IDENTIFICATION OF DROUGHT TOLERANT COCOA TYPES

By
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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

Department of Agronomy
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR
KERALA, INDIA
2005

DECLARATION

I,Binimol B.hereby declare that the thesis entitled **"Identification of Drought Tolerant Cocoa Types"** 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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16 - 4 - 2005

CERTIFICATE

Certified that the thesis entitled **"Identification of Drought Tolerant Cocoa Types"**is a record of research work done independently by **Mrs. Binimol B.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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We, the undersigned members of the advisory committee of **Mrs. Binimol B.**, a candidate for the degree of **Master of Science in Agriculture**, with major field in Agronomy, agree that the thesis entitled **"Identification of Drought Tolerant Cocoa Types"** may be submitted by Miss Binimol.B, in partial fulfilment of the requirement for the degree.

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Dedicated To

My Family

Introduction

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.), an evergreen tropical tree of the plant family Sterculiaceae, is a native of Amazon River basin. From there it spread to all over the tropical regions of the world during 18th and 19th centuries. Commercial cultivation of cocoa was commenced in Ghana in 1879. From Ghana, it was introduced into other African countries; the most important of which are Ivory Coast, Nigeria and Cameroon. It is the third important beverage crop next to coffee and tea and is primarily an important raw material for confectionary industries. The cocoa of commerce is the cured dry beans, which contain 57.0 per cent fat, 7.0 per cent protein, 7.0 per cent carbohydrates and 1.7 percent theobromine.

Cocoa was introduced to India in the first half of the twentieth century. However, cultivation on commercial scale started in the 1960's only. As cocoa is a tropical crop, the tropical and sub tropical regions of India are suitable for growing cocoa. The cocoa growing states in India are Kerala, Karnataka, Goa, Maharashtra, Andra Pradesh, Tamil Nadu, Pondicherry, Orissa and West Bengal. In the late part of 1980's, there was a major expansion in the area under cocoa in the southern states of Kerala, Karnataka and Tamil Nadu, where cocoa is grown as a intercrop along with coconut and arecanut.

The growth and yield of cocoa is influenced by a number of environmental factors, particularly rainfall, temperature and water stress. The harvest of cocoa pods is spread over several months but peak harvesting is normally done during the months of July-August and November-December.

In India, cocoa is mainly grown under coconut and arecanut gardens. However, in the rainfed areas, drought is the major constraint limiting the productivity of the crop. When moisture is not sufficient, the plant suffers. Cocoa is very sensitive to drought. Rainfall in the range of 1250-3000mm per annum, preferably between 1500 to 2000mm, with a dry season of not more than three months with less than

100mm rainfall per month and mean maximum temperature varying between 30° to 32°C and mean minimum between 18° to 21°C with an absolute minimum of 10°C are the climatic conditions desirable for cocoa (Wood and Lass, 1985). Water stress affects the most important determinants of yield – canopy architecture, photosynthesis and partitioning of assimilates.

The drought intensity is more pronounced in the Northern regions of Kerala and Coastal Karnataka. The rainfall pattern in Southern Kerala is some what even whereas in Northern Kerala, 85 per cent of rainfall is received during the southwest monsoon, while 7.5 per cent is received during the northeast monsoon, (October to November) and the remaining 8.5 per cent is received as non-seasonal rainfall. The rainless period ranges from 5 to 7 months in northern Kerala, so that the non-availability of water towards the summer exposes the plants to stress.

Efforts to increase the crop area under irrigation have several limitations, and the major one being water. Being a perennial crop, the water requirement of cocoa is fairly high. The approach under such a situation should be to utilize the available water sources for high use efficiency. Therefore, it is important to identify the varieties, which can withstand moisture stress conditions in the field, and to evolve management strategies for conserving soil water in order to mitigate adverse effects of drought.

In the circumstances discussed, it was felt worthwhile to investigate the drought governance characteristics of the most promising cocoa varieties and accessions. It is hoped that by identifying cocoa cultivars/accessions that are able to tide over the drought period, the crop can be introduced into more rainfed areas without sacrificing yield. However, as cocoa is a cross-pollinated crop, the progenies from seeds may not be exhibiting the characteristics of their parents. Now-a-days, vegetative propagation in coca is common and farmers are increasingly planting clones produced through patch budding. Hence, in the present investigation, both seedling progenies and budling progenies of the same varieties/accessions were selected and screened. The main objectives of the study were to screen and identify drought tolerant cocoa cultivars/accessions suitable for rainfed conditions and to study the physiological and biochemical attributes of drought tolerance in cocoa.

The investigation involved a two-stage screening of seedlings and budlings and monitoring of their field grown plants. A preliminary screening was done on ten cocoa cultivars released from Kerala Agricultural University and twenty promising accessions as the first step. A secondary screening of cocoa cultivars/accessions selected from the preliminary screening was then conducted. Simultaneously, field monitoring of cocoa trees included in the secondary screening was also done.

Review of Literature

2. REVIEW OF LITERATURE

Cocoa is regarded as a sensitive crop to water stress. Water stress affects several physiological processes leading to reduction in crop yield. Cocoa is grown mainly as an intercrop in coconut and arecanut gardens in Kerala. However, in rainfed gardens, a continuous drought period of about 4 to 6 months affects its growth drastically. Available literature on drought tolerance in cocoa as well as related crops is reviewed in this chapter.

Stocker (1943) defined drought tolerance as the capacity of a plant to develop normally in dry habitats yielding maximum crops. For evaluating drought tolerance in plants, Sullivan (1971) suggested certain criteria, which include high leaf water potential, stomatal resistance to water loss and tolerance to heat. A rapid method of screening for drought tolerance by measuring the leaf water potential was used in cacao accessions by Balasimha and Daniel (1988). In cocoa, chlorophyll stability index (Ravindran and Menon, 1981) and nitrate reductase activity (Balasimha, 1982) were the other major parameters used for screening for drought tolerance. Thick leaf, higher wax content, efficient stomatal closure and high tissue elasticity were responsible for better adaptation of plants to drought conditions (Balasimha *et al.*, 1988). Free proline accumulation in leaves was used as one of the parameters for screening for drought tolerance (Sharma and Aravindkumar, 1991). The characters such as chlorophyll-carotenoid ratio, stomatal resistance, leaf water potential, transpiration rate, relative water content, specific leaf weight, proline content, sugars and nitrate reductase activity were suggested for screening drought tolerance in black pepper varieties (Vasantha *et al.*, 1989). Rajagopal *et al.*, (1990) screened coconut genotypes for drought tolerance by assessing leaf stomatal resistance, transpiration rate, leaf water potential and relative water content. Diffusive resistance, transpiration rate, leaf water potential, chlorophyll stability index, proline content and nitrate reductase activity were used for screening drought tolerance in cashew seedlings by Latha (1998). Chandrasekhar (1977) used leaf water potential at pre drawn hours as an index of drought tolerance in rubber.

2.1 WATER STRESS ON GROWTH

The growth and yield of crops are influenced by a number of environmental factors particularly rainfall, temperature and water stress. Moisture stress is the major factor that decides the distribution of plants and their productivity (Fisher and Turner, 1978). Reduction in plant size is the general effect of water stress (Kramer, 1983). Water stress affects the most important physiological determinants of yield-canopy architecture, photosynthesis and partitioning of assimilates (Balasimha, 1999). Rajagopal and Balasimha (1994) observed that coconut trees under regular watering had a biomass of 150 kg per palm, but when grown under moisture stress, it was only 100 kg per palm. According to Rajesh (1996), irrigated seedlings of *Acacia mangium* (1 year old) were 138.5 cm tall whereas moisture stressed seedlings were only 81.5 cm tall. He also reported a similar decrease in plant height due to moisture stress in seedlings of *Swietenia macrophylla*. Moisture stress reduced the height, number of leaves and leaf area of cashew seedlings (Latha, 1998); and height, girth, number of leaves and leaf area in blackpepper (Thankamony, 2000). The stomatal frequency and its behaviour play a major role in water conservation in many plants.

2.2 WATER STRESS ON PHYSIOLOGICAL CHARACTERS

2.2.1 Relative leaf water content (RLWC)

Relative leaf water content is the ratio of fresh weight of leaves to oven dry weight expressed as percentage. RLWC is an alternative measure of plant water status (Sinclair and Ludlow, 1985). The drought tolerant accessions of cocoa had higher RLWC (82.35%) compared to susceptible ones (79.43%)(Balasimha *et al.*, 1988). A high RLWC was maintained by stress tolerant hybrids of coconut like Laccadive Ordinary x Gangabondom and Laccadive Ordinary x Chowghat Orange Dwarf and local varieties like West Coast Tall, under simulated water stress (Voleti *et al.*, 1990). The relative water content in drought tolerant and susceptible genotypes of coconut were 82 and 79 per cent respectively in plants grown under water stress (Rajagopal and Balasmiha, 1994). Latha (1998) observed high RLWC (49%) in regularly irrigated cashew seedlings and low RLWC in plants under life saving irrigation (33%). The decrease in RLWC under water stress is also reported in pepper (Thankamony, 2000). Suryakumari *et al.*, 2000 reported a decrease in RLWC under water stress in black gram genotypes.

2.2.2 Dry weight fraction (DWF)

Helkvis *et al* (1974) related the dry weight ratio of leaf lamina to its turgid weight and drought tolerance. DWF increased with increase in duration of moisture stress in cashew seedlings (Latha, 1998).

2.2.3 Transpiration rate

Water stress, in general, reduces transpiration rate of plants. The drought tolerant accessions of cocoa (NC 23, NC 29 and NC 39) showed 54 to 59 per cent decrease in transpiration under stress compared to plants under irrigation (Balasimha *et al.*, 1988). In coconut also, drought tolerant hybrid Laccadive Ordinary X Chowghat Dwarf Orange had lower transpiration rate compared to the sensitive hybrid, Chowghat Dwarf Orange X West Coast Tall and the values reduced from 3.45 to 1.89 μ mol $cm^{-2}s^{-1}$ and from 4.58 to 3.30 μ mol $cm^{-2}s^{-1}$ respectively during water stress (Rajagopal and Balasimha, 1994). Transpiration rate was the highest (4.75 m mol $m^{-2}s^{-1}$) in cashew seedlings stressed for two days, and it declined to 2.11 m mol $m^{-2}s^{-1}$ in plants stressed for five days (Latha, 1998). Bhatt *et al.* (1998) observed a decrease in transpiration rate under water stress in oats.

2.2.4 Stomatal conductance

High diffusive resistance (low stomatal conductance) was observed in drought tolerant coconut varieties as compared to the susceptible variety, Chowghat Orange Dwarf under water stress (Kasthuri bai *et al.*, 1988; Rajagopal *et al.*, 1990). Balasimha *et al.*, (1988) reported effective stomatal regulation in cacao clones NC-23, NC-29 and NC-31 resulting in decrease of transpiration. The soil moisture stress reduced stomatal conductance in sorghum, maize and pearl millet (Singh and Singh, 1994). In sugar cane, plants under normal soil moisture condition possessed lower stomatal diffusive resistance and higher transpiration, whereas under soil moisture deficient condition, they exhibited greater stomatal diffusive resistance and less transpiration (Srivastava *et al.*, 1996). Latha (1998) reported a decrease in stomatalconductance with increase in reduction of water stress in cashew seedlings and the values were 183 m mol $m^{-2}s^{-1}$ when water stressed for two days, and 61 m mol $m^{-2}s^{-1}$ when water stressed for 10 days. The stomatal conductance of bush pepper decreased considerably due to higher levels of water stress indicating efficient stomatal regulation in response to water stress (Thankamony, 2000).

2.2.5 Leaf water potential (LWP)

Leaf water potential is an important quantitative character used to assess water status of plants. Though variation exists in water potential, a plateau in water potential is reached once the stoma close beyond -1.5 bars (Hucheon, 1975). Under moisture stress condition, drought tolerant accessions of cocoa showed leaf water potential of -0.91 bars whereas in susceptible ones it was 0.93 bars (Balasimha and Daniel, 1988). It was reported that water potential measurement could be successfully used to screen field grown sorghum genotypes for drought tolerance (Blum, 1990). Singh *et al.*, (1990) observed significant differences in water potential among wheat genotypes under drought stress. Latha (1998) observed a decrease in water potential of cashew seedlings with increase in duration of stress and it was the highest two days after stress (-2.62 bars) and decreased to -3.08 bars at five days after stress and further decreased to -3. 42 bars, 10 days after stress.

2.2.6 Chlorophyll

Water stress often decreases the chlorophyll content of leaves (Hsiao, 1973). The chlorophyll 'a', 'b' and total chlorophyll contents of cashew leaves (13 years old trees) were 0.39, 0.48 and 0.76 mg g^{-1} leaf tissue respectively (Latha, 1992). The leaf chlorophyll content of cocoa accessions were low in plants under water stress compared to irrigated plants (Balasimha *et al.*, 1998). Chlorophyll content was high in regularly irrigated cashew seedlings than under life saving irrigation (Latha, 1998). Deka and Baruah (1994) reported that the rice cultivars, which exhibited the highest drought resistance, had maximum chlorophyll content and yield when stress was imposed at three stages of growth, viz; tillering, panicle initiation and ripening. The chlorophyll content decreased considerably due to severe water stress in black pepper (Thankamony, 2000).

2.2.7 Chlorophyll stability index (CSI)

Chlorophyll stability under stress is one of the factors, which contribute drought tolerance in plants. Chlorophyll stability index was used for *in vitro* screening for drought tolerance in cacao (Ravindran and Menon, 1981). In cashew, chlorophyll stability index of drought tolerant accessions were higher than sensitive varieties (Latha, 1998).

2.2.8 Relative Injury (RI)

According to Silva *et al.*, (1974), the leaf membrane stability is disturbed due to moisture stress and stability is indirectly measured by relative injury. Clarke and Mc Graig (1982a) used membrane stability to evaluate drought tolerance. In coconut, the drought tolerant genotypes showed electrolyte leakage of 20.19 per cent where as in susceptible ones, it was 27.66 per cent under water stress (Rajagopal and Balasmiha, 1994).

2.2.9 Membrane stability

The membrane damage and solute leakage under stress are measures of cell membrane stability (Blum and Ebercon, 1981). Kasthuri bai *et al.*, (1988) studied membrane damage in coconut by measuring the leakage of electrolytes from leaf discs of water stressed plants and found that it was less in two hybrids and more in two dwarfs, the highest being in Malayan Yellow Dwarf. The drought tolerant cocoa accessions had lower electrolytic leakage due to increased wax and lipid fractions in the water stress as compared to susceptible accessions (Bhat *et al.*, 1990). Rajagopal and Balasimha (1994) observed that the electrolyte leakage of drought tolerant coconut genotypes was lower in susceptible ones, due to water stress. Membrane damage increased significantly with increase in water stress.

2.3 WATER STRESS ON BIOCHEMICAL CHARACTERS

2.3.1 Proline

Proline, an amino acid, is known to accumulate in plants under stress conditions (Hsiao, 1973). Proline seems to aid in drought tolerance (Gardner *et al.*, 1985). Proline accumulation during water stress is due to its synthesis from glutamase as well as due to decreased rate of proline oxidation (Kramer, 1983). Proline acts as a storage pool for nitrogen and as a solute molecule reducing the osmotic potential of the cytoplasm. Free proline accumulation in leaves was used as one of the parameters for screening the crop varieties for relative drought tolerance (Sharma and Aravindkumar, 1991). In cocoa, water stress increased the proline content of seedlings from 57 to 333 μ mol g^{-1} (Rajagopal and Balasimha, 1994). Proline accumulation was found to be similar in many cultivars of water stressed plants such as barley (Singh *et al.*, 1972), Sorghum (Jones and Turner, 1978), Soybean (Sarkar, 1992) and Mulberry (Kumari and Veeranjaneyulu, 1996). Proline accumulation under drought is also reported in other crops like cluster beans (Garg *et al.*, 1998), sorghum (Sathbai *et al.*, 1998), and black gram (Suryakumari *et al.*, 2000).

2.3.2 Nitrate reductase activity (NRA)

Nitrate reductase is the key enzyme, which catalyses the reduction of nitrate to nitrite, the first step in nitrate assimilation by plants (Bhaskar, 1997). A decrease in NRA at stress condition in rice was reported by Sairam and Dube (1984) and Deka and Baruah (1994). Seasonal differences also affect NRA. Higher NRA was observed from February to April, but it was low during rainy season. At the same time, in irrigated plants, the NR activity was low during dry period (Balasimha *et al.*, 1991). NR stability under drought was 0.59 and 0.53 in tolerant and susceptible species of cocoa respectively (Balasimha and Daniel, 1988). Garg *et al.*, (1998) indicated that NR activity decreased and proline increased under water stress in cluster bean genotypes. A reduction in NR activity due to stress was reported in maize by Foyer *et al.*, (1998), and wheat by Yadav *et al.*, (1998).

