in vanilla. The salient findings of the study are summarized below:

1. **Soil moisture stress altered the physiological and biochemical parameters in vanilla**

   - The colour of the vanilla leaf became faded and turned light green due to soil moisture stress.
   - Wilting symptoms appeared in vanilla as paling of leaves and vines, development of lines on leaves and vines, reduction in succulent nature of leaves and girth of vines.
   - Thickness of leaves decreased when plants were starved for moisture.
   - Relative leaf water content in plants decreased.
   - Cell membrane stability decreased in plants.
   - Total chlorophyll content decreased.
   - Soluble protein content decreased.
   - Epicuticular wax content increased.
   - Total free aminoacid content increased.
   - Accumulation of proline in leaf increased.
   - Peroxidase enzyme activity increased.
   - Total sugar content increased.
   - Potassium concentration in tissues increased.
   - Soil moisture content decreased.

2. Soil moisture stress induced flowering in vanilla

- Flower initiation in vanilla was observed in December-February months.
- Maximum flowering (80 %) was observed in plants stressed for moisture for a period of one month during November-December.
- The number of inflorescence emerged in one month, 1½ month and two months stress treatments were on par.
- A stress period of 2½ months recorded significantly lower number of inflorescences.
- Mean number of buds / inflorescence were on par in various stress treatments.
- Duration of flowering in vanilla varied from 69 to 74 days in plants stressed for different periods of moisture stress. Peak flowering in vanilla was observed in the month of March and maximum flowers opened during 1st fortnight of March.

3. Micro meteorological factors of the garden influenced flowering in vanilla

- The micro meteorological situations at the two locations studied varied with respect to temperature, light and relative humidity.
- The maximum temperature during January to May varied from 33°C -37.8°C in the locations which is detrimental to the crop.
- The diurnal variation was found more in Plantation Crops and Spices farm as compared to farmer's field.
- The relative humidity ranged from 32 to 88 percent in the locations, which is far below the requirement.
- The ideal climatic requirements for vanilla were not met in the two locations studied.
- Flowering in vanilla was found positively correlated with maximum temperature and negatively with relative humidity and minimum temperature.
- Flowering showed highly significant positive correlation with light received from North and South directions and percentage of light infiltration.

References

REFERENCES

Agarwal, S. and Pandey, V. 2002. Effect of water stress on carbohydrate metabolism of senna (*Cassia angustifolia* Vahl.) plants. *Curr. Agric.* 26(1-2): 47-50.

Alhamadani, S.H. and Barger, T.W. 2003. Influence of water stress on selected physiological responses of three sorghum genotypes. *Italian J. Agron.* 7(1): 15-22.

Alvim, P. De T. 1960a. Physiology of growth and flowering in coffee. *Coffee* (Turrialba). 2: 57-62.

Alvim, P. De T. 1960b. Moisture stress as a requirement for flowering of coffee. *Sci.* 132: 354.

Alvim, P. De T. 1977. Factors affecting flowering of coffee. *J. Coffee Res.*7(1): 15-25.

Armitage, A.M. 1995. Photoperiod, irradiance and temperature influence of flowering of *Hamelia patens* (Texas firebush). *Hort Sci.* 30(2): 255-256.

Baker, E.A. 1974. The influence of environment on leaf wax development in *Brassica oleracea* var. gemmifera. *New Phytol.* 73: 955-966.

Balasimha, S. 1999. Stress Physiology of cocoa. *J. Plantn Crops.* 27(1): 1-8.

Bally, I.S.E., Harris, M., Whiley, A.W., Subhadrabandhu, S. 2000. Effect of water stress on flowering and yield of 'Kensington Pride' mango (*Mangifera indica* L.). In: Pichakum. (ed.), *Proceedings of the Sixth International Symposium on Mango*; 6-9 April, 1999; Pattaya City, Thailand. Volume 1. *Acta Hort.* 509: pp.277-281.

Barnett, N.M. and Naylor, A.W. 1966. Aminoacid and protein metabolism in bermuda grass during water stress. *Plant physiol*. 41: 1222-1230.

Bates, L.S. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*. 39: 205-207.

Bedse, D.R., Sorte, N.V., Kamble, P.S., Lende, S., Budanwar, P.D., and Ranjan, R. 2007. Physiological, chemical and biochemical studies in maize genotypes as influenced by moisture stress. *J. Soils Crops*. 17(1): 139-144.