Materials and Methods

3. MATERIALS AND METHODS

The experiment "Identification of drought tolerant cocoa types" was conducted in the farm of Cadbury- KAU Co-operative Cocoa Research Project (CCRP) attached to the College of Horticulture, Kerala Agricultural University, Thrissur during 2003-2004.

The experiment involved a preliminary screening of 10 cultivars and 20 promising accessions of cocoa, a detailed secondary screening of selected cultivars/accessions, and field monitoring of trees of the selected cultivars/accessions.

3.1 GENERAL DETAILS

Location

The experiment was conducted in the CCRP farm, Vellanikkara. Vellanikkara is situated at $10^0 31'$ North latitude, $76^0 13'$ East longitude and at an altitude of 40.3m above mean sea level.

Soil

Preliminary screening and secondary screening were done using seedlings/budlings of cocoa. These were raised in potting medium prepared with garden soil, sand and cow dung at 1:1:1 ratio. In general, the soil of the farm was sandy clay loam in texture (order Ultisol). Important physical and chemical properties of the soil in the farm are given in Table 1.

Table 1. Important physical and chemical properties of the soil in the farm

Physical properties	Value	Method used
a) Mechanical composition		
Sand (%)	55.3	International pipette method (Piper, 1942)
Silt (%)	13.4	
Clay (%)	31.3	
Textural class	**Sandy clay** loam	
b) Chemical properties		
Organic carbon (%)	0.57	Walkley and Black rapid titration method (Jackson, 1958)
Total Nitrogen (%)	0.04	Microkjeldhal method (Jackson, 1958)
Available phosphorus(kg ha^{-1})	22.5	Ascorbic acid reduced molybdophosphoric blue colour method (Watanabae and Olsen, 1965)
Available potassium (kg ha^{-1})	139.6	Flame photometry, Neutral normal ammonium acetate extraction (Jackson, 1958)

Seeds and seedlings

Seeds obtained from well-matured pods collected from the field of CCRP were used for sowing. From these pods, uniform sized beans were selected and sown in polythene bags and seedlings were raised (Plate 1 and Plate 2).

Weather

Weather data on atmospheric temperature (maximum and minimum), rainfall, evaporation, relative humidity and sunshine hours during the period of experiments were collected from the Department of Agricultural Meteorology, College of Horticulture, Vellanikkara and presented in Appendix 1.

Varieties/accessions

The main objective of the experiment was to identify drought tolerant cocoa cultivars/accessions for rainfed conditions. For this, 30 cocoa cultivars/accessions from the germplasm collection maintained at Cadbury – KAU Co-operative Cocoa (CCRP) project located at the main campus of the Kerala Agricultural University, Vellanikkara, were screened (Table 2). Screening was done at three stages viz, preliminary, secondary and field monitoring. Screening was done in two sets of plants, seedlings and budded plants.

3.2 PRELIMINARY SCREENING

The preliminary screening was conducted during February to April using six-month-old seedlings raised in polythene bags.

Design - CRD

Replication - 3

The seeds of 30 cultivars were collected during September 2003 from the germplasm collections of CCRP. Twenty seeds of each cultivars were sown in white polythene bags (30 cm x 25 cm) containing a mixture of garden soil, sand and cow dung

Plate 1. Six month old cocoa seedlings

Plate 2. Six month old cocoa budlings

Table 2. Details of varieties and accessions used for the experiment

Sl. No.	Name of Accessions	Released Varieties
1	M 16.9	CCRP 1
2	M 13.12	CCRP 2
3	GI 5.9	CCRP 3
4	GII 19.5	CCRP 4
5	GIV 18.5	CCRP 5
6	GVI 55	CCRP 6
7	GVI 56	CCRP 7
8	P_1 1.21	CCRP 8
9	S_1 7.1	CCRP 9
10	S_2 4.13	CCRP 10
11	M 9.16	
12	GI 4.8	
13	GI 9.6	
14	GI 10.3	
15	GI 15.5	
16	GII 12.3	
17	GII 20.4	
18	GIII 1.2	
19	GIII 4.1	
20	GIV 2.5	
21	GIV 32.5	
22	GVI 50	
23	GVI 51	
24	GVI 54	
25	GVI 59	
26	GVI 60	
27	GVI 61	
28	GVI 64	
29	GVI 68	
30	GIV 35.7	

at 1:1:1 ratio and kept in a shade house with regular watering for six months. The seedlings were budded with the selected cultivars/accessions, when they attained pencil thickness. When the budded seedlings were ready for planting, five seedlings, which showed uniform growth, were selected and subjected to moisture stress by withholding irrigation till all the seedlings dried.

In another set, seedling progenies of the above 30 types (without budding) ready for planting were also screened. The following observations were made:

3.2.1 Relative leaf water content (RLWC) and dry weight fraction (DWF)

Ten leaf discs of one-centimetre diameter were taken from the youngest fully matured leaf (third leaf from the top) using a cork borer and their fresh weight recorded to 0.1 mg accuracy with the help of an electronic balance. The discs were floated in water, in covered petridishes, for four hours at room temperature and ambient light .The discs were then gently bloated with tissue paper and the turgid weight recorded .The leaf discs were then oven dried for six hours at 85^0c and the dry weight recorded. RLWC and DWF were calculated as follows.

$$\text{RLWC} = \frac{(\text{Fresh weight- dry weight})}{(\text{Turgid weight-dry weight})} \times 100$$

(Barrs, 1968)

$$\text{DWF} = \frac{\text{Dry weight}}{\text{Turgid weight}}$$

(Helkvis *et al.*, 1974)

3.2.2 Percentage of dried leaves and number of days taken for complete drying

Observations on percentage of dried leaves at 15 days after withholding irrigation and the number of days took for complete drying of the plant were recorded. The number of leaves dried and total number of leaves present per plant were counted at 15 days after withholding irrigation and the percentage of leaves dried was calculated.

Based on the data on RLWC, DWF, percentage of dried leaves and number of days took for complete drying, seven apparently tolerant cultivars and three apparently sensitive cultivars were selected for secondary screening after doing DMRT and cluster analysis.

3.3 SECONDARY SCREENING

Based on the preliminary screening, seven apparently tolerant and three apparently sensitive cultivars were identified. These 10 cultivars were subjected to a secondary screening during June to September 2004 at three soil moisture regimes, regular watering (daily), watering once in five days (life saving irrigation) and no watering. Seedlings/budlings ready for planting were observed for one month after imposing the treatments .The experiment was laid out taking moisture regimes as main plots and accessions as sub plots.

Observations on relative leaf water content, dry weight fraction, percentage of dried leaves and number of days taken for complete drying were also recorded, as done during the preliminary screening. Other characters included for observations were:

Physiological parameters

3.3.1 Transpiration rate

Transpiration rates were recorded at 1100 hrs and 1400 hrs using a Steady State Porometer (Model LI-1600, LI-COR, Nebraska, USA).

3.3.2 Stomatal conductance

A Steady State Porometer was used to measure the leaf diffusive resistance. Measurements were taken at 1100 hrs and 1400 hrs on the abaxial surface of physiologically matured leaves of plants from each treatment. The mean diffusive resistance was converted to conductance and expressed in m mol m^{-2} s^{-1}.

3.3.3 Leaf water potential

A scholander - pressure chamber (Soil moisture equipment corporation, Ohio, USA) was used for finding out the leaf water potential. The leaf water potential was measured at 0600 hrs from the youngest fully matured leaf (3^{rd} leaf from the top). The leaf water potential was measured from the plants and mean values worked out and expressed in bars.

3.3.4 Leaf area per plant

The total leaf area was estimated using the emperical relation, LA= 0.64 l x w (Gopinathan, 1981) and by summing the areas of individual leaves, where LA=leaf area, l= length of leaves and w = width of leaves.

3.3.5 Chlorophyll content of leaves

Chlorophyll of the leaves was extracted by dimethyl sulfoxide (DMSO) and estimated spectrophotometrically, following the methods of Shoaf and Lium (1976). Chlorophyll 'a', chlorophyll 'b' and total chlorophyll of each sample were calculated using the following formulae.

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

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

$$\text{Total Chlorophyll} = 20.2\ (\text{OD at 645 nm}) + 8.02\ (\text{OD at 663 nm}) \times \frac{V}{1000Xw}$$

Where,

OD =Optical density

V =Final volume of dimethyl sulfoxide extract in ml

W =Fresh weight of tissue in g

3.3.6 Chlorophyll stability index (CSI)

Two fresh leaf samples (0.1g) were weighed separately and kept in two test tubes containing 7ml of DMSO. One sample was subjected to a temperature of 55^0 C for 30 minutes by keeping on hot water bath (treated) and the other sample was kept at room temperature (control). The samples were removed after 30 minutes. The extract was made up to 10 ml (V) with DMSO.The procedure was same as that of chlorophyll. The absorbance at 652 nm (A_{652}) was recorded. The chlorophyll contents (mg g^{-1} of fresh tissue) of the two samples (control and treated) were estimated as shown below.

$$\text{Chlorophyll content} = \frac{A_{652}/ 34.2 \times 1000 \times V}{1000XW}$$

The chlorophyll stability index was worked out using the following formulae (Kaloyereas, 1958)

$$\text{CSI} = \frac{(\text{Chlorophyll in control} - \text{Chlorophyll in treated})}{\text{Chlorophyll in control}}$$

3.3.7 Relative injury (RI)

Forty leaf discs (size 1 cm^2) were taken and washed three times with distilled water to wash out the contents of the cut cells at the peripherals of the leaf discs. Twenty leaf discs each were kept in two test tubes containing 20 ml of distilled water .One test tube was kept in a water bath at 45°C for 30 minutes, cooled to room temperature quickly with the tap water and kept as such at 6-10°C in a temperature controlled refrigerator for 18 hrs (for diffusion of electrolyte into medium). Then, it was kept in water bath at 25°C for one hour and electrical conductivity of the medium was measured at this temperature (T_1). This test tube was then boiled at 100°C for 20 minutes, cooled to room temparature, volume adjusted to 20ml and EC of the medium was measured at 25°C (T_2).

The other test tube containing 20 leaf discs was kept at 25°C for 30 minutes (instead of 45°C for the other test tube) and the EC was measured at 25°C (C_1). Following the same steps adopted with the first test tube, EC at 100°C (C_2) of the medium in the second tube was also recorded. RI was calculated as follows (Clarke and McGraig, 1982a).

$$\text{Relative Injury (RI)} = 1 - \frac{(1 - T_1/T_2)}{(1 - C_1/C_2)}$$

T_1 = EC of the medium at 45°C

T_2 = EC of the medium at 100°C

C_1 = EC of the medium at 25°C

C_2 = EC of the medium at 100°C

3.3.8 Membrane stability

The cell membrane stability was studied by observing the leakage of the membrane under water stress. For this, leaf discs (0.1g) were floated in 15ml-distilled water for three hours .The leaf discs were removed and the electrical conductivity of the solution was measured. After the initial measurements, leaf discs were returned to original solution and boiled in distilled water for 10 minutes. Leaf discs were removed and the solution was cooled. The electrical conductivity of the solution was determined again.

$$\text{Membrane stability} = \frac{\text{Initial electrical conductivity}}{\text{Final electrical conductivity}}$$

Growth characters

3.3.9 Plant height

The height of the plant (cm) was measured from ground level to the tip of the topmost leaf using a metre scale.

3.3.10 Collar girth

The girth of the plant (cm) was measured at 10cm above the ground level using a thread and the length of the thread was measured using a metre scale.

3.3.11 Number of leaves

The numbers of leaves on the plant were counted at initial and final stage of treatments.

3.3.12 Total biomass

After recording the observations, the plants were uprooted carefully with least damage to the root system. The root portion is washed well and after allowing the free water to go, the fresh weight of the plants was recorded (g). The plants were then separated into shoot and roots and kept in hot air oven at 70°C for 48 hours and dry weight recorded to constant weight (g).

Biochemical characters

3.3.13 Proline

The proline content of the leaves was estimated by the method of Bates *et al.*, (1973). Fresh leaves (500mg) from the plants in each treatment were homogenized with 10ml of 3 per cent aqueous sulfosalicylic acid, centrifuged at 3000 rpm for 10 minutes. Two milliliters of the supernatant liquid was taken and 2ml of glacial acetic acid, 2ml of acid ninhydrin mixture and 2ml of 6N orthophosphoric acid were added. The content allowed to react at 100^0C for 1hour and the reactions were terminated in an ice bath for 10 minutes. The reaction mixture was mixed vigorously with 4ml of toluene for 10-20 seconds. The upper chromophore containing toluene was aspirated from the aqueous phase and warmed at room temperature and the OD was read at 520nm in a spectrophotometer. The proline content was determined from a standard curve of pure proline and expressed μ g $g\text{-}^1$ fresh weight.

3.3.14 Nitrate Reductive Activity (NRA)

The NRA of leaves was estimated by the method of (Malik and Singh, 1980) .The leaf samples collected in ice bucket were cleaned with distilled water and blotting paper. One gram leaf discs of approximately 1cm diameter were suspended in a five milliliter reaction mixture (5% propanol and 0.02 %potassium nitrate in 0.1% potassium phosphate buffer of p^H 7.5) in a capped test tube and incubated in a flask at 30^0 C for 2 hrs. After that 0.4 ml of reaction mixture was taken in a test tube and added 0.2 ml of 1% Sulphanilamide (prepared in 3 N HCL) and 0.2 ml of 0.2 per cent N-napthyl ethylene diamene dihydrochloride. After 20 minutes, 4 ml of distilled water was added to the test tube and the intensity of the pink colour developed was read at 570 nm in a spectronic 20 spectrophotometer. The enzyme activity was estimated from the standard curve prepared by using different concentrations of potassium nitrite. The activity was expressed in terms of milli moles of nitrite formed per gram fresh weight of leaf per hour.

3.4 FIELD MONITORING OF COCOA TREES

Secondary screening of cultivars using six month old seedlings/budlings in the green house enabled to identify certain tolerant and sensitive varieties to moisture stress .To asses the performance of field established trees of these cultivars to moisture stress, some physiological and anatomical features were measured during summer months. For this purpose, existing 16-year-old clonal plantation containing these tolerant and sensitive varieties at CCRP, Vellanikkara (three trees each) were monitored. The trees were planted during 1980. All the plants were raised and maintained well as per the package of practices, recommendations of Kerala Agriculture University (KAU, 1996). Physiological characters such as transpiration rate, stomatal conductance, and leaf water potential were measured as explained in the secondary screening during the summer months. The index leaf (fully matured youngest leaf of the current season flush) was used for the above measurements. The following observations were also made.

3.4.1 Stomatal frequency

The number of stomata present in the abaxial surface of the leaf was counted by making surface impressions of leaves using glue. Stomata present on a number of fields were counted and mean worked out. The microscopic field estimated using a stage micrometry and the field area was calculated.

3.4.2 Leaf thickness

The leaf thickness of the youngest fully matured leaf from the top (third or fourth leaf from the top) was measured by using vernier calipers.

3.4.3 Bark thickness

Sections from the stem portion adjacent to the youngest fully matured leaf (third or fourth leaf from the top) were taken and the bark thickness measured with the help of vernier calipers and expressed in millimeters

3.4.4 Soil moisture content

Soil samples were drawn from around the trees (0-30 cm depth) and the soil moisture content was measured gravimetrically.

3.5 STATISTICAL ANALYSIS

The data collected for preliminary screening were tabulated and subjected to analysis of variance techniques (Gomez and Gomez, 1984) .The means were subjected to DMRT and cluster analysis was done for selecting varieties for secondary screening. The means were ranked by DMRT. Non Hierarchical Euclidean Model cluster analysis (Chatfield and Collins, 1980) was done for further selection of varieties for secondary screening.

Results

4. RESULTS

4.1 PRELIMINARY SCREENING OF SEEDLINGS

Six month-old seedlings of 30 cocoa genotypes were subjected to moisture stress by withholding water. Observations on RWC and DWF (at 3days interval), percentage of dried leaves at 15 days after withholding water and number of days taken for complete drying were recorded. The results are presented in this chapter.

4.1.1 Relative leaf water content (RLWC)

The RLWC varied considerably between varieties and duration of stress (Table3). The mean RLWC was the lowest with the variety GVI 61 (59.38%) and the highest with the variety GVI 56(86.07%). The RLWC of seven varieties (GVI 56,GII 19.5,M 9.16,GII 15.5,M13.12,GI 4.8,GIV 18.5) were above 80 per cent and those of eight varieties (GVI 59, S 27.10,GIV 35.7,GVI 68,GVI 50,GVI 51,GIV 18.5,GVI 61) were below 65 per cent.

4.1.2 Dry Weight Fraction (DWF)

The DWF varied significantly between varieties and duration of stress (Table 4). Between varieties, the DWF varied from 0.16 (G VI 51) to 0.63 (G VI 55). The DWF of GVI 55,GI 5.9, G II 19/5, G II 12/3,M 16/9 were above 0.5 and those of GVI 61,GIV 35/7,GVI 64,GVI 54,GVI 51 were less than 0.25.The DWF of the remaining 20 varieties ranged between 0.25 and 0.50.