Blum, A. and Ebercon, A. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci*. 21: 43-47.

Bowler, C., Vanmontagu, M., and Inze, D. 1992. Superoxide dismutase and stress tolerance . *A. Rev. Plant Physiol. Plant Mol. Biol* . 43: 83-116.

*Boyer, J. 1973. Etude particuliere des facteurs hydriques de la croissance des cacaoyers. Cafe, Cacao. 15: 189-201.

Brown, P.H. and Menary, R.C. 1994. Flowering in pyrethrum (*Tanacetum cinerariaefolium* L.). 1. Environmental requirements. *J. Hort. Sci*. 69(5): 877-884.

Cakmak, I. and Engels, C. 1999. Role of mineral nutrients in photosynthesis and yield formation. In: Rengel, Z.(ed.), *Mineral Nutrition of Crops* : *Mechanisms and Implications* . The Haworth Press , New York , USA , pp 141-168.

Chacko, E.K. and Randhawa, G.S. 1971. Towards an understanding of the factors affecting flowering in mango. *Andhra Agric. J*. 18(6): 227-234.

Chandraparnik, S., Hiranpradit, H., Poonnachit, U., and Salakpetch, S. 1992. Paclobutrazol influences flower induction in durian, *Durio zibethinus* Murr. *Acta Hort*. 321: 282-290.

Charugupta. 2006. Relationship between plant growth, biomass and seed yield under moisture stress and non stress conditions in mustard (*Brassica campestris* cv. Varuna). *Int. J. Plant Sci.* 1(2): 142-144.

Cortes, P.M. and Sinclair, T.R. 1987. Osmotic potential and starch accumulation in leaves of field grown sorghum. *Crop Sci*. 27(1): 80-84.

Cremer, F., Havelange, A., Saedler, H., and Huijser, P. 1998. Environmental control of flowering time in *Antirrhinum majus*. *Physiologia Plant*. 104(3): 345-350.

Dalal, M. and Chopra, K. 2001. Differential response of antioxidant enzymes in leaves of necrotic wheat hybrids and their parents . *Physiol. Plant* . 111: 297-304.

DASD.2008. *Area and Production Statistics of Arecanut and Spices*.Directorate of Arecanut and Spices development, Calicut, Kerala, 74p.

Duan, L. 1992. Investigations on the physiology of drought resistance of tea plants. Effects of water stress on the growth of tea plants. *Tea. Sci. Tech. Bull*. 1: 12-15.

Dubois, M.K., Gilles, J.K., Hamilton, P.A., Rebers, K., and Sonith, F. 1951. A calorimetric method for determination of sugars. *Nature* 167-168.

Duysen, M.E. and Freeman, T.P.1974. Effects of water deficit(stress) on wheat seedling growth and plastid pigment development. *Physiol. Plant*. 31:262-266.

Ebercon, A., Blum, A., and Jordhan, W.A. 1977. A rapid calorimetric method for epicuticular wax content of sorghum leaves. *Crop Sci*. 17: 179-180.

Esnault, F., Tromeur, C., Kermarrec, M.P., and Pouliquen, R. 2005. Effect of temperature on growth and flowering ability of three jersey shallot (*Allium cepa* L. group Aggregatum) cultivars. *J.Hort. Sci. and Biotech*. 80(4): 413-420.

Freend, D.J.C. 1981. Effect of night temperature on flowering and fruit size in pineapple (*Ananas comosus* L.). *Bot. Gaz*. 142(2): 188-190.

Genkel, P.A., Satarova, N.A., and Tvorus, E.K. 1971. Effect of drought on protein synthesis and the state of ribosomes in plants. *Fiziol. Rast*. 14: 898- 907.

Ghadekar, S.R., Ladole, S.S., Bagde, T.R. and Matte, A.D. 1993. Study of moisture stress pattern before monsoon flowering in citrus orchard. *J. Soils and Crops*. 3(1): 6-10.

Ghosh, P. and Pal, P. 2004. Influence of temperature and humidity on the flowering of *Polianthes tuberosa* L. var. Double. *Hort. J*. 17(1): 73-78.

Greenway, H. and Munns, R.1980. Mechanism of salt tolerance in non- halophytes. *Ann. Rev. Plant Physiol*. 31: 149-190.