Table 3. Effect of duration of stress on RLWC of cocoa varieties/ accessions (Six month old seedlings)

Varieties/ accessions	RLWC (%) at different duration of stress			
	3DAWW	6DAWW	9DAWW	Mean
M 16.9	81.15^{d}	75.85^{abcdef}	$69.44^{abcdefg}$	75.48
M 13.12	85.35^{c}	82.86^{abc}	83.01^{a}	83.74
GI 5.9	81.42^{d}	76.64^{abcde}	$65.00^{abcdefg}$	74.35
G II 19.5	87.92^{b}	87.38^{a}	82.75^{a}	86.01
G IV 18.5	84.79^{c}	84.04^{ab}	$71.53^{abcdefg}$	80.12
G VI 55	66.24^{l}	$63.18^{fghijkl}$	50.00^{h}	59.80
G VI 56	90.07^{a}	85.61^{ab}	82.53^{a}	86.07
P_1 1.21	90.54^{a}	$74.76^{abcdefg}$	80.63^{ab}	81.97
S_2 4.13	81.15^{d}	$64.03^{efghijkl}$	56.17^{efgh}	67.11
S_1 7.1	52.96^{m}	58.16^{jkl}	80.57^{ab}	63.89
M 9.16	89.83^{a}	84.47^{ab}	77.29^{abcd}	83.86
GI 4.8	89.02^{ab}	85.80^{ab}	74.22^{abcdef}	83.01
GI 9.6	82.48^{d}	77.14^{abcd}	57.87^{defgh}	72.49
GI 10.3	85.36^{c}	$74.01^{bcdefgh}$	53.68^{fgh}	71.01
GII 15.5	89.09^{ab}	86.78^{ab}	75.57^{abcde}	83.81
GII 12.13	74.57^{g}	$69.81^{defghij}$	54.85^{efgh}	66.41
GII 20.4	74.57^{g}	$69.17^{defghij}$	$63.10^{abcdefgh}$	68.94
GIII 1.2	79.29^{e}	$66.91^{defghijk}$	$60.80^{bcdefgh}$	69.00
GIII 4.1	79.21^{e}	$74.53^{abcdefgh}$	$60.83^{bcdefgh}$	71.52
GIV 2.5	73.47^{gh}	$66.41^{defghijkl}$	$60.80^{bcdefgh}$	66.89
GIV 32.5	73.21^{gh}	63.92^{l}	79.70^{abc}	72.27
GIV 35.7	70.73^{i}	$65.51^{defghijkl}$	55.41^{efgh}	63.88
GVI 50	67.83^{kl}	59.48^{ijkl}	55.73^{efgh}	61.01
GVI 51	67.52^{l}	59.88^{ijkl}	52.23gh	59.87
GVI 54	71.79^{hi}	$71.34^{cdefghi}$	$60.83^{bcdefgh}$	67.98
GVI 59	72.49^{h}	62.01^{ghijkl}	59.01^{defgh}	64.50
GVI 60	76.42^{f}	62.01^{ghijkl}	$65.91^{abcdefg}$	68.11
GVI 61	69.34^{jk}	55.39^{kl}	53.41^{fgh}	59.38
GVI 64	70.60^{ij}	$64.56^{defghijkl}$	$72.45^{abcdefg}$	69.20
GVI 68	69.80^{j}	61.64^{hijkl}	59.36^{cdefgh}	63.60

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. DAWW- Days after with holding water

Table 4. DWF in relation to cocoa varieties/accessions and duration of stress (six month old seedlings)

Varieties / accessions	3DAWW	6 DAWW	9DAWW	Mean
M 16.9	0.46^{cdef}	0.58^{ab}	0.48^{bcdefg}	0.51
M 13.12	0.59^{b}	0.54^{abc}	0.34^{hijk}	0.49
GI 5.9	0.50^{cd}	0.44^{bcdefg}	0.70^{a}	0.55
G II 19.5	0.45^{def}	0.58^{ab}	0.50^{bcde}	0.51
G IV 18.5	0.27^{b}	0.51^{abcd}	0.53^{bcd}	0.44
G VI 55	0.64^{a}	0.54^{abc}	0.70^{a}	0.63
G VI 56	0.48^{cde}	0.50^{abcde}	0.49^{bcdef}	0.49
P_1 1.21	0.42^{efg}	0.48^{abcde}	$0.40^{defghij}$	0.43
S_2 4.13	0.40^{fg}	0.56^{ab}	0.54^{bc}	0.50
S_1 7.1	0.67^{a}	0.46^{abcdef}	$0.37^{efghijk}$	0.50
M 9.16	0.16^{jkl}	0.39^{cdefgh}	0.35^{hijk}	0.30
G1 4.8	0.16^{jkl}	0.29^{ghi}	0.48^{bcdefg}	0.31
G1 9.6	0.37^{g}	0.38^{cdefgh}	0.26^{jkln}	0.34
G1 10.3	0.24^{hi}	0.46^{abcdef}	0.33^{hijk}	0.34
GII 15.5	0.08^{m}	0.17^{i}	0.54^{bc}	0.26
G11 12.13	0.51^{c}	0.50^{abcde}	0.60^{ab}	0.54
G11 20.4	0.49^{cd}	0.46^{abcdef}	0.24^{klm}	0.40
GIII 1.2	0.51^{c}	0.42^{bcdefg}	0.36^{fghijk}	0.43
GIII 4.1	0.23^{hi}	0.47^{abcde}	0.27^{jklm}	0.32
GIV 2.5	0.47^{cde}	0.62^{a}	$0.41^{cdefghi}$	0.50
GIV 32.5	0.46^{cde}	0.34^{efgh}	0.16^{mn}	0.32
GIV 35.7	0.14^{kl}	0.37^{defgh}	0.11^{n}	0.21
GVI 50	0.25^{hi}	0.30^{fghi}	0.35^{ghijk}	0.30
GVI 51	0.10^{lm}	0.19^{i}	0.29^{ijklm}	0.16
GVI 54	0.17^{jk}	0.25^{hi}	0.18^{lmn}	0.20
GVI 59	0.20^{ij}	0.29^{ghi}	0.30^{ijkl}	0.26
GVI 60	0.15^{kl}	0.29^{ghi}	$0.42^{cdefghi}$	0.29
GVI 61	0.13^{klm}	0.18^{i}	0.38^{efghij}	0.23
GVI 64	0.12^{klm}	0.17^{i}	0.32^{ijk}	0.20
GVI 68	0.28^{h}	0.18^{i}	0.46^{cdefgh}	0.31

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. DAWW- Days after with holding water

The DWF increased with the increase in duration of moisture stress. The DWF was the lowest (0.33) at 3DAWW and the highest (0.39) at 9 DAWW.

4.2 PRELIMINARY SCREENING WITH BUDDED PLANTS

4.2.1 Relative leaf water content (RLWC)

The RLWC varied considerably between varieties and duration of water stress (Table 5). The mean RLWC was the lowest with the variety G VI 54 (17.37%) and the highest with the variety G VI 68 (83.46%). The RLWC decreased with the increase in the duration of stress. RLWC of seven varieties (G VI 68, G IV 2.5, S_2 4.13, GII 12.3, GIII 4.1, GVI 51, G IV 32.5) were above 50 per cent. The highest RLWC was at 3 DAWW and the lowest at 9 DAWW.

4.2.2 Dry weight fraction (DWF)

The DWF showed significant differences between varieties and duration of stress (Table 6). Between varieties, the DWF varied from 0.312 (GI 4.8) to 0.529 (S_2 4.13). The DWF of five varieties showed values above 0.45 (S_2 4.13, GIII 4.1, GVI 59, GVI 64, GVI 54) and two varieties (S_1 7.1, GI 4.8) showed dry weight fraction less than 0.32.

4.2.3 Leaf drying percentage

There were significant variations in the leaf drying percentage recorded at 15 DAWW. The leaf drying percentage ranged from 22.22 (GI 10.3) to 100.0 per cent (M 16.9). Seven varieties (GI 5.9, GVI 56, GVI 60, GVI 51, GIV 2.5, GI 10.3) had leaf-drying percentage less than 65 per cent. In six varieties, over 90 per cent of leaves dried (M 16.9, M 9.16, GIII 4.1, GI 15.5, GVI 61). The lowest leaf drying (22.22%) was noticed in GI 10.3 and the maximum (100%) with M 16.9. The variety with the lower leaf drying percentage is considered as apparently tolerant varieties to moisture stress.

Table 5. RLWC (%) in relation to cocoa varieties/ accessions and duration of stress (six month old budlings)

Varieties/ accessions	3 DAWW	6 DAWW	9 DAWW	Mean
M 16.9	68.10^{c}	36.09^{i}	10.32^{qr}	38.17
M 13.12	45.84^{j}	38.55^{i}	14.64^{mno}	33.01
GI 5.9	30.38^{p}	28.31^{k}	20.29^{hi}	26.32
G II 19.5	43.29^{kl}	27.88^{k}	21.58^{gh}	30.92
G IV 18.5	45.91^{j}	44.24^{h}	29.06^{e}	39.74
G VI 55	46.28^{j}	36.76^{i}	11.88^{pq}	31.64
G VI 56	45.07^{jk}	37.72^{i}	16.60^{lmn}	33.13
S_2 4.13	72.00^{d}	68.28^{b}	55.56^{a}	65.28
S_1 7.1	26.59^{qr}	20.56^{n}	29.32^{e}	25.49
M 9.16	51.33^{h}	32.21^{j}	14.09^{nop}	32.54
G1 4.8	38.89^{m}	36.84^{i}	18.33^{ijkl}	31.35
G1 9.6	27.79^{q}	44.94^{gh}	13.20^{op}	28.46
G1 10.3	25.23^{r}	23.28^{m}	10.10^{qr}	19.54
GII 15.5	62.21^{f}	53.78^{e}	17.08^{klm}	44.36
G11 12.13	80.01^{b}	64.27^{c}	34.34^{c}	59.54
G11 20.4	34.41^{o}	26.77^{kl}	19.88^{hij}	27.02
GIII 1.2	57.15^{g}	51.04^{f}	19.00^{hijkl}	42.40
GIII 4.1	66.86^{e}	58.06^{d}	31.94^{cd}	52.29
GIV 2.5	84.06^{a}	81.92^{a}	32.73^{cd}	66.24
GIV 32.5	76.02^{c}	58.97^{d}	17.24^{jklm}	50.74
GIV 35.7	35.28^{no}	24.87^{lm}	23.10^{fg}	27.75
GVI 50	48.38^{i}	46.85^{g}	43.88^{b}	46.37
GVI 51	56.32^{g}	54.89^{e}	45.06^{b}	52.09
GVI 54	24.60^{r}	15.20^{o}	12.31^{opq}	17.37
GVI 59	68.51^{c}	36.95^{i}	16.98^{klm}	40.81
GVI 60	36.84^{n}	26.28^{kl}	19.43^{hijk}	27.52
GVI 61	30.65^{p}	26.71^{kl}	25.36^{f}	27.57
GVI 64	42.85^{l}	37.32^{i}	13.37^{de}	37.18
GVI 68	85.41^{a}	82.17^{a}	82.80^{r}	83.46

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. DAWW- Days after with holding water

Table 6. Dry weight fraction (DWF) in relation to cocoa varieties/ accessions and duration of stress (six month old budlings)

Varieties/ accessions	3 DAWW	6DAWW	9DAWW	Mean
M 16.9	0.329^{ijkl}	0.574^{b}	0.408^{cdef}	0.370
M 13.12	0.278^{l}	0.286^{k}	0.418^{cde}	0.327
GI 5.9	0.333^{ijkl}	0.489^{cd}	0.429^{cd}	0.417
G II 19.5	0.413^{defgh}	0.487^{cd}	0.231^{k}	0.377
G IV 18.5	0.421^{defg}	0.404^{efgh}	0.439^{c}	0.421
G VI 55	0.423^{defg}	0.285^{k}	0.358^{efghi}	0.355
G VI 56	0.354^{hijk}	0.422^{ef}	0.305^{ij}	0.360
S_2 4.13	0.552^{b}	0.520^{bc}	0.515^{b}	0.529
S_1 7.1	0.327^{ijkl}	0.313^{ijk}	0.309^{ij}	0.316
M 9.16	0.378^{fghij}	0.305^{ijk}	0.409^{cde}	0.364
GI 4.8	0.407^{defgh}	0.411^{efg}	0.378^{defg}	0.399
GI 9.6	0.312^{kl}	0.348^{hij}	0.278^{jk}	0.312
GI 10.3	0.334^{ijkl}	0.425^{ef}	0.310^{ij}	0.356
GII 15.5	0.400^{efgh}	0.540^{bc}	0.285^{jk}	0.408
GII 12.13	0.605^{a}	0.314^{ijk}	0.375^{defgh}	0.431
GII 20.4	0.286^{l}	0.269^{k}	0.416^{cde}	0.323
GIII 1.2	0.384^{fghi}	0.328^{ijk}	0.363^{efghi}	0.358
GIII 4.1	0.473^{cde}	0.461^{de}	0.697^{a}	0.528
GIV 2.5	0.498^{c}	0.399^{fgh}	0.360^{efghi}	0.419
GIV 32.5	0.426^{defg}	0.358^{ghi}	0.364^{efghi}	0.382
GIV 35.7	0.367^{ghijk}	0.304^{ijk}	0.348^{fghi}	0.339
GVI 50	0.490^{c}	0.501^{cd}	0.305^{ij}	0.428
GVI 51	0.461^{cde}	0.334^{ghij}	0.319^{ghij}	0.378
GVI 54	0.357^{hijk}	0.567^{b}	0.461^{c}	0.461
GVI 59	0.467^{cd}	0.658^{a}	0.432^{cd}	0.519
GVI 60	0.317^{jkl}	0.450^{def}	0.316^{hij}	0.361
GVI 61	0.403^{efgh}	0.454^{def}	0.323^{ghij}	0.386
GVI 64	0.454^{cde}	0.576^{b}	0.366^{efghi}	0.465
GVI 68	0.429^{def}	0.296^{jk}	0.264^{jk}	0.327

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. DAWW- Days after with holding water

4.2.4 Number of days taken for complete drying of plants

The number of days taken for complete drying of budlings varied considerably between varieties. Four varieties (GII19.5, M16.9, GI 4.8, GVI 61) took less than 25 days to die whereas; six varieties (G I 10.3,GVI 56,GII 18.5,GVI 51,GIV 2.5,GVI 55,GI 5.9) took more than 30 days for complete drying. The longest duration of life under moisture stress was 34 days with the variety GI 10.3.The variety GVI 56 was also similar in this regard with variety G I 10.3(34.33 days). The variety GVI 61 was the first one to show drying symptoms (24 days).

Based on the data on RWC, DWF, leaf drying percentage and number of days taken for complete drying; the varieties were short listed by doing DMRT and cluster analysis. Varieties were selected as in the case of seedlings. The apparently tolerant varieties were GII 12.3,GIII 4.1,GIV 2.5,GIV 32.5, GVI 50,GVI 51 and GVI 68 and the apparently sensitive varieties were M 16.9, GI 4.8, M 13.12.

4.3 SECONDARY SCREENING OF SEEDLINGS

Six month-old seedlings of seven apparently tolerant and three apparently sensitive varieties identified in the preliminary screening were subjected to moisture stress by withholding water. Observations on physiological parameters, growth characters and biochemical characters were recorded. The results obtained are presented below.

4.3.1 Physiological parameters

Relative leaf water content (RLWC)

Relative leaf water content (RLWC) of 10 cocoa varieties/ accessions grown under three soil moisture regimes, i.e. no irrigation (T_1), watering once in five days (T_2) and regular watering (T_3) are shown in (Table7). There were considerable

Table 7. Relative leaf water content (RLWC) of cocoa seedlings (six month old) in relation to soil moisture regimes and varieties

a) Soil moisture regimes

Treatments		RLWC (%)
T_1	No irrigation	71.76^{a}
T_2	Irrigation once in 5 days	46.97^{b}
T_3	Daily irrigation	72.45^{a}

b) Varieties / accessions

Varieties / accessions	RLWC (%)
GI 5.9	43.45^{h}
G I 10.3	57.15^{e}
G VI 50	66.11^{c}
G II 20.4	85.62^{a}
G VI 51	54.19^{f}
G I 4.8	75.8^{b}
M 13.12	48.02^{g}
G VI 55	58.60^{e}
G VI 61	87.52^{a}
G II 19.5	61.4^{d}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level.

differences in RLWC in between varieties and due to soil moisture regimes. RLWC values of GVI 61 (87.52%) and GII 20.4 (85.62%) were on par followed by GI 4.8 (75.08%). The lowest RLWC was in GI 5.9 (43.45%).

The seedlings raised under regular irrigation (72.45%) had high RLWC and those under life saving irrigation (46.97%) had the lowest RLWC.