Gribanovskji, A.P. 1969. On the lateral fruit buds in apples. *Trudy Centgen Lab. I. V. Micurnia*, 10: 256-259.

Hall, H.M. and Jones, R.L. 1961. Physiological significance of surface wax on leaves. *Nature* 191: 95-96.

Hare, P.D., Cress, W.A., and Vanstaden, J. 1998. Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ*. 21: 535-553.

Herrera, A., Fernandez, M.D., and Taisma, M.A. 2000. Effects of drought on CAM and water relations in plants of *Peperomia carnevalii*. *Ann. Bot*. 86: 511-517.

Hsiao, T.C. 1970. Rapid changes in the levels of polyribosomes in *Zea mays* in response to water stress. *Plant Physiol*. 46: 281-285.

Hsiao, T.C. 1973. Plant responses to water stress. *Ann. Rev. Plant Physiol*. 24: 519-570.

Hutcheon, W.V., Smith, R.W., and Asomaniang, E.J.A. 1973. Effects of irrigation on the yield and physiological behaviour of mature Amelonado cocoa in Ghana. *Trop. Agric*. 50(4): 261-272.

Jackson, M.L. 1973. *Soil chemical analysis*. Prentice Hall of India Pvt. Ltd., New Delhi, 385p.

Jadoski, S.O., Salvador, E.D., Klar, A.E., and Lima, J.P.P. 2000. Peroxidase enzyme (EC.1.11.1.7) activity as an indicator of water stress in sweet pepper plants. *Acta Hort*. 697.

Jerway, C., Nan, C.C., Zen, C.I., Yung, C.C., Chang, J.W., Chao, C.N., Chen, I.Z., Cheng, C.Y., and Yungwu, C. 1997. Effect of temperature and water stress on flowering of litchi (*Litchi chinensis* Sonn.). In: Linren, C. (ed.), *Proceedings of a Symposium on Enhancing Competitiveness of Fruit Industry*; 20-21 March, 1997; Taichung, Taiwan. 38: pp.97-102.

Jeyaramraja, P.R., Manian, S., and Pius, P.K. 2004. Hormonal regulation and osmotic adjustment as mechanisms for drought tolerance in field grown tea (*Camellia* spp.) plants. *Trop. Agric. (Trin.)*. 81(3): 157-161.

Jha, B.N. and Singh, R.A. 1997. Physiological responses of rice varieties to different levels of moisture stress. *Indian J. Plant Physiol.* 2(2): 81-84.

Joseph, C.V. and Yelenosky, G. 1988. Water deficit and associated changes in some photosynthetic parameters in leaves of 'Valencia' Orange (*Citrus sinensis* (L.) Osbeck). *Plant Physiol.* 88: 375-378.

Kamata,S. 1992. Effects of meteorological factors on flowering day of apple. *J. Jpn. Soc. Hort. Sci.* 61(1): 17-24.

Kasturibai, K.V. and Rajagopal, V. 2004. Is stress induced proline accumulation an indicator of leaf water stress in coconut? *J. Plantn Crops*. 32(Suppl.): 1-4.

Kasturibai, K.V., Voleti, S.R., and Rajagopal, V. 1988. Water relations of coconut palms as influenced by environmental variables. *Agric. For. Met*. 43: 193-199.

KAU (Kerala Agricultural University). 2007. *Package of Practices Recommendations: Crops* (13th ed.). Kerala Agricultural University, Thrissur, pp.109-111.

Kirnak, H., Kaya, C., Ismail, T.A.S., and Higgs, D. 2001. The influence of water deficit on vegetative growth, physiology, fruit yield and quality in egg plants. *Bulg. J. Plant Physiol.* 27(3-4): 34-46.

Koshita, Y. and Takahara, T. 2004. Effect of water stress on flower bud formation and plant hormone content of satsuma mandarin (*Citrus unshiu* Marc.). *Scientia Hort.* 99: 301-307.

Krajewski, A.J. and Rabe, E. 1995. Citrus flowering: a critical evaluation. *J. Hort. Sci.* 70(3): 357-374.