The interaction between moisture regime and variety was significant with respect to this character (Table 9). The variety, GI 4.8 showed the highest RLWC under regular watering (91.57%); GVI 61 (92.36%) under life saving irrigation and GVI 55, G50 and GVI 61 under no irrigation. The varieties showed considerable variations in their RLWC both in water stressed as well as in daily-irrigated plants. The highest RLWC under no irrigation was in GVI 61 (94.76%), which was on par with GVI 55 (93.57%) and G50 (93.94).

Dry weight fraction (DWF)

The DWF differed considerably between varieties (Table8). GII 20.4 had the highest DWF (0.387) followed by GI 5.9 (0. 383) and the lowest in GI 4.8 (0.300), which was on par with GVI 51(0.307).

Interaction between moisture regime and variety was significant (Table 9). GII 20.4 (0.447) showed the highest DWF under no irrigation; GII 19.5 (0.532) showed the highest DWF under the life saving irrigation and GVI 61 (0.394) the highest values under regular irrigation.

Leaf drying percentage

The leaf drying percentage increased with increase in moisture stress (Table 10). The seedlings grown under regular irrigation had the lowest leaf drying percentage (28.35%). The leaf drying percentage was the highest in seedlings raised under no irrigation (48.63%).

Table 8. Dry weight fraction (DWF) in Cocoa varieties (six month old seedlings)

Varieties/accessions	DWF (%)
GI 5.9	0.383^{ab}
G I 10.3	0.358^{abcd}
G VI 50	0.349^{cd}
G II 20.4	0.387^{a}
G VI 51	0.307^{e}
G I 4.8	0.300^{e}
M 13.12	0.351^{bcd}
G VI 55	0.326^{de}
G VI 61	0.363^{abc}
G II 19.5	0.382^{abc}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level.

Table 9. Effect of water stress on relative leaf water content (RLWC) and dry weight fraction (DWF) of cocoa varieties/accessions

Varieties/ accessions	RLWC (%)			DWF (%)		
	Water Stress Levels			Water Stress Levels		
	T_1	T_2	T_3	T_1	T_2	T_3
GI 5.9	25.80^{k}	47.50^{i}	57.06^{gh}	0.429^{bc}	0.344^{efghi}	0.376^{cdefg}
G I 10.3	54.41^{h}	34.13^{j}	82.93^{c}	0.394^{bcde}	$0.333^{efghijk}$	0.349^{efghi}
G VI 50	93.94^{a}	32.65^{j}	71.74^{f}	0.326^{fghijk}	0.349^{efghi}	0.372^{cdefg}
G II 20.4	88.44^{b}	88.24^{b}	80.18^{cd}	0.447^{b}	0.349^{efghi}	0.365^{defg}
G VI 51	82.03^{c}	20.55^{l}	60.00^{g}	0.283^{jkl}	0.287^{ijkl}	0.350^{defgh}
G I 4.8	53.46^{h}	80.21^{cd}	91.57^{ab}	0.317^{ghijk}	0.283^{jkl}	0.299^{hijkl}
M 13.12	47.40^{i}	19.80^{l}	76.87^{de}	0.324^{ghijk}	0.411^{bcd}	0.319^{ghijk}
G VI 55	93.57^{a}	28.65^{k}	53.58^{h}	0.274^{kl}	0.388^{cdef}	0.317^{ghijk}
G VI 61	94.76^{a}	92.36^{a}	75.44^{ef}	0.393^{bcde}	0.300^{hijkl}	0.394^{bcde}
G II 19.5	83.84^{c}	25.64^{k}	75.13^{ef}	0.365^{defg}	0.532^{a}	0.248^{l}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 10. Effect of moisture regimes on leaf drying percentage in cocoa seedlings

Moisture regimes	*Leaf drying percentage*
T_1	48.63[a]
T_2	32.77[b]
T_3	28.35[b]

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

There was no significant variation between varieties, and interaction between moisture regimes and variety was absent.

Number of days took for complete drying

The number of days took for complete drying was worked out in no irrigation treatments. There was no significant relation between varieties in number of days took for complete drying.

Transpiration rate

The transpiration rate differed between levels of stress. At 11 00 hrs, it was the highest in regular irrigation (47.05 $\mu g\ cm^{-2}s^{-1}$) and the lowest in no irrigation (20.16$\mu g\ cm^{-2}s^{-1}$) (Table 11) (Fig. 1).

At 14 00 hrs also, transpiration rate was the highest in regular irrigation, however, life saving irrigation and no irrigation showed on par values (Table 12). The interactions were not significant.

Stomatal conductance

There was drastic reduction in stomatal conductance with increase in stress at 11.00 hrs (Table11) (Fig. 2). It was the highest in regularly irrigated seedlings (12.90 m mol $m^{-2}s^{-1}$) and decreased to 5.17 m mol $m^{-2}s^{-1}$ in life saving irrigation and further decreased to 4.71 m mol $m^{-2}s^{-1}$ under no irrigation.

The interaction between moisture regimes and varieties was not significant.

At 14 00 hrs, stomatal conductance showed further decrease to 6.75 m mol $m^{-2}s^{-1}$ under regular irrigation and life saving irrigation (3.63 m mol $m^{-2}s^{-1}$) was on par with no irrigation (3.61 m mol $m^{-2}s^{-1}$)(Table 12). The interaction was not significant.

Leaf water potential (ψ_w)

The ψ_w varied between moisture regime and varieties (Table13). The leaf water potential differed according to the changes in soil moisture regime. At 3DAWW, the highest ψ_w was noticed in plants under regular irrigation and this was consistently seen at 6 DAWW also. Leaf water potential decreased with the increase in soil moisture stress and it was the lowest in plants raised under life saving irrigation (T_2).

At 3 DAWW, the highest ψ_w was noticed in seedlings of GII 19.5 (-2.911 bars) followed by GI 4.8 (-2.933 bars) and GVI 50 (-3.256 bars) and all these three were on par. The ψ_w was lowest with the variety GI 5.9 (-5.833 bars). At 6 DAWW, the variety GI 10.3 (-3.44 bars) showed the highest ψ_w followed by M 13.12 (-3.46 bars) and GII 19.5 (-3.5 bars) and all were on par. The lowest was with the variety GVI 50 (- 9.32 bars). The variety GII 19.5 had the highest mean ψ_w (-3.20 bars) followed by GI 4.8(-3.42 bars). The lowest mean ψ_w (-5.06 bars) was with the variety GI 5.9.

The interaction between moisture regime and varieties was also significant (Table 14). At 3 DAWW, the variety GVI 51 showed high value (-3.03 bars) under no irrigation, GVI 50 (-2.06 bars) under life saving irrigation; and GII 19.5 (-2.03 bars) and GVI 51(-2.16 bars) with on par values under regular irrigation.

At 6 DAWW, GI 10.3 (-2.03 bars) had high LWP under no irrigation, GII 19.5 (-3.93 bars) under life saving irrigation and GII 20.4 (-2.96 bars) under regular irrigation.

Table 11. Stomatal conductance and Transpiration rate in cocoa seedlings (six month old) in relation to moisture regimes at (11 00 hrs)

Moisture regimes	Stomatal conductance (m mol $m^{-2}s^{-1}$)	Transpiration rate ($\mu g\ cm^{-2}\ s^{-1}$)
T_1	4.71^c	27.16^c
T_2	5.17^b	28.25^b
T_3	12.90^a	47.05^a

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table12. Stomatal conductance and Transpiration rate in cocoa seedlings (six month old) in relation to moisture regimes at (14 00 hrs)

Moisture regimes	Stomatal conductance (m mol $m^{-2}s^{-1}$)	Transpiration rate ($\mu g\ cm^{-2}\ s^{-1}$)
T_1	3.606^b	28.53^b
T_2	3.628^b	29.05^b
T_3	6.749^a	47.81^a

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

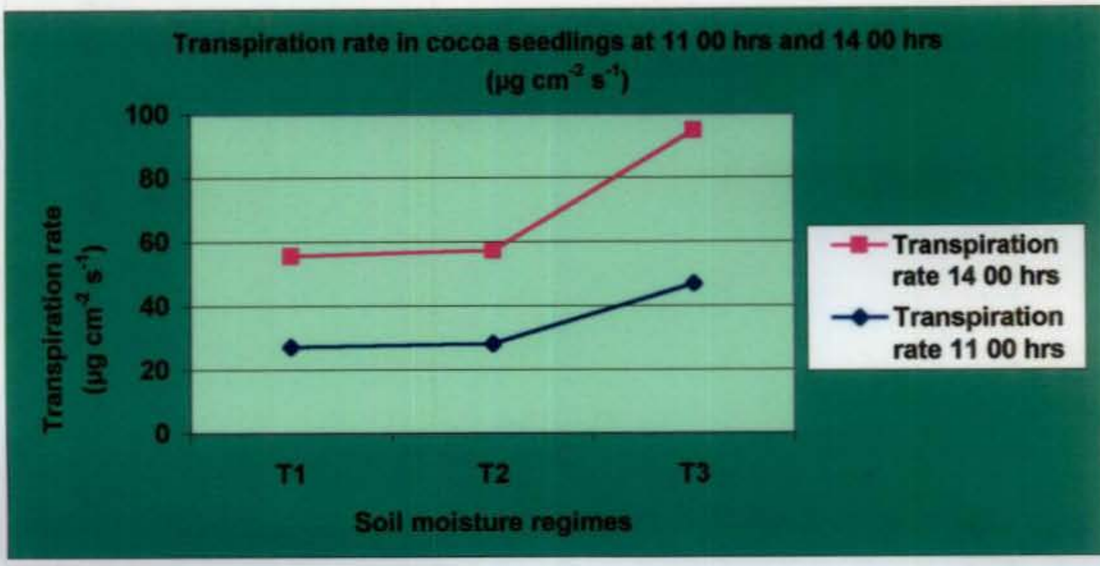

Fig. 1. Transpiration rate in cocoa seedlings (six month old) in relation to soil moisture regimes at 11 00 hrs and 14 00 hrs

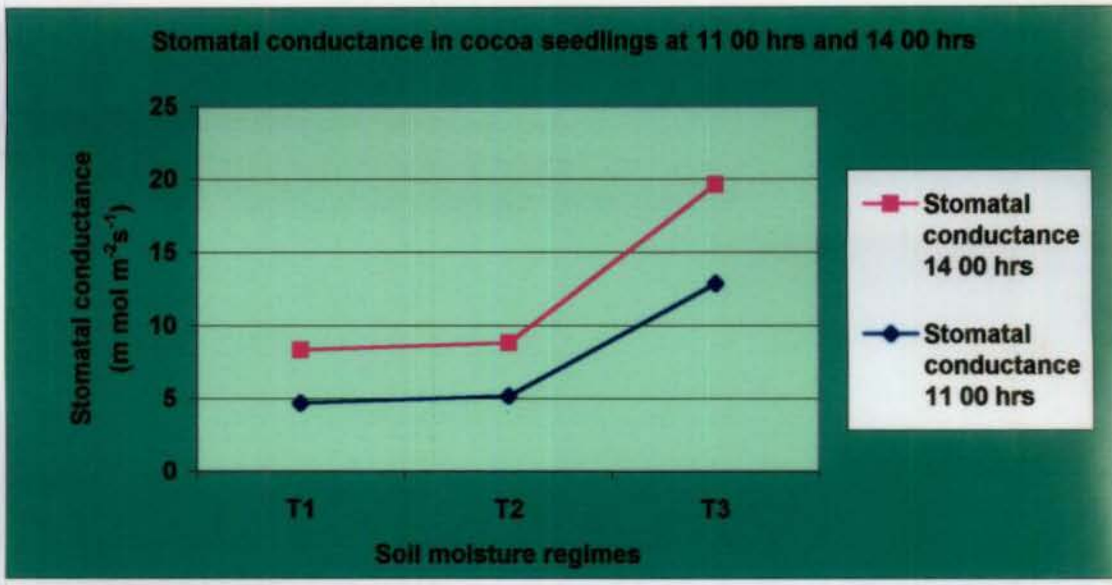

Fig. 2. Stomatal conductance in cocoa seedlings at 11 00 hrs and 14 00 hrs

Table 13. Leaf water potential (bars) in cocoa seedlings (six month old) in relation to soil moisture regimes and varieties

Treatments	3DAWW	6DAWW	Mean
a) Soil moisture regimes			
T_1	-4.483[b]	-3.840[b]	-4.16
T_2	-4.667[a]	-4.803[a]	-4.73
T_3	-2.960[c]	-3.780[c]	-3.37
Mean	-4.037	-4.141	
b) Varieties			
GI 5.9	-5.833[a]	-4.289[b]	-5.061
G I 10.3	-3.767[d]	-3.444[d]	-3.605
G VI 50	-3.256[e]	-4.900[a]	-4.078
G II 20.4	-4.700[c]	-3.867[c]	-4.283
G VI 51	-3.744[d]	-4.55[c]	-4.147
G I 4.8	-2.933[e]	-3.922[c]	-3.427
M 13.12	-4.389[c]	-3.467[d]	-3.928
G VI 55	-5.156[b]	-4.544[b]	-4.85
G VI 61	-3.678[d]	-4.922[a]	-4.345
G II 19.5	-2.911[e]	-3.500[d]	-3.205
Mean	-4.036	-4.140	

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. DAWW- Days after with holding water in no irrigation. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 14. Effect of water stress on leaf water potential (bars) of six month old cocoa seedlings

Varieties/ accessions	3DAWW			6DAWW		
	Water stress levels					
	T_1	T_2	T_3	T_1	T_2	T_3
GI 5.9	-7.93^{a}	-6.13^{c}	-3.43^{fg}	-3.46^{jkl}	-5.20^{bcd}	-4.20^{fghi}
G I 10.3	-4.96^{d}	-3.30^{fgh}	-3.03^{fgh}	-2.03^{n}	-4.86^{cde}	-3.43^{jkl}
G VI 50	-4.33^{de}	-2.06^{ij}	-3.36^{fgh}	-5.20^{bcd}	-4.83^{cde}	-4.66^{def}
G II 20.4	-3.86^{ef}	-7.03^{b}	-3.20^{fgh}	-3.80^{ijk}	-4.83^{cde}	-2.96^{lm}
G VI 51	-3.03^{fgh}	-6.03^{c}	-2.16^{i}	-4.56^{efg}	-4.43^{efgh}	-4.66^{def}
G I 4.8	-3.30^{fgh}	-2.56^{hi}	-2.93^{gh}	-3.16^{lm}	-5.33^{abc}	-3.26^{klm}
M 13.12	-4.96^{d}	-5.00^{d}	-3.20^{fgh}	-2.83^{m}	-4.16^{fghi}	-3.40^{jkl}
G VI 55	-5.9^{c}	-6.33^{bc}	-3.23^{fgh}	-4.76^{de}	4.80^{cde}	-4.06^{ghi}
G VI 61	-3.1^{fgh}	-4.93^{d}	-3.00^{gh}	-5.73^{a}	5.63^{ab}	-3.40^{jkl}
G II 19.5	-3.43^{fg}	-3.26^{fgh}	-2.03^{i}	2.83m	3.93^{hij}	-3.73^{ijk}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. DAWW - Days after with holding water in no irrigation. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Leaf area per seedling

The leaf area per seedling varied between varieties (Table15). Seedlings of GI 4.8 had the highest leaf area per seedling (4224 cm^2) on par with M 13.12 (4219 $cm^{2)}$ and the lowest with the variety GVI 51 (4118 cm^2). There were no significant differences between moisture regimes.

The interaction between varieties and moisture regime was significant (Table16). The variety M 13.12 had the highest leaf area per seedling under regular irrigation (4245cm ^{2}), GVI 55 (4232 cm^2) under life saving irrigation, GI 4.8 (4229 cm^2) under no irrigation.

Chlorophyll 'a' content of leaves

The variation in leaf chlorophyll 'a' content between varieties and soil moisture regimes was not significant.

The interaction between variety and moisture regime was significant. (Table 18) Under regular irrigation, chlorophyll 'a' content was the highest in GVI 50 (0.393), which was on par with GII 10.3 and GI 5.9. The highest chlorophyll 'a' content under the life saving irrigation was in GII 19.5 (0.617), which was on par with GVI 55 (0.613). GI 4.8 showed the highest chlorophyll content under no irrigation (0.568) followed by GVI 61 (0.545) and the lowest was in GII 20/4 (0.347).

Chlorophyll 'b' content of leaves

There was variation in chlorophyll 'b' content between varieties (Table17). The chlorophyll 'b' content was the highest in seedlings of GII 19.5 (0.657 mg g^{-1} tissue) which was on par with GI 5.9 (0.655 mg g^{-1} tissue) and the lowest in GII 20.4 (0.419 mg g $^{-1}$ tissue). The variation in chlorophyll 'b' content between soil moisture regimes was not significant.

Table 15. Leaf area per seedling in cocoa varieties/ accessions

Varieties/accessions	Leaf area per seedling (cm^2)
GI 5.9	4163^{c}
GI 10.3	4180^{d}
GVI 50	4185^{d}
GII 20.4	4140^{f}
GVI 51	4118^{g}
GI 4.8	4224^{a}
M 13.12	4219^{a}
GVI 55	42.6^{bc}
GVI 61	4200^{c}
GII 19.5	4215^{ab}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level.

Table 16. Effect of water stress on leaf area per seedling in cocoa Varieties/ accessions

Varieties/ accessions	Leaf area per seedling (cm^2) Water stress levels		
	T_1	T_2	T_3
GI 5.9	4182^{ijk}	4144^{mno}	4163^{klm}
G I 10.3	4193^{fghi}	4162^{klm}	4185^{hij}
G VI 50	4167^{jkl}	4200^{efghi}	4189^{ghi}
G II 20.4	4128^{op}	4155^{lmn}	4137^{no}
G VI 51	4140^{no}	4116^{pq}	4097^{q}
G I 4.8	4229^{abc}	4207^{defgh}	4237^{ab}
M 13.12	4194^{fghi}	4219^{bcde}	4245^{a}
G VI 55	4189^{ghi}	4232^{abc}	4197^{fghi}
G VI 61	4223^{bcd}	4182^{ijk}	4194^{fghi}
G II 19.5	4211^{cdefg}	4220^{bcde}	4213^{cdef}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

The interaction between variety and soil moisture regime was significant (Table18). In varieties, the chlorophyll 'b' content in general decreased with increase in moisture stress. However a definite trend was lacking. GI 10.3 (0.663 mg g^{-1} tissue) had high chlorophyll ' b' content under regular irrigation, GVI 55 (0.949 mg g^{-1} tissue) and GII 19.5 (0.926 mg g^{-1} tissue) had high values under life saving irrigation and GVI 61 (0.810 mg g^{-1} tissue) under no irrigation.

Total chlorophyll content

The total chlorophyll content varied between varieties (Table17). Seedlings of GI 5.9 had the highest total chlorophyll (1.329 mg g^{-1} tissue) followed by GII 19.5 (1.305 mg g^{-1} tissue) and, GI 10.3 (1.261 mg g^{-1} tissue) and the lowest with GII 20.4 (0.952 mg g^{-1} tissue). The variation in total chlorophyll content between moisture regimes was not significant.

The interaction between variety and moisture regime was significant (Table18). In general, in most varieties, total chlorophyll content decreased under water stress. The variety GI 10.3 showed the highest total chlorophyll in regular irrigation (1.485 mg g^{-1} tissue). The varieties GII 19.5 (1.789 mg g^{-1} tissue), which was on par with GVI 55 (1.804 mg g^{-1} tissue), GI 5.9 (1.571 mg g^{-1} tissue) showed higher total chlorophyll contents under life saving irrigation. GVI 61 (1.579 mg g^{-1} tissue) had the highest total chlorophyll content under no irrigation.

Chlorophyll stability index (CSI)

There was no significant relation between moisture regimes and varieties. The interaction was also found to be not significant.

Table 17. Variation of chlorophyll content in cocoa varieties (six month old seedlings)

Varieties/accessions	Chlorophyll 'b' (mg g^{-1} tissue)	Total Chlorophyll (mg g^{-1} issue)
GI 5.9	0.655^{a}	1.329^{a}
G I 10.3	0.681^{ab}	1.261^{b}
G VI 50	$0.552b^{cd}$	1.155^{c}
G II 20.4	0.419^{e}	0.952^{d}
G VI 51	0.569^{bc}	1.179^{c}
G I 4.8	0.563^{bcd}	1.155^{c}
M 13.12	0.525^{cd}	1.125^{c}
G VI 55	0.585^{abc}	1.155^{c}
G VI 61	0.479^{de}	0.990^{d}
G II 19.5	0.657^{a}	1.305^{ab}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level.

Table 18. Effect of water stress on chlorophyll content in cocoa seedlings (six month old)

Varieties/ accessions	Chlorophyll 'a' (mg g^{-1} tissue)			Chlorophyll 'b' (mg g^{-1} tissue)			Total chlorophyll (mg g^{-1} tissue)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
GI 5.9	0.358^{cdefgh}	0.519^{abcd}	$0.382^{bcdefgh}$	0.525^{ghijk}	0.846^{ab}	0.594^{efghi}	1.024^{hi}	1.571^{b}	1.391^{cde}
G I 10.3	0.511^{abcd}	0.284^{fghij}	$0.3865^{bcdefgh}$	0.739^{bcde}	0.451^{hijk}	0.663^{cdefg}	1.449^{cd}	0.848^{lm}	1.487^{bc}
G VI 50	0.479^{abcde}	0.270^{fghij}	$0.393^{bcdefgh}$	0.706^{bcdef}	0.398^{k}	0.553^{fghijk}	1.37^{def}	0.771^{mn}	1.323^{ef}
G II 20.4	0.347^{defgh}	$0.386^{bcdefgh}$	0.303^{efghi}	0.515^{ghijk}	0.212^{l}	0.531^{ghijk}	0.995^{hij}	0.673^{n}	1.188^{g}
G VI 51	0.422^{bcdefg}	0.351^{defgh}	0.370^{cdefgh}	0.613^{efgh}	0.521^{ghijk}	0.572^{fghij}	1.197^{g}	1.010^{hij}	1.329^{ef}
G I 4.8	0.568^{ab}	0.525^{abcd}	0.133^{ij}	0.713^{dcdef}	0.775^{bcd}	0.201^{l}	1.495^{bc}	1.505^{bc}	0.464^{o}
M 13.12	0.519^{abcd}	0.366^{cdefgh}	0.292^{fghij}	0.589^{efghi}	0.517^{ghijk}	0.470^{hijk}	1.273^{fg}	1.021^{hi}	1.081^{h}
G VI 55	0.250^{ghij}	0.613^{a}	0.231^{hij}	0.410^{jk}	0.949^{a}	0.397^{k}	0.760^{mn}	1.804^{a}	1.899^{jkl}
G VI 61	0.545^{abc}	0.244^{ghij}	0.112^{j}	0.810^{abc}	0.437^{jk}	0.189^{l}	1.579^{b}	0.963^{ijk}	0.430^{o}
G II 19.5	0.441^{abcdef}	0.617^{a}	0.212^{hij}	0.652^{defg}	0.926^{a}	0.392^{k}	1.264^{fg}	1.789^{a}	0.861^{klm}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Relative injury (RI)

There was significant relation between moisture regimes and varieties. RI under life saving irrigation was 11.10 per cent and 12.39 per cent under regular irrigation.

The variety GI 5.9 had the highest RI (22.18%) followed by GVI 50 (17.84%), GII 19.5(14.92%) and the lowest RI was shown by GVI 51(6.12%). (Table 19).

The interaction between moisture regime and varieties was also significant. (Table20). The variety GI 5.9 (36.78%) had high RI during regular irrigation and life saving irrigation (19.53%) and GVI 50 (19.83%) had high RI under no irrigation.

Membrane stability

Membrane stability showed significant relation between moisture regimes (Table 21). Membrane stability was the highest in no irrigation (0.80) and lowest in regular irrigation (0.49). There was no significant relation between varieties. The interaction between moisture regimes and varieties was not significant.

4.3.2 Growth characters

Seedling height

Seedling height differed with variety as well as soil moisture regimes (Table 22). The seedlings of GVI 55 were the tallest (98.47 cm), which was on par with GVI 61 (96.67 cm). The seedlings of GI 4.8 were the shortest (76.44 cm).

The seedlings raised under life saving irrigation were the tallest (92.47 cm). The shortest seedlings were noticed with plants kept under no irrigation (80.92 cm).

Table 19. Variation in relative injury in seedlings (six month old) of cocoa varieties/accessions

Varieties/accessions	Relative injury (%)
GI 5.9	22.18^{a}
GI 10.3	13.53^{cd}
GVI 50	17.84^{b}
GII 20.4	9.99^{e}
GVI 51	6.12^{f}
GI 4.8	11.27^{de}
M 13.12	10.14^{e}
GVI 55	12.78^{cd}
GVI 61	8.90^{e}
GII 19.5	14.92^{c}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level.

Table 20. Effect of water stress on relative injury of cocoa varieties (six month old seedlings)

Varieties/accessions	Relative injury (%)		
	Water stress levels		
	T_1	T_2	T_3
GI 5.9	10.24^{hijkl}	19.53^{bc}	36.78^{a}
GI 10.3	15.69^{cdef}	14.23^{defghi}	10.67^{ghijkl}
GVI 50	19.83^{bc}	11.41^{fghijk}	22.28^{b}
GII 20.4	6.95^{kl}	10.51^{ghijkl}	$12.51e^{fghi}$
GVI 51	9.72^{ijkl}	6.62^{l}	2.02^{m}
GI 4.8	14.47^{defgh}	11.61^{fghij}	7.71^{jkl}
M 13.12	10.87^{ghijkl}	9.70^{ijkl}	9.84^{ijkl}
GVI 55	9.74^{ijkl}	11.29^{fghijk}	17.31^{cd}
GVI 61	13.13^{defghi}	1.06^{m}	$12.51e^{fghi}$
GII 19.5	13.19^{defghi}	14.99^{defg}	16.57^{cde}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 21. Variation in membrane stability of cocoa seedlings (six month old) in relation to moisture regimes

Moisture regimes	Membrane stability
T_1	0.80^a
T_2	0.70^b
T_3	0.49^c

Table 22. Growth characters of cocoa seedlings (six month old) in relation to soil moisture regimes and varieties

Treatments	Height (cm)	Girth (cm)	No: of leaves	Total biomass (g pl^{-1})
a)Soil moisture regime				
T_1	80.92^c	3.203^b	12.23^b	78.79^c
T_2	92.47^a	4.393^a	15.27^a	92.85^b
T_3	89.77^b	4.363^a	15.03^a	94.63^c
b)Varieties				
GI 5.9	86.89^{bcd}	3.922^{cd}	13.89^d	90.52^{ab}
GI 10.3	89.00^{bc}	4.011^c	12.89^c	88.71^{ab}
GVI 50	81.44^{de}	4.211^b	14.33^{cd}	89.65^{ab}
GII 20.4	89.22^{bc}	3.856^d	14.22^d	88.99^{ab}
GVI 51	84.11^{cd}	4.011^c	10.33^f	88.57^{ab}
GI 4.8	79.44^e	3.689^e	17.78^a	82.38^b
M 13.12	83.78^{cd}	3.967^c	14.00^d	91.73^a
GVI 55	98.44^a	4.333^a	13.89^d	89.34^{ab}
GVI 61	96.67^a	3.900^{cd}	15.11^{bc}	87.89^{ab}
GII 19.5	91.17^b	3.967^c	13.33^b	89.80^{ab}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

The interaction between varieties and soil moisture regimes was significant (Table 23). GVI 61 (106.7cm) had high value under regular irrigation, GVI 55 (113.7 cm) under life saving irrigation and GI 5.9 (90.00 cm) under no irrigation.

Collar girth

There were considerable differences in seedling girth between varieties (Table 22). Seedlings of GVI 55 had the highest girth (4.33 cm) and those of GI 4.8 had the lowest (3.68 cm). Variation in soil moisture regime was significant. Regular irrigation (4.36 cm) was on par with life saving irrigation (4.39 cm) seedling girth under no irrigation was the lowest (3.20 cm).

The interaction between varieties and moisture regime was significant (Table 24). GI 10.3 (5.07 cm) had high collar girth under regular irrigation, GVI 55 (5.10 cm) under life saving irrigation and GII 19.5 (3.83 cm) under no irrigation.

Number of leaves

The number of leaves was significant between soil moisture regimes and varieties (Table 22). The highest number of leaves were produced in life saving irrigation (15.27). The daily irrigation is on par with life saving irrigation (15.03). The lowest number of leaves was observed in no irrigation (12.23).

The leaf production was the highest (17.78) with seedling GI 4.8 followed by GII 19.5 (15.33). The lowest leaf production was shown by GVI 51 (10.33).

The interaction between varieties and moisture regime was significant with this character (Table 25). The number of leaves decreased with increase in levels of leaves of moisture stress. The variety GI 4.8 had the highest number of leaves under regular irrigation, GVI 50 (19.67) under life saving irrigation and GVI 61(16.67) under no irrigation.

Table 23. Effect of water stress on height of cocoa varieties (six month old seedlings)

Varieties	Height (cm) Water Stress Levels		
	T_1	T_2	T_3
GI 5.9	90.00^{defgh}	86.33^{defghi}	84.33^{efghi}
G I 10.3	85.67^{defghi}	85.33^{defghi}	96.00^{bcd}
G VI 50	83.33^{fghij}	87.67^{defghi}	73.33^{jkl}
G II 20.4	77.33^{ijk}	86.00^{defghi}	104.3^{abc}
G VI 51	67.67^{klm}	88.67^{defgh}	96.00^{bcd}
G I 4.8	79.00^{hij}	88.00^{defghi}	62.33^{m}
M 13.12	64.67^{lm}	107.3^{a}	79.33^{ghij}
G VI 55	91.33^{def}	113.7^{a}	90.33^{defg}
G VI 61	88.67^{defgh}	94.67^{cde}	106.7^{a}
G II 19.5	81.50^{fghij}	87.00^{defghi}	105.0^{ab}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 24. Effect of water stress on collar girth of cocoa varieties/accessions (six-month-old seedlings)

Varieties/accessions	Collar Girth (cm) Water stress level		
	T_1	T_2	T_3
GI 5.9	3.233^{l}	3.967^{ij}	4.567^{c}
G I 10.3	2.900^{mn}	4.067^{hi}	5.067^{a}
G VI 50	3.067^{lm}	5.000^{a}	4.567^{c}
G II 20.4	3.167^{l}	4.033^{hi}	4.367^{de}
G VI 51	3.167^{l}	4.300^{def}	3.567^{c}
G I 4.8	2.867^{n}	4.100^{ghi}	4.100^{ghi}
M 13.12	3.133^{l}	4.800^{b}	3.967^{ij}
G VI 55	3.433^{k}	5.100^{a}	4.467^{cd}
G VI 61	3.233^{l}	4.300^{def}	4.167^{fgh}
G II 19.5	3.833^{j}	4.267^{efg}	3.800^{j}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Total biomass

The total biomass production in relation to moisture regime and varieties was significant (Table 22).Total biomass decreased with moisture stress. Regular irrigation had the highest total biomass (94.63) and no irrigation had the lowest (78.79). The seedlings of M 13.12 showed the highest biomass production (91.73).

The interaction effect was also significant (Table 26).Under regular irrigation, M 13.12 (94.94) is on par with GII 20.4 (94.78) showed highest values. Under life saving irrigation, M 13.12 (85.92), GI 5.9 (84.34), GVI 50(83.06) and GVI 55 (80.79) all have on par values. Under no irrigation, M13.12 (91.73) had the highest value.

4.3.3 Biochemical characters

Proline content

The proline content of leaves varied between varieties (Table 27 and Fig. 3). It was the highest in the variety GVI 51(369.7μ g g^{-1} leaf) followed by M 13.12 (366 μ g g^{-1} leaf) and GII 20.4 (340.9 μg g^{-1} leaf) and the lowest in GI 5.9 (144.5 μ g g^{-1} leaf).

The variation between moisture regimes was found to be not significant. The interaction effect between moisture regimes and varieties was significantly different (Table 28 and Fig. 4). GVI 55 (392 μg g^{-1} leaf) had high proline under regular irrigation and GI 4.8 (439 μ g g^{-1} leaf) under life saving irrigation and M 13.12 (487 μg g^{-1} leaf) under no irrigation.

Nitrate reductase activity (NRA)

NRA of leaves differed considerably between moisture regimes. The NRA was the highest in plants grown under regular watering (27.17m mol NO_2 per gram per

2 hr at 30^{0}C). NRA of leaves between varieties and interaction effect was found to be not significant.

4.4 CLUSTER ANALYSIS

Non-hierarchical Euclidean Cluster Analysis (Chatfield and Collins, 1980)) was done for selection of drought tolerant and sensitive varieties.

The parameters selected for clustering were relative leaf water content, dry weight fraction, stomatal conductance, transpiration rate, leaf area per seedlings, total chlorophyll content, leaf water potential and proline content. The varieties with high RWC, DWF, total chlorophyll content, leaf water potential and proline content; low stomatal conductance, transpiration rate and leaf area during water stress is considered as drought tolerant and with opposite characters as drought sensitive.

Based on these criteria, the varieties M 13.12, GII 19.5 and GVI 55 were identified as drought tolerant and GVI 61, GV 50 and GI 4.8 as drought sensitive under no irrigation (T_1). The varieties M 13.12, GVI 61, GI 5.9, GVI 50 and GI 4.8 were selected as drought tolerant and GI 10.3, GVI 51 and GVI 55 drought sensitive varieties under life saving irrigation

4.5 SECONDARY SCREENING WITH BUDDED PLANTS

Six month old budlings of seven apparently tolerant and three apparently sensitive varieties/accessions identified in the preliminary screening were subjected to moisture stress as done in the case of seedlings. Observations on physiological parameters, Growth characters and biochemical characters were recorded. The results obtained are presented below.