Krishnamurthy, K.S., Gowda, S.J.A., and George, J. 1998. Impact of water stress on some physiological parameters in black pepper. In: Adanandan, A.K., Krishnamurthy, K.S., Kandiannan, K., and Korikanthimath, V.S. (eds.), *Water and Nutrient Management for sustainable Production and Quality of spices*. Proceedings of a national seminar, 5-6 October, 1997; Madikeri, Karnataka, India, pp 153-157.

Kuhad, M.S. and Sheoran, I.S. 1986. Physiological and biochemical changes in cluster bean (*Cyamopsis tetragonoloba* L.) genotypes under water stress. *Indian J. Plant Physiol*. XXIX(1): 46-52.

Kurup, V.V.G.K., Voleti, S.R., and Rajagopal, V. 1993. Influence of weather variables on the content and composition of leaf surface wax in coconut. *J. Plantn Crops*. 21(2): 71-80.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem*. 193: 265-275.

Lutts, S., Kinet, J. M., and Bouharmont, J. 1995. Changes in plant response to Nacl during development of rice varieties differing in salinity resistance. *J. Exp. Bot*. 46: 1843-1852.

Madhu, P. 1984. Pollination, podset and compatability studies in open pollinated progenies of cocoa var. Forastero. M.Sc(Ag) thesis, Kerala Agricultural University, Thrissur, 83p.

Marschner, H. 1995. *Mineral nutrition of higher plants*(2nd ed.). Academic Press., San Diego, USA, 360p.

Matsui, N. and Yoneda, K. 1999. Effects of shading and temperature on the growth and flowering of Miltonia. *J. Jpn. Soc. Hort. Sci.* 68(3): 608-613.

Mengel, K. and Kirkby, E.A. 2001. *Principles of Plant Nutrition* (5th ed.). Kluwer Academic Publishers., Dordrecht, 367p.

Menzel, C.M., Rasmussen, T.S., and Simpson, D.R. 1989. Effects of temperature and leaf water stress on growth and flowering of litchi (*Litchi chinensis* Sonn.). *J. Hort. Sci.* 64(6): 739-752.

Molina, R.V., Valero, M., Navarro, Y., Guardiola, J.L., and Garcialuis, A. 2005. Temperature effects on flower formation in saffron (*Crocus sativus* L.). *Scientia Hort*. 103(3):361-379.

Moradshahi, A., Eskandari, B.S., and Kholdebarin, B. 2004. Some physiological responses of canola (*Brassica napus* L.) to water deficit stress under laboratory conditions . *Indian J. Sci. Technol.*, Transaction A. 28(A1): 43-50.

More, S.R., Lad, B.L., Jamadagani, B.M., and Wagh, R.S. 2004. Effect of potassium levels on growth and yield of maize under moisture stress at different phenophases. *A. Plant Physiol*. 18(1): 13-16.

Morgan, A. and Sedgley, M. 2002. Environmental control of bud formation and flowering of clonal *Acacia bacleyana* F. Muell. for ornamental horticulture. *Aust. J. Exp. Agric.* 42(2): 211-216.

Nahar, K. and Gretzmacher, R. 2002. Effect of water stress on nutrient uptake, yield and quality of tomato (*Lycopersicon esculentum* Mill.) under subtropical conditions. *Die Bodenkultur*. 53(1): 45-51.

Nakata, S. and Sueihisa, R.1969. Growth and development of *Litchi chinensis* as affected by soil moisture stress. *Am. J. Bot*. 56(10): 1121-1126.

Nalini, P.V.1983. Flower bud differentiation in pepper (*Piper nigrum* L.). M.Sc. (Hort) thesis, Kerala Agricultural University , Thrissur , 83p.

Nawazish, S., Hameed, M., and Naurin, S. 2006. Leaf anatomical adaptations of *Cenchrus ciliaris* L. from the salt range, Pakistan against drought stress. *Pakist. J. Bot.* 38(5): 1723-1730.

Nayar, H. and Kaushal, S.K. 2002. Chilling induced oxidative stress in germinating wheat grains as affected by water stress and calcium. *Biol. Plant.* 45: 601-604.

Nir, I., Goren, R., and Leshen, B. 1972. Effects of water stress, gibberellic acid and 2-chloroethyltrimethyl ammonium chloride (CCC) on flower differentiation in Eureka Lemon Trees. *J. Am. Soc. Hort. Sci.*, Alexandria. 97(6): 774-778.