Table 25. Effect of water stress on number of leaves of cocoa varieties/ accessions (six month old seedlings)

Varieties/accessions	Number of Leaves		
	Water Stress Levels		
	T_1	T_2	T_3
GI 5.9	11.67^{nop}	14.00^{ijkl}	16.00^{fgh}
G I 10.3	11.00^{opqr}	11.33^{nopq}	16.33^{efgh}
G VI 50	10.00^{qr}	19.67^{a}	13.33^{klm}
G II 20/4	9.667^{r}	18.00^{bcd}	15.00^{hij}
G VI 51	7.333^{s}	12.67^{lmn}	11.00^{opqr}
G I 408	19.33^{ab}	15.33^{ghi}	18.67^{abc}
M 13.12	12.00^{mno}	14.00^{ijkl}	16.00^{fgh}
G VI 55	10.33^{pqr}	17.67^{cde}	13.67^{jkl}
G VI 61	16.67^{defg}	12.67^{lmn}	16.00^{fgh}
G II 19.5	14.33^{ijk}	17.33^{cdef}	14.33^{ijk}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 26. Effect of water stress on total biomass of cocoa cultivars/varieties (six months old seedlings)

Varieties	Total Biomass (g pl^{-1})		
	Water Stress Levels		
	T_1	T_2	T_3
GI 5.9	90.52^{abcde}	84.34^{abcde}	93.89^{ab}
G I 10.3	88.71^{abcde}	78.12^{de}	292.92^{abc}
G VI 50	89.65^{abcde}	83.06^{abcde}	93.98^{ab}
G II 20.4	88.99^{abcde}	76.87e	94.78^{a}
G VI 51	88.57^{abcde}	80.00^{bcde}	92.44^{abcd}
G I 4.8	82.38^{abcde}	60.80^{f}	92.70^{abc}
M 13.12	91.73^{abcd}	85.92^{abcde}	94.94^{a}
G VI 55	89.34^{abcde}	80.79^{abcde}	91.43^{abcd}
G VI 61	87.89^{abcde}	80.51^{abcde}	87.85^{abcde}
G II 19.5	89.90^{abcde}	78.90^{cde}	93.57^{ab}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 27. Proline content in cocoa varieties/accessions (six month old seedlings)

Varieties/accessions	Proline ($\mu g\ g^{-1}$ leaf)
GI 5.9	144^{d}
GI 10.3	206^{cd}
GVI 50	267^{bc}
GII 20.4	340^{a}
GVI 51	369^{a}
GI 4.8	311^{ab}
M 13.12	366^{a}
GVI 55	207^{cd}
GVI 61	156^{d}
GII 19.5	205^{cd}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level.

Table 28. Effect of water stress on proline content of cocoa varieties/accessions (six month old seedling)

Varieties/accessions	Proline content ($\mu g\ g^{-1}$ leaf)		
	Water stress levels		
	T_1	T_2	T_3
GI 5.9	139^{jk}	158^{ijk}	136^{jk}
GI 10.3	243^{efghi}	159^{ijk}	216^{fghij}
GVI 50	$274^{defghij}$	$268^{defghij}$	$259^{defghij}$
GII 20.4	384^{abcd}	327^{cdefgh}	$311^{cdefghij}$
GVI 51	463^{ab}	296^{defghi}	380^{bcdef}
GI 4.8	298^{defgh}	439^{abc}	197^{ghij}
M 13.12	487^{a}	236^{fghij}	374^{abcde}
GVI 55	211^{ghij}	219^{fghij}	392^{ghijk}
GVI 61	204^{ghij}	61^{k}	204^{ghij}
GII 19.5	239^{efghij}	160^{hijk}	216^{fghij}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

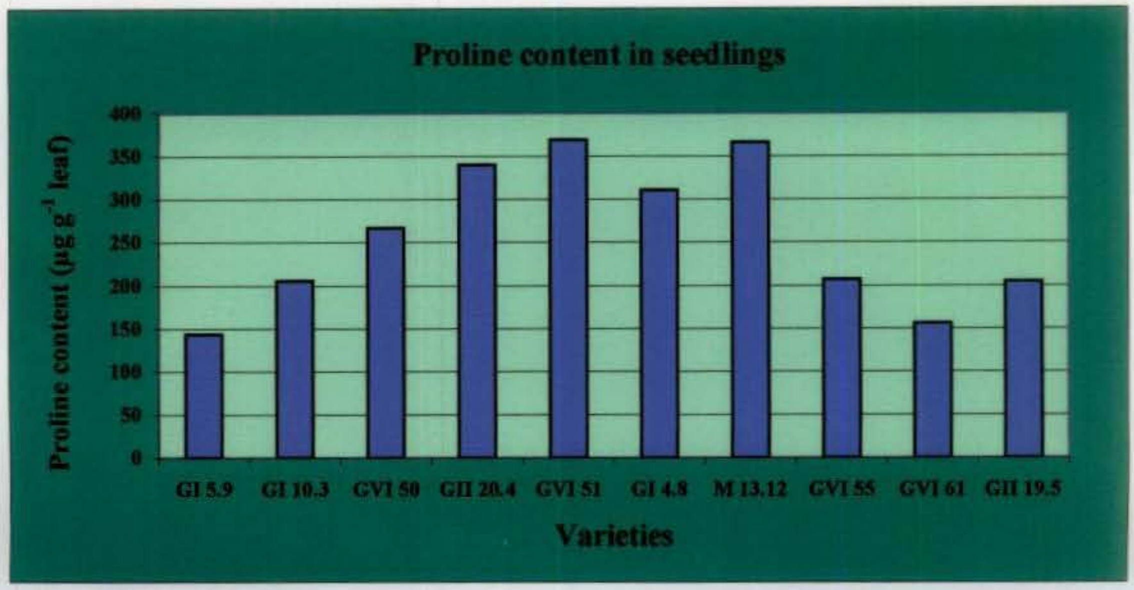

Fig. 3. Proline content (µ g g^{-1} leaf) in six month old cocoa seedlings in relation to varieties/accessions

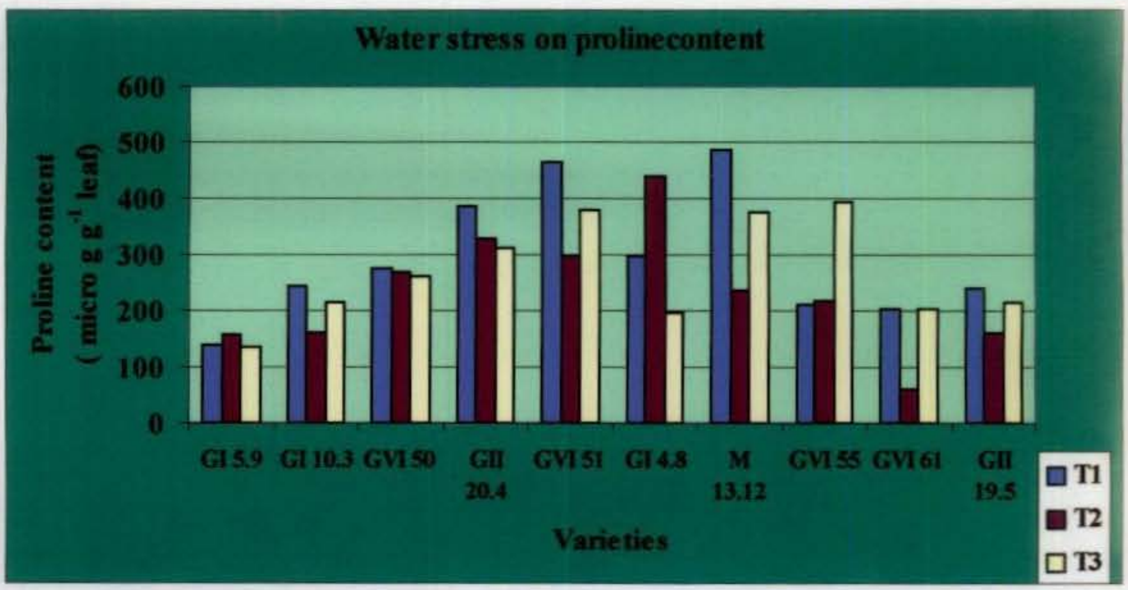

Fig. 4. Effect of water stress on proline content (µg g^{-1} leaf) in six month old cocoa seedlings between varieties/accessions

4.5.1 Physiological parameters

Relative leaf water content (RLWC)

Relative leaf water content (RLWC) of six month old budlings of ten clones of cocoa grown under three soil moisture regimes, i.e. no irrigation (T_1), watering once in five days (T_2) and regular watering (T_3) were shown in Table 29. Clones and moisture regimes significantly influenced RLWC. RLWC was the highest under life saving irrigation (25.70 %). The variety GVI 50 showed the highest RLWC (28.98%) followed by GVI 68 (26.98%) and GVI 51(25.87%). The lowest RLWC was shown by GI 4.8 (14.25%).

The interaction between moisture regime and varieties was also significant (Table 30). The variety GVI 50 showed the highest RLWC under no irrigation (36.64%) and life saving irrigation (35.43%). The variety GVI 51 showed the highest RLWC under regular irrigation (31.57%).

Dry weight fraction (DWF)

The DWF differed considerably between moisture regime and varieties (Table 29). The DWF of budlings grown under no irrigation (0.37%) was on par with life saving irrigation (0.36%). The lowest DWF was noticed in regularly irrigated budlings (0.32%).

The DWF was the highest in GIII 4.1 (0.41%) followed by GVI 68 (0.3%), GIV 2.5 (0.36%) and M 13.12 (0.35%) and the lowest in GI 4.8 (0.31%).

The interaction was also significant (Table 30). In regular watering GVI 68 (0.394) had the high values, in life saving irrigation GII 4.1 (0.464), in no irrigation M 13.12 (0.416) had high values.

Leaf drying percentage

There was no significant relation between moisture regimes and varieties. Interaction was also absent.

Number of days taken for complete drying

There was no significant relation between varieties in number of days took for complete drying under no irrigation.

Transpiration rate

The transpiration rate showed differences between the levels of moisture stress (Table 31 and Fig. 5). It was the highest in regular irrigation (44.66 μg $cm^{-2}s^{-1}$). The life saving irrigation (41.07 μg $cm^{-2}s^{-1}$) was on par with no irrigation (38.83 μg $cm^{-2}s^{-1}$).

Varietal variation was also found to be significant. M 13.12 showed high value (4.75 μg $cm^{-2}s^{-1}$) and the lowest value by GI 4.8 (37.33 μg $cm^{-2}s^{-1}$).

The interaction between moisture regimes and varieties were also significant (Table 32). M 13.12 (48.02 μg $cm^{-2}s^{-1}$) had the highest and GIV 32.5 (42.08 μg $cm^{-2}s^{-1}$) had the lowest transpiration rate under regular watering. M13.12 was found (38.06 μg $cm^{-2}s^{-1}$) on par with GIV 32.5 (38.07 μg $cm^{-2}s^{-1}$) which had the high values under life saving irrigation and GVI 68 (35.31 μg $cm^{-2}s^{-1}$) and GI 4.8 (35.34 μg $cm^{-2}s^{-1}$) had the lowest values. M 13.12 (36.17 μg $cm^{-2}s^{-1}$) had the highest transpiration rate and GI 4.8 (33.03 μg $cm^{-2}s^{-1}$) had the lowest value under no irrigation.

Stomatal conductance

Decreased stomatal conductance was observed with increase in levels of water stress (Table 31). Regularly irrigated budlings showed the highest stomatal conductance (7.30 m mol $m^{-2}s^{-1}$) and the lowest by no irrigation (4.31 m mol $m^{-2}s^{-1}$).

Table 29. Physiological characters in relation to soil moisture regimes and varieties /accessions (six month old budlings)

Treatments	RWC (%)	DWF (%)	Leaf area per budlings (cm^2)
Soil moisture regimes			
T_1	23.25^{b}	0.370^{a}	740^{c}
T_2	25.70a	0.363^{a}	776^{b}
T_3	20.11^{c}	0.326^{b}	784^{a}
Varieties/accessions			
GI 4.8	14.25^{e}	0.314^{ef}	794^{a}
M 16.9	26.03^{b}	0.345^{d}	757^{d}
GIII 4.1	22.82^{c}	0.415^{a}	755^{d}
GVI 50	28.98^{a}	0.342^{d}	738^{f}
GVI 51	25.87^{b}	0.305^{f}	756^{d}
GII 12.3	20.31^{d}	0.380^{b}	770^{c}
GIV 32.5	21.57^{cd}	0.321^{e}	768^{c}
GIV 2.5	22.63^{cd}	0.364^{c}	745^{c}
GVI 68	26.98^{ab}	0.384^{b}	781^{b}
M 13.12	20.77^{cd}	0.356^{c}	793^{a}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 30. Effect of water stress on relative leaf water content (RLWC) and dry weight fraction (DWF) of cocoa varieties/cultivars (six month old budlings)

Varieties/ accessions	RLWC (%)			DWF (%)		
	Water stress levels			Water stress levels		
	T_1	T_2	T_3	T_1	T_2	T_3
GI 4.8	11.74^{o}	12.25^{o}	18.75^{klmn}	0.316^{l}	0.300^{m}	0.326^{kl}
M 16.9	26.60^{defg}	24.40^{efghi}	27.07^{cdef}	0.372^{fgh}	0.323^{l}	0.341^{jk}
GIII 4.1	17.87^{lmn}	26.28^{defgh}	24.30^{efghi}	0.401^{bcd}	0.464^{a}	0.380^{ef}
GVI 50	36.64^{a}	35.43^{a}	14.87^{no}	0.385^{cdef}	0.384^{def}	0.258^{p}
GVI 51	19.75^{jklm}	26.28^{defgh}	31.57^{b}	0.372^{fgh}	0.277^{no}	0.266^{op}
GII 12.3	15.43^{no}	28.56^{bcde}	16.95^{mn}	0.378^{efg}	0.403^{bc}	0.358^{hij}
GIV 32.5	28.94^{bcd}	20.93^{ijklm}	14.85^{no}	0.280^{no}	0.352^{ij}	0.330^{kl}
GIV 2.5	21.93^{ijkl}	23.77^{fghij}	22.19^{hijk}	0.389^{cdef}	0.384^{def}	0.318^{l}
GVI 68	30.93^{bc}	31.11^{bc}	18.89^{klmn}	0.387^{cdef}	0.380^{ef}	0.394^{cde}
M 13.12	22.64^{ghijk}	27.99^{bcdef}	11.68^{o}	0.416^{b}	0.361^{ghi}	0.290^{mn}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

The variation between varieties was significant. The variety GIII 4.1 had the highest stomatal conductance (8.77 m mol $m^{-2}s^{-1}$) followed by GVI 68 (7.55 m mol $m^{-2}s^{-1}$). Stomatal conductance was the lowest in GIV 2.5 (3.48 m mol $m^{-2}s^{-1}$).

The interaction was also found to be significant (Table 33). GIII 4.1 (10.18 m mol $m^{-2}s^{-1}$) had the highest and GI 4.8 (3.2 m mol $m^{-2}s^{-1}$) had the lowest transpiration rate under regular irrigation. Under life saving irrigation GVI 68 (7.47 m mol $m^{-2}s^{-1}$) and M 13.12 (1.45 m mol $m^{-2}s^{-1}$) had the highest and lowest stomatal conductance. GIII 4.1 (7.55 m mol $m^{-2}s^{-1}$) and GII 12.3(1.27 m mol $m^{-2}s^{-1}$) had the highest and lowest transpiration rate under no irrigation.

Leaf water potential (ψ_w)

The (ψ_w) showed significant variation between moisture regime and varieties (Table 31). The highest leaf water potential (ψ_w) was observed in plants grown under regular watering (-3.78 bars) and lowest in plants raised under life saving irrigation (-4.91 bars).

GVI 50 showed the highest ψ_w (-3.35 bars) followed by GII 12.3 (-3.66 bars) and GVI 51 (-3.86 bars). The ψ_w was the lowest with the variety GIV 2.5 (-5.47 bars).

Interaction was also significant (Table 34). Under regular irrigation, GII 12.3 (-3.05 bars) had the highest ψ_w, GVI 50 (-3.50 bars) under life saving irrigation, and GVI 50 (-3.3 bars) under no irrigation.

Leaf area per budling

Leaf area per budlings showed significant variation between moisture regime and varieties (Table 29). The budlings under regular irrigation showed the highest leaf area (784 cm^2) and the lowest leaf area was under no irrigation (740 cm^2).

Table 31. **Physiological characters in relation to soil moisture regimes and varieties /accessions (six month old budlings)**

Treatments	Stomatal conductance (m mol $m^{-2}s^{-1}$)	Leaf water potential (bars)	Transpiration rate (μg $cm^{-2}s^{-1}$)
a) Soil moisture regimes			
T_1	4.31^{c}	-4.67^{b}	34.45^{c}
T_2	4.75^{b}	-4.91^{a}	36.74^{b}
T_3	7.30^{a}	-3.78^{c}	44.60^{a}
b) Varieties/accessions			
GI 4.8	3.52^{i}	5.37^{a}	37.33^{f}
M 16.9	3.88^{h}	4.79^{b}	39.10^{b}
GIII 4.1	877^{a}	4.49^{bc}	38.27^{c}
GVI 50	5.67^{e}	3.35^{e}	39.31^{b}
GVI 51	6.16^{c}	3.86^{d}	37.74^{e}
GII 12.3	5.25^{f}	3.66^{de}	39.19^{d}
GIV 32.5	5.73^{d}	4.76^{d}	37.96^{d}
GIV 2.5	3.48^{i}	5.47^{a}	39.11^{b}
GVI 68	7.55^{b}	4.55^{bc}	37.39^{f}
M 13.12	4.50^{g}	4.26^{c}	40.75^{a}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

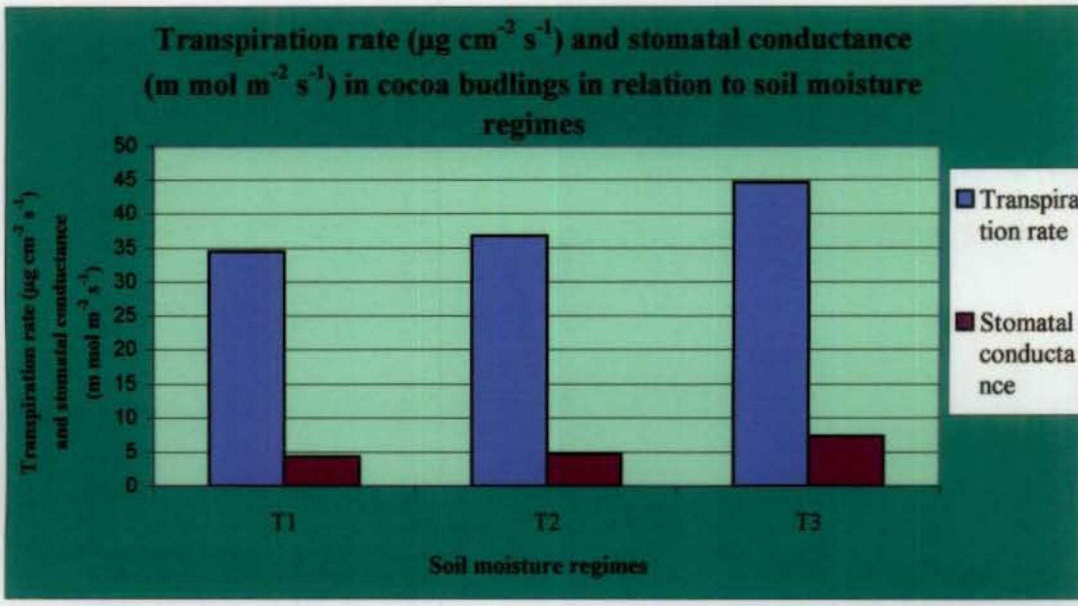

Fig. 5. Effect of moisture stress on transpiration rate and stomatal conductance in cocoa budlings (six month old)

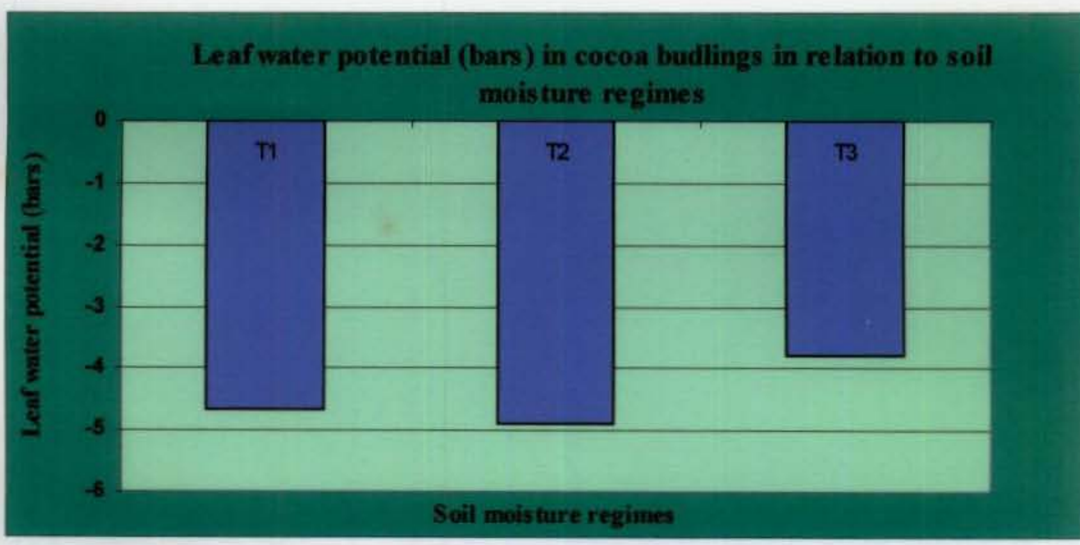

Fig. 6. Effect of moisture stress on leaf water potential (bars) in cocoa budlings

Table 32. Effect of water stress on transpiration rate of cocoa varieties/accessions (six month old budlings)

Varieties/accessions	Transpiration rate (μg $cm^{-2}s^{-1}$)		
	Water stress levels		
	T_1	T_2	T_3
GI 4.8	33.03^{s}	35.34^{n}	43.61^{f}
M 16.9	34.85^{o}	37.15^{j}	45.28^{d}
GIII 4.1	35.02^{o}	37.60^{i}	42.18^{g}
GVI 50	35.36^{n}	37.66^{i}	44.37^{e}
GVI 51	33.26^{r}	35.66^{m}	44.30^{e}
GII 12.3	35.43^{n}	36.10^{l}	46.05^{c}
GIV 32.5	33.73^{q}	38.07^{h}	42.08^{g}
GIV 2.5	34.47^{p}	36.45^{k}	46.40^{b}
GVI 68	33.15^{rs}	35.31^{n}	43.71^{f}
M 13.12	36.17^{l}	38.08^{h}	48.02^{a}

Table 33. Effect of water stress on stomatal conductance of cocoa varieties/accessions (six month old budlings)

Varieties/accessions	Stomatal conductance (m mol $m^{-2}s^{-1}$)		
	Water stress levels		
	T_1	T_2	T_3
GI 4.8	3.42^{q}	3.88^{p}	3.26^{r}
M 16.9	3.47^{q}	3.86^{p}	4.32^{m}
GIII 4.1	7.55^{g}	8.57^{d}	10.18^{a}
GVI 50	4.04^{o}	5.05^{i}	7.92^{f}
GVI 51	5.14^{k}	3.95^{op}	9.40^{c}
GII 12.3	1.27^{w}	6.95^{i}	7.53^{g}
GIV 32.5	6.00^{j}	3.09^{s}	8.11^{e}
GIV 2.5	2.99^{t}	3.25^{r}	4.21^{n}
GVI 68	7.10^{h}	7.47^{g}	8.10^{e}
M 13.12	2.09^{u}	1.45^{v}	9.97^{b}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 34. Water stress on leaf water potential of cocoa varieties/ accessions (six month old budlings)

Varieties/accessions	Leaf water potential (bars) Water stress levels		
	T_1	T_2	T_3
GI 4.8	-6.13^{a}	-6.03^{ab}	-3.96^{ijkl}
M 16.9	-5.28^{d}	-5.43^{bcd}	-3.66^{jklmn}
GIII 4.1	-4.86^{defg}	-5.13^{de}	-3.48^{klmn}
GVI 50	-3.36^{lmn}	-3.50^{klmn}	-3.18^{mn}
GVI 51	-4.00^{ijkl}	-4.23^{ghij}	-3.36^{lmn}
GII 12.3	-3.76^{jklm}	-4.16^{hij}	-3.05^{n}
GIV 32.5	-4.90^{def}	-5.33^{cd}	-4.06^{ijk}
GIV 2.5	-5.36^{cd}	-5.93^{abc}	-5.13^{de}
GVI 68	-4.83^{defg}	-4.60^{efghi}	-4.23^{ghij}
M 13.12	-4.26^{fghij}	-4.80^{defgh}	-3.73^{jklm}

Table 35. Water stress on leaf area per budlings of cocoa varieties/ accessions (six month old)

Varieties/accessions	Leaf area per budlings (cm^2) Water stress levels		
	T_1	T_2	T_3
GI 4.8	748^{n}	880^{c}	754^{m}
M 16.9	733^{q}	749^{n}	790^{h}
GIII 4.1	703^{u}	679^{w}	892^{a}
GVI 50	808^{i}	711^{t}	694^{v}
GVI 51	710^{t}	820^{e}	738^{p}
GII 12.3	803^{g}	742^{o}	766^{k}
GIV 32.5	715^{s}	780^{i}	810^{f}
GIV 2.5	638^{x}	874^{d}	723^{r}
GVI 68	809^{i}	771^{j}	788^{h}
M 13.12	736^{pq}	758^{l}	886^{b}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

The variety GI 4.8 had the highest leaf area (794 cm^2) followed by M 13.12 (793 cm^2) and the lowest by GVI 50(738 cm^2).

The interaction effect was also significant (Table 35). Under regular irrigation GIII 4.1 (892 cm^2) had the highest leaf area, GI 4.8 (880 cm^2) under life saving irrigation. GVI 50 (808 cm^2) under no irrigation.

Chlorophyll 'a' content of leaves

The leaf chlorophyll 'a' content was observed significant (Table 36). It was the highest in budlings under no irrigation (0.981 mg g^{-1} leaf tissue).

GII 12.3 had the highest chlorophyll 'a'(1.53 mg g^{-1} leaf tissue) and GIV 32.5 showed the lowest chlorophyll 'a'(0.36 mg g^{-1} leaf tissue).

The interaction was also found to be significant (Table 37). Under regular irrigation GII 12.3 (1.41 mg g^{-1} leaf tissue), GII 12.3 (1.54 mg g^{-1} leaf tissue) it is on par with GIII 4.1 (1.52 mg g^{-1} leaf tissue) under life saving irrigation, GII 12.3 (1.63 mg g^{-1} leaf tissue) had high values under no irrigation.

Chlorophyll 'b' content of leaves

The leaf chlorophyll 'b' content was observed significant (Table 36). Chlorophyll 'b' was the highest in life saving irrigation (0.241 mg g^{-1} leaf tissue).

Chlorophyll 'b' was the highest in GIV 32.5 (0.371 mg g^{-1} leaf tissue) and the lowest in GII 12.3 (0.121 mg g^{-1} leaf tissue). There was significant interaction between moisture regimes and varieties (Table 38). Under regular (0.39 mg g^{-1} leaf tissue) and life saving irrigation (0.36 mg g^{-1} leaf tissue) GIV 32.5 had the highest chlorophyll 'b' content. Under no irrigation M 16.9 (0.35 mg g^{-1} leaf tissue) had the highest chlorophyll 'b' content.

Total chlorophyll

Significant differences were noticed between moisture regimes and varieties in total chlorophyll content (Table 36). GII 12.3 showed the highest content of total chlorophyll (1.66 mg g^{-1} leaf tissue) and the lowest by GIV 32.5 (0.80 mg g^{-1} leaf tissue).

The interaction was also significant (Table 39). Under regular irrigation (1.55 mg g^{-1} leaf tissue) and no irrigation (1.76 mg g^{-1} leaf tissue) GII 12.3 had the highest total chlorophyll content and GIII 4.1 (1.76 mg g^{-1} leaf tissue) had high total chlorophyll content under life saving irrigation.

Chlorophyll stability index

The CSI of leaves varied between soil moisture regimes and varieties (Table 36). The CSI was the highest in budlings under regular irrigation (44.66%).

The CSI was the highest in budlings of M 16.9 (55.84%) followed by GIV 32.5 (54.57%) and the lowest in M 13.12 (33.79%).

The interaction effects between moisture regimes and varieties was significant (Table 40). In regular irrigation GVI 68 (60.43%), GIV 2.5 (60.85%) under life saving irrigation and M 16.9 (52.81%) which is on par with GIV 32.5 (52.31%) had high values under no irrigation.

Relative injury

The RI of budlings showed no significant relation between soil moisture regimes and varieties.

Interactions were not significant.

Table 36. Chlorophyll 'a', 'b', and total chlorophyll, and chlorophyll stability index in relation to soil moisture regimes and varieties (six month old budlings)

Treatments	Chlorophyll 'a' (mg g^{-1} leaf tissue)	Chlorophyll 'b' (mg g^{-1} leaf tissue)	Total chlorophyll (mg g^{-1} leaf tissue)	Chlorophyll stability index
a) Soil moisture regimes				
T_1	**0.98a**	**0.20c**	**1.30a**	**38.83b**
T_2	0.80b	0.24a	1.13b	41.07b
T_3	0.71c	0.23b	1.02c	44.66a
b) Varieties/cultivars				
GI 4.8	1.08^{c}	0.18^{f}	1.34^{d}	39.84^{c}
M 16.9	0.45^{i}	0.31^{b}	0.87^{h}	55.84^{a}
GIII 4.1	1.22^{b}	0.17^{g}	1.55^{b}	35.60^{c}
GVI 50	0.83^{e}	0.27^{c}	1.36^{c}	35.91^{c}
GVI 51	0.64^{g}	0.22^{d}	0.93^{g}	34.87^{c}
GII 12.3	1.53^{a}	0.12^{h}	1.66^{a}	33.97^{c}
GIV 32.5	0.36^{j}	0.37^{a}	0.80^{j}	54.57^{ab}
GIV 2.5	0.79^{f}	0.16^{g}	0.95^{f}	47.52^{ab}
GVI 68	0.56^{h}	0.22^{d}	0.84^{i}	46.29^{b}
M 13.12	0.85^{d}	0.19^{e}	1.19^{e}	33.79^{c}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 37. Effect of water stress on chlorophyll 'a' of cocoa varieties/accessions (six month old budlings)

Varieties/accessions	Chlorophyll 'a' (mg g^{-1} leaf tissue)		
	Water stress levels		
	T_1	T_2	T_3
GI 4.8	1.48^{c}	1.31^{e}	0.46^{s}
M 16.9	0.42^{u}	0.44^{t}	0.50^{pq}
GIII 4.1	1.28^{f}	1.52^{b}	0.85^{k}
GVI 50	1.22^{g}	0.55^{o}	0.71^{m}
GVI 51	0.94^{i}	0.49^{r}	0.49^{qr}
GII 12.3	1.63^{a}	1.54^{b}	1.41^{d}
GIV 32.5	0.43^{tu}	0.24^{v}	0.41^{u}
GIV 2.5	0.74^{l}	0.54^{o}	1.08^{h}
GVI 68	0.68^{n}	0.49^{qr}	0.52^{p}
M 13.12	0.93^{ij}	0.91^{j}	0.71^{m}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 38. Effect of Water stress on chlorophyll 'b' of cocoa varieties/ accessions (six month old budlings)

Varieties/accessions	Chlorophyll 'b' (mg g^{-1} leaf tissue)		
	Water stress levels		
	T_1	T_2	T_3
GI 4.8	0.13^{nop}	0.14^{no}	0.27^{de}
M 16.9	0.35^{b}	0.33^{c}	0.26^{ef}
GIII 4.1	0.18^{jk}	0.13^{no}	0.18^{jk}
GVI 50	0.18^{jk}	0.35^{b}	0.28^{d}
GVI 51	0.15^{mn}	0.24^{g}	0.28^{d}
GII 12.3	0.11^{p}	0.12^{p}	0.12^{op}
GIV 32.5	0.34^{bc}	0.36^{b}	0.39^{a}
GIV 2.5	0.16^{lm}	0.26^{ef}	0.08^{q}
GVI 68	0.19^{j}	0.25^{fg}	0.22^{h}
M 13.12	0.21^{hi}	0.19^{ij}	0.17^{kl}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 39. Effect of water stress on total chlorophyll of cocoa varieties/ accessions (six month old budlings)

Varieties/accessions	Total chlorophyll (mg g^{-1} leaf tissue) Water stress levels		
	T_1	T_2	T_3
GI 4.8	1.69^{c}	1.53^{f}	0.80^{t}
M 16.9	0.88^{qr}	0.88^{qr}	0.85^{s}
GIII 4.1	1.73^{b}	1.76^{a}	1.16^{j}
GVI 50	1.66^{d}	1.15^{j}	1.27^{i}
GVI 51	1.13^{k}	0.77^{u}	0.88^{r}
GII 12.3	1.76^{a}	1.66^{d}	1.55^{e}
GIV 32.5	0.90^{pq}	0.53^{v}	0.97^{m}
GIV 2.5	0.93^{o}	0.91^{op}	1.01^{l}
GVI 68	0.95^{n}	0.80^{t}	0.77^{u}
M 13.12	1.36^{g}	1.28^{h}	0.92^{o}

Table 40. Effect of Water stress on chlorophyll stability index of cocoa varieties/ accessions (six month old budlings)

Varieties/accessions	Chlorophyll stability index Water stress levels		
	T_1	T_2	T_3
GI 4.8	34.79^{defghi}	39.23^{cdefg}	36.20^{defgh}
M 16.9	52.81^{abcd}	59.68^{ab}	55.04^{abc}
GIII 4.1	29.44^{fghi}	18.06^{i}	59.29^{ab}
GVI 50	34.73^{defghi}	40.71^{cdefg}	32.28^{efghi}
GVI 51	37.18^{cdefgh}	20.30^{hi}	47.12^{abcdef}
GII 12.3	29.32^{fghi}	40.75^{cdefg}	31.84^{efghi}
GIV 32.5	52.31^{abcd}	46.75^{abcdef}	64.66^{a}
GIV 2.5	48.87^{abcde}	60.85^{a}	32.84^{efghi}
GVI 68	26.79^{ghi}	51.65^{abcd}	60.43^{a}
M 13.12	$42.08b^{cdefg}$	32.69^{efghi}	26.59^{ghi}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level. T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Membrane stability

Membrane stability showed significant variations between varieties (Table 41).GIV 32.5 had the highest membrane stability (0.56) followed by M 16.9 (0.47) and the lowest by GII 12.3 (0.18). Moisture regime was not significant.