Okuda, H., Noda, K., Hirabayashi, T., and Yonemoto, J.Y. 2004. Relationship between floral evocation and bud dormancy in satsuma mandarin. *Scientia Horticulturae* 102(2): 213-219.

Pandey, N. 2004. Ascendancy of genotype and stress temperature on flowering behaviour in long duration pigeonpea. *Legume Res.* 27(3): 177-182.

Panse, V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers*.(2nded.). Indian Council of Agricultural Research, New Delhi, 325p.

Prakash, M. and Ramachandran, K. 2000. Effects of moisture stress and anti-transpirants on leaf chlorophyll, soluble protein and photosynthetic rate in brinjal plants. *J. Agron. Crop Sci.* 184(3): 153-156.

Preez, R.J., Welgemoed, C.P., and Dupreez, R.J. 1994. Flowering of the surinam cherry. In lightings bulletin - Institunt-Vir-Tropiese-en-Subtropiese-Gewasse. 257, 10; 1 p.

Rajan, P.S. 1985. Flower bud differentiation in *Piper* sp. MSc(Ag) thesis, Kerala Agricultural University, Thrissur.

Rameshwar, A. 1988. Mango flowering – Stress induced. *Acta Hort*. 231: 433-439.

Rao, G.S.L.H.V.P. 2003. *Agricultural Meteorology*. Kerala Agricultural University, Thrissur, Kerala, India, 326p.

Rao, N.K.S., Bhatt, R.M., Anitha, N., Mascarenhas, J.B.D., and Naren, A. 1999. Influence of moisture stress on leaf water status, osmotic potential, chlorophyll fluorescence and solute accumulation in field grown tomato cultivars. *Vegetable Sci.* 26(2):129-132.

*Roberts, G.W. and Knoerr, K.R. 1977. Components of water potential estimated from xylem pressure in five tree species. *Ocecologia*. 28: 191-202.

Sadasivam, S. and Manikam, A. 1992. *Biochemical methods for Agricultural Sciences.* Wiley Eastern Ltd., New Delhi, 240p.

Sairam, R.K. and Saxena, D.C. 2000. Oxidative stress and antioxidants in wheat genotypes: Possible mechanism of water stress tolerance. *J. Agron. Crop Sci*. 184(1): 55-61.

*Salakpetch, S. and Poonnachit, U. 2006. Soil moisture stress and irrigation management promote mangosteen (*Garcinia mangostana* L.) flowering. *J. Hawaiian Pacif. Agric*. 13: 35-41.

Sale, P.J.M. 1970. Growth, flowering and fruiting of cocoa under controlled soil moisture conditions. *J. Hort. Sci.* 45:99-118.

Samdur, M.Y., Manivel, P., Jain, V.K., Chikani, B.M., Gor, H.K., Desai, S., and Misra, J.B. 2003. Genotypic differences and water deficit induced enhancement in epicuticular wax load in pea nut. *Crop Sci*. 43: 1294-1299.

Sankar, B., Jaleel, C.A., Manivannan, P., Kishorekumar, A., Somasundaram, R., and Panneerselvam, R. 2007. Drought induced biochemical modifications and proline accumulation in bhindi varieties. *Acta Bot. Croat*. 66(1): 43-56.

Sarker, B.C., Hara, M., and Uemura, M. 2005. Proline synthesis, physiological responses and biomass yield of egg plants during and after repetitive soil moisture stress. *Scientia Horticulturae*. 103: 387-402.

Schonherr, 1976. Water permeability of isolated culicular membranes. The effect of cuticular waxes on diffusion of water. *Planta*. 131: 159-164.

*Sergent, E., Casanova, E., and Leal, F.1993. Effect of some climatic and nutritional factors on flowering in mango (*Mangifera indica* L.) yield. *Revista-de-la-Facultad - de – Agronomia*. 10(3): 287-296.

Sharma, B.B., Mageed, K.I.A., and Watlal, P.N. 1987. Studies on water stress in Kinnow as influenced by irrigation schedules and nitrogen applications. *Indian J. Hort*. 4: 52-56.

Shinde, A.K., Patil, B.P., Godse, S.K., Jadhav, B.B., and Kandalkar, M.P. 2005. Weather relations on floral sex expression in Alphonso mango. *J. Agromet*. 7(1): 135-139.

Sinclair, T.R. and Ludlow, M.M. 1985. Who taught plants thermodynamics? The unfulfilled potential of water potential. *Aust. J. Plant Physiol*. 12: 213-217.

Singh, G., Rajan, S., Pandey, D., and Singh, A.K. 1997. Effect of soil moisture stress on water relation by plant and cropping behaviour in guava (*Psidium guajava*). *Indian J. Agric. Sci.* 67(7): 303-306.

Singh, N., Chhokar, V., Sharma, K.D., and Kuhad, M.S. 1997. Effect of potassium on water relations, CO_2 exchange and plant growth under quantified water stress in chickpea. *Indian J. Plant Physiol*. 2(3): 202-206.

Smith , R.W. 1964. The establishment of cocoa under different soil moisture regimes. *Emp. J. Exp. Agric.* 32(127): 249-256.

Sofo, A., Dichio, B., Xiloyannis, C., and Masia, A. 2004. Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress. *Physiologia Plant*. 121: 58-65.

Song, L.R., En, S.H., Fen, L.S., Chung, C.C., Lin, R.S., and Shyn, H.E. 1998. Effect of light intensity and photoperiodism on flowering and flower quality of *Oncidium* spp. *Chinese J. Agromet.* 5(4): 193-202.

Southwick, S.M. and Davenport, T.L. 1986. Characterization of water stress and low temperature effects on flower induction in citrus. *Physiol.* 81: 26-29.

Spices Board. 2003. *Vanilla- The prince of spices,* Thomas, J. and Rao, Y.S (eds.), Spices Board, Cochin, 2p.

Starner, W.J. and Hardley, H.H. 1967. Chlorophyll content of various strains of soyabeans. *Crop Sci*. 5: 9-11.

Stephenson, R.A., Ko, H.L., and Gallagher, E.C. 1989. Plant water relations of stressed, non-bearing macadamia trees. *Scientia Hort*. 39: 41-53.

Stoyanova, D., Tehakalova, E., and Yordana, I. 2002. Influence of different soil moisture on anatomy of maize leaves and ultrastructure of chloroplasts. *Bulg. J. Plant Physiol.* 28(1-2): 11-20.

*Suzuki, H. and Tanno, S. 1971. Studies on blossoming and fruit bearing of apple trees. II. Forecast of bud break and flowering times based on the average minimum temperature during early March in eleven areas. *Bull. Aketa Fruit Exp. Stn* 4: 33-35.

Taiz, L. and Zeiger, E. 2003. *Plant Physiology*(3rd ed.). Panima Publishing Corporation., New Delhi, 1405p.

Thakur, P.S., Singh, G., and Rai, V.K. 1981. Peroxidase isozymes in relation to developing water deficits in two *Zea mays* L. cultivars. *New Phytol.* 89: 25-32.

Thankamani, C.K. 2000. Influence of soil moisture regimes on growth and yield in bush pepper (*Piper nigrum* L.). MSc(Ag) thesis, Kerala Agricultural University, Thrissur.

Thankamani, C.K. and Ashokan, P.K. 2004. Solute accumulation in black pepper (*Piper nigrum* L.) varieties in response to water stress. *Indian J. Hort*. 61(1): 74-77.

Thomas, J., Rajkumar, R., and Pius, P.K. 2004. Screening of tea germplasm under soil moisture stress for productivity. *J. Plantn Crops*. 32 (Suppl.): 50-53.

Tyagi, A., Kumar, N., and Sairam, R.K. 1999. Efficacy of RWC, membrane stability, osmotic potential, endogenous ABA and root biomass as indices for selection against water stress in rice. *Indian J. Plant Physiol.* 4(4): 302-306.

Upreti, K.K. and Murti, G.S.R. 2004. Changes in electrolyte leakage, chlorophyll concentration, endogenous hormones and bulb weight in onion in response to water stress. *Trop. Agric. (Trin.).* 81(2): 127-131.

Veeraraghavan, P.G. and Vasavan, M.G. 1979. Influence of rainfall on the productivity of cashew. *Indian Cashew J.* 12(2):23-24.

Velu, G. and Palanisami, K. 2002. Impact of moisture stress and ameliorants on growth and yield of sun flower. *Madras Agric. J.* 88(10/12): 660-665.

Vincent, S., Rajarathinam, S., and Jayakumar, P. 2005. Studies on the physiological parameters as a selection criteria for drought tolerance among hybrid coconut accessions. *Madras Agric. J.* 92(10-12): 699-704.

Vishal, N., Bhargava, R., and Nath, V. 2000. Flowering scenario in commercial cultivars of jujube (*Ziziphus mauritiana* Lamk.) under Indian arid ecosystem. *J. Appl. Hort.* 2(2): 98-101.

Voleti, S.R., Kasturibai, K.V., Rajagopal, V., and Shivashankar, S. 1990. Relative water content and proline accumulation in coconut genotypes under moisture stress. *J. Plantn Crops.* 18(2): 88-95.

Woodhams, D.H. and Kozlowski, T.T. 1954. Effects of soil moisture stress on carbohydrate development and growth in plants. *Am. J. Bot.* 41: 316-320.

Yadav, R.S. and Chandrabhushan. 2001. Effect of moisture stress on growth and yield in rice genotypes. *Indian J. Agric. Res.* 35(2): 104-107.

Yadav, S. K., Jyothilakshmi, N., Maheswari, M., Vanaja, M., and Venkateswarlu, B. 2005. Influence of water deficit at vegetative, anthesis and grain filling stages on water relation and grain yield in sorghum. *Indian J. Plant Physiol*. 10: 20-24.

Yasuyoshi, H., Toshiko, T., Satoru, K., and Koichi, I. 1998. Effect of water stress on growth, sugar and nitrogen content in tomato cherry fruit. *J. Jpn. Soc. Hort. Sci.* 67(5): 759-766.

Yonemoto, Y., Inoue, H., Majikina, M., Okuda, H., and Higuchi, H. 2005. Effect of temperature on floral induction and development of floral organs in white sapote (*Casimiroa edulis* Llave and Lex.) cv. Florida. *Jpn. J.Trop. Agric*. 49(1): 8-13.

* Originals not seen

INFLUENCE OF MICRO METEOROLOGICALFACTORS ON FLOWERING IN VANILLA (*Vanilla planifolia* Andrews)

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ABSTRACT OF THE THESIS

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ABSTRACT

Investigations on “Influence of micro meteorological factors on flowering in vanilla” were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University during 2005-2007. The objective of the study was to find out the effect of soil moisture stress and micro - meteorological factors on flowering in vanilla. The studies were carried out in five year old vanilla plants maintained in Department of Plantation Crops and Spices farm and in a selected farmer’s field at Thrissur district.

Moisture stress was induced in vanilla gardens by withholding irrigation at four levels viz. one month, 1½ month, two months and 2½ months. The influence of soil moisture stress on flowering in vanilla and the changes in physiological and biochemical parameters in vanilla due to moisture stress and influence of micro meteorological parameters on flowering in vanilla were studied in the present investigations.

Soil moisture stress induced flowering in vanilla. Maximum flowering of 80 per cent was observed in plants stressed for moisture for a period of one month followed by 60 per cent flowering in 1½ month stress period. Hence soil moisture stress for a period of 1-1½ month during November-December is sufficient to induce flowering in vanilla.

Soil moisture stress altered various physiological and biochemical parameters in vanilla. Leaf thickness, relative leaf water content, membrane stability, soluble protein and total chlorophyll content decreased due to soil moisture stress. The content of epicuticular wax, total free amino acids, accumulation of proline, activity of peroxidase enzyme, total sugar content and K concentration in tissues increased due to stress. The changes in physiological and biochemical parameters were more pronounced as the intensity of stress increased.

The micro-meteorological parameters of the garden also influenced flowering in vanilla. Flower opening in vanilla was found positively correlated with maximum

temperature and negatively with relative humidity and minimum temperature. Flower opening showed highly significant positive correlation with light received from North and South directions and percentage of light infiltration.

Soil moisture stress for a period of 1-1 ½ month during November- December is sufficient to induce flowering in vanilla. Changes in physiological parameters due to moisture stress could be used to visually assess the extent of moisture stress in the garden. The biochemical parameters recorded at ideal stress period could be used as indices to assess the extent of moisture stress in plants more precisely in high tech / precision farming systems. Manipulation of microclimate with respect to temperature, light and relative humidity is essential for getting proper flowering in vanilla.