The interactions were found to be significant (Table 42). In regular irrigation GIV 32.5 (0.73) had the highest value, in life saving irrigation M 16.9 (0.59), in no irrigation GIV 32.5 (0.57) had the highest membrane stability.

4.5.2 Growth characters

Height of budlings

The height of budlings differed with soil moisture regimes as well as varieties (Table 43). The budlings raised under regular irrigation were the tallest (80.80 cm). The height was lesser with plants kept under no irrigation (67.87 cm).

The budlings of GI 4.8 were the tallest (85.56 cm) followed by M 16.9 (79.69 cm). The budlings of GVI 50 were the shortest (64.00 cm).

The interaction between soil moisture regimes and varieties was significant (Table 44). In regular irrigation (92.67 cm), life saving irrigation (88.00 cm) and no irrigation (76.00 cm) GI 4.8 showed highest values.

Girth of budlings

There were considerable differences in budlings girth between soil moisture regimes and varieties (Table 43). Regular irrigation showed the highest girth (4.51 cm). Budling girth under no irrigation was the minimum (3.90 cm).

Table 41. Variation in membrane stability among varieties. (Six month old budlings)

Varieties /accessions	Membrane stability
GI 4.8	0.31^{cd}
M 16.9	0.48^{b}
GIII 4.1	0.29^{cd}
GVI 50	0.36^{c}
GVI 51	0.33^{cd}
GII 12.3	0.19^{e}
GIV 32.5	0.57^{a}
GIV 2.5	0.32^{cd}
GVI 68	0.37^{c}
M 13.12	0.23^{de}

Table 42. Water stress on membrane stability of cocoa varieties / accessions (six month old budlings)

Varieties/accessions	Membrane stability		
	Water stress levels		
	T_1	T_2	T_3
GI 4.8	0.31^{defghi}	0.32^{j}	0.28^{fghi}
M 16.9	0.48^{bcd}	0.59^{ab}	0.54^{bc}
GIII 4.1	0.28^{efghi}	$0.37^{cdefghi}$	0.33^{defghi}
GVI 50	$0.36^{cdefghi}$	0.38^{cdefgh}	0.47^{bcdef}
GVI 51	0.33^{defghi}	0.32^{defghi}	0.33^{defghi}
GII 12.3	0.19^{ij}	0.20^{hi}	0.28^{fghi}
GIV 32.5	0.57^{b}	0.57^{b}	0.73^{a}
GIV 2.5	0.33^{defghi}	0.42^{bcdefg}	0.47^{bcde}
GVI 68	$0.38^{cdefghi}$	0.45^{bcdef}	0.38^{cdefgh}
M 13.12	0.23^{hi}	0.26^{ghi}	0.25^{ghi}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level .T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 43. Growth characters in relation to soil moisture regimes and varieties (six month old budlings)

Treatments	Height	Girth
Soil moisture regimes		
T_1	67.88^{c}	3.90^{C}
T_2	76.57^{b}	4.38^{b}
T_3	80.80^{a}	4.51^{a}
Varieties/accessions		
GI 4.8	85.56^{a}	4.27^{cd}
M 16.9	79.67^{b}	4.10^{ef}
GIII 4.1	75.11^{d}	4.14^{e}
GVI 50	64.00^{g}	4.28^{d}
GVI 51	77.00^{c}	4.20^{de}
GII 12.3	72.22^{f}	4.00^{g}
GIV 32.5	72.67^{f}	4.78^{a}
GIV 2.5	73.78^{e}	4.10^{ef}
GVI 68	75.44^{d}	4.40^{b}
M 13.12	75.33^{d}	4.36^{c}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level .T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Table 44. Water stress on height of cocoa varieties/accessions (six month old budlings)

Varieties/accessions	Height (cm) Water stress levels		
	T_1	T_2	T_3
GI 4.8	76.00^{i}	88.00^{a}	92.67^{a}
M 16.9	72.00^{h}	78.67^{c}	88.33^{b}
GIII 4.1	66.67^{ij}	77.33^{cf}	81.33^{d}
GVI 50	55.33^{k}	67.67^{i}	69.00^{i}
GVI 51	70.67^{hi}	85.33^{b}	75.00^{fg}
GII 12.3	67.33^{ij}	73.00^{gh}	76.33^{f}
GIV 32.5	64.00^{j}	74.00^{g}	80.00^{d}
GIV 2.5	70.00^{hi}	73.00^{gh}	78.33^{e}
GVI 68	70.66^{hi}	76.00^{f}	79.67^{de}
M 13.12	66.00^{ij}	72.67^{gh}	87.33^{c}

Table 45. Water stress on girth of cocoa varieties/ accessions (six month old budlings)

Varieties/accessions	Girth (cm) Water stress levels		
	T_1	T_2	T_3
GI 4.8	4.20^{i}	4.20^{i}	4.40^{h}
M 16.9	3.53^{kl}	4.27^{hi}	4.50^{g}
GIII 4.1	4.10^{i}	4.10^{j}	4.23^{hi}
GVI 50	4.00^{jk}	4.43^{h}	4.40^{h}
GVI 51	4.00^{jk}	4.10^{j}	4.50^{g}
GII 12.3	3.76^{k}	4.10^{j}	4.13^{j}
GIV 32.5	4.20^{hi}	4.93^{c}	5.20^{a}
GIV 2.5	4.10^{i}	4.00^{jk}	4.20^{i}
GVI 68	3.30^{l}	5.03^{b}	4.86^{d}
M 13.12	3.77^{k}	4.60^{f}	4.70^{e}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level .T_1- No irrigationT_2- Life saving irrigation (once in five days); T_3- Daily irrigation

Budlings of GIV 32.5 had the highest girth (4.78cm) and those of GII 12.3 had the lowest (4.00 cm).

The interaction with moisture regimes and varieties were significant (Table 45). Under regular irrigation GIV 32.5 (5.20 cm), under life saving irrigation GVI 68 (5.03 cm) and under no irrigation GIV 32.5 (4.20 cm) which is on par with GI 4.8 (4.20 cm) had the highest values.

4.5.3 Biochemical characters

Proline content

The proline content of leaves varied between moisture regimes and varieties (Table 46). Proline content increased with increase in water stress. It was the highest under life saving irrigation (423.79μ g g^{-1} leaf) and the lowest in regular irrigation.(324.41 μ g g^{-1} leaf) (Fig. 7).

M 13.12 had the highest proline content under stress (516.86 μ g g^{-1} leaf) followed by GVI 51 (490.52 μ g g^{-1} leaf) and GII 12.3 (490.09 μ g g^{-1} leaf) and the lowest in GI 4.8 (240.96 μ g g^{-1} leaf) (Fig. 8).

The interaction effect was significant (Table 47). Under regular irrigation M 13.12 (469μ g g^{-1} leaf), under life saving irrigation GVI 51 (610 μ g g^{-1} leaf) and under no irrigation GII 12.3 (495 μ g g^{-1} leaf) had high values.

Nitrate reductase activity (NRA)

NRA of leaves differed considerably between moisture regimes and varieties (Table 46). The NRA was the highest in plants grown under regular watering (26.45m mol NO_2 per gram per 2hr at 30^0C) and the lowest in no irrigation (13.20 m mol NO_2 per gram per 2hr at 30^0C).

The variety GI 4.8 showed the highest NRA (29.89 m mol NO_2 per gram per 2hr at 30^0C) followed by GIV 2.5(25.58 m mol NO_2 per gram per 2hr at 30^0C) and the lowest NRA of leaves was found in GVI 50(13.62 m mol NO_2 per gram per 2hr at 30^0C).

The interaction between moisture regimes and varieties was significant (Table 48). Under regular irrigation GI 4.8(62.03 m mol NO_2 per gram per 2hr at 30^0C), under life saving irrigation M 13.12 (51.51 m mol NO_2 per gram per 2hr at 30^0C) and under no irrigation GVI 51 (18.90 m mol NO_2 per gram per 2hr at 30^0C) had high values.

4.5.4 Cluster analysis

Non-hierarchical Euclidean Cluster Analysis (Chatfield and Collins, 1980) was done for selection of drought tolerant and sensitive varieties.

The parameters selected for clustering were relative leaf water content, dry weight fraction, stomatal conductance, transpiration rate, leaf area per seedlings, total chlorophyll content, leaf water potential and proline content. The varieties with high RWC, DWF, total chlorophyll content, leaf water potential and proline content; low stomatal conductance, transpiration rate and leaf area during water stress is considered as drought tolerant and with opposite characters as drought sensitive.

Based on these criteria the varieties GI 4.8 and GII 19.5 were selected as drought tolerant and GIII 4.1, GVI 51 and GII 12.3 as drought sensitive under no irrigation (T_1). No variety was identified as drought tolerant under life saving irrigation. The varieties GI 4.8, M 16.9, GVI 50, GVI 51, GIV 2.5 and M 13.12 were identified as drought sensitive.

Table 46. **Proline and NRA content of leaves in relation to soil moisture regimes and varieties (six month old budlings)**

Treatments	Proline ($\mu g\ g^{-1}$ leaf)	Nitrate reductase activity (m mol NO_2 per gram per two hour at 30° C)
a) Soil moisture regimes		
T_1	356.92^{b}	13.20^{c}
T_2	423.79^{a}	20.35^{b}
T_3	324.41^{c}	26.45^{a}
b) Varieties/ accessions		
GI 4.8	240.96^{b}	29.89^{a}
M 16.9	319.66^{c}	18.71^{c}
GIII 4.1	353.43^{d}	17.35^{f}
GVI 50	301.49^{f}	13.62^{h}
GVI 51	490.52^{b}	17.95^{f}
GII 12.3	490.09^{b}	16.92^{g}
GIV 32.5	404.61^{c}	19.58^{d}
GIV 2.5	284.93^{f}	25.58^{h}
GVI 68	281.17^{g}	16.12^{g}
M 13.12	516.86^{a}	24.28^{c}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level .T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

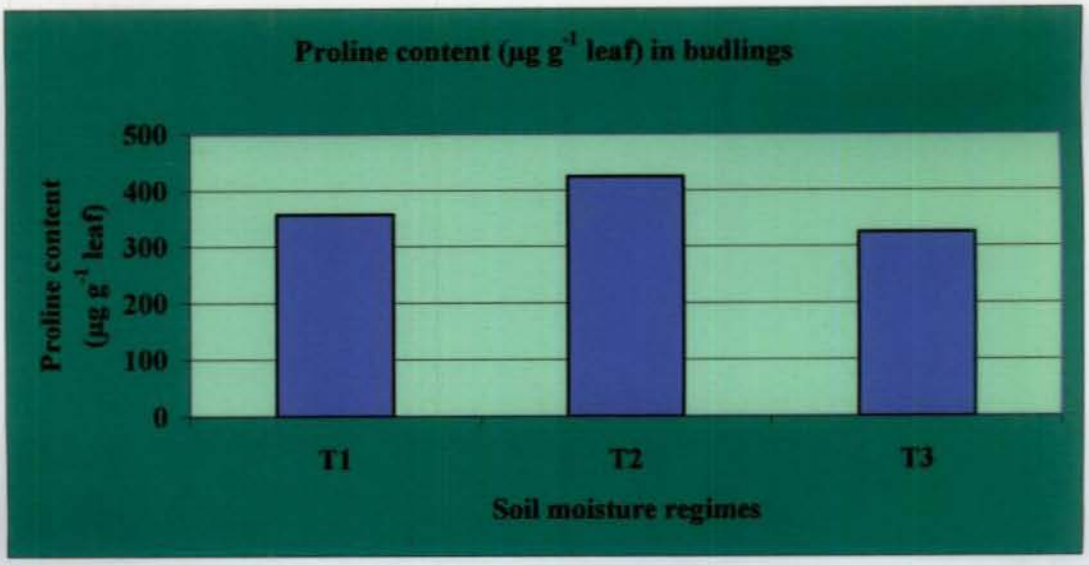

Fig. 7. Effect of moisture stress on proline content in cocoa budlings (six month old)

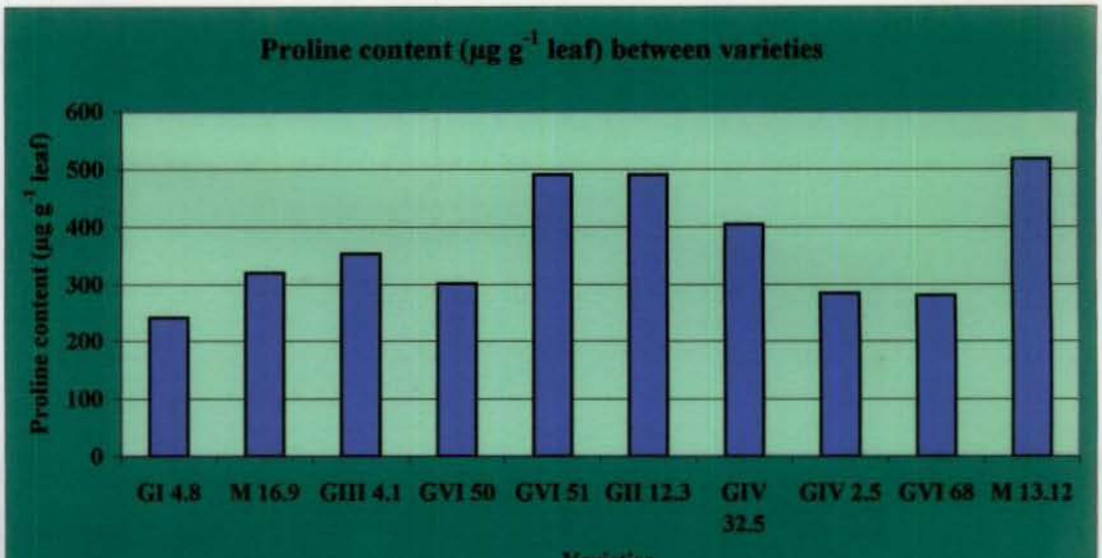

Fig. 8. Proline content between varieties (six month old cocoa budlings)

Table 47. Water stress on proline content (six month old budlings) of cocoa varieties/ accessions

Varieties/accessions	Proline ($\mu g\ g^{-1}$ leaf) Water stress levels		
	T_1	T_2	T_3
GI 4.8	238.70^{pq}	277.41^{o}	206.77^{q}
M 16.9	302.25^{n}	379.98^{k}	276.76^{o}
GIII 4.1	348.70^{lm}	392.89^{jk}	318.70^{m}
GVI 50	286.76^{no}	363.21^{l}	254.51^{p}
GVI 51	450.95^{g}	610.62^{q}	409.98^{i}
GII 12.3	495.79^{d}	561.91^{c}	412.56^{hi}
GIV 32.5	396.76^{j}	447.72^{gh}	369.34^{kl}
GIV 2.5	287.41^{no}	309.99^{mn}	257.41^{p}
GVI 68	271.60^{d}	303.86^{h}	268.05^{op}
M 13.12	490.30^{e}	590.30^{b}	469.98^{f}

Table 48. Water stress on nitrate reductase activity (six month old budlings) of cocoa varieties/ accessions

Varieties/accessions	Nitrate reductase activity (m mol NO_2 per gram per two hour at 30° C) Water stress levels		
	T_1	T_2	T_3
GI 4.8	10.18l	17.46fg	62.03a
M 16.9	12.19k	25.98de	17.97fg
GIII 4.1	12.81k	13.96ij	25.28de
GVI 50	13.74ij	12.58k	14.53i
GVI 51	18.90f	10.52l	24.40de
GII 12.3	8.75n	19.21f	22.79ef
GIV 32.5	17.91fg	13.74ij	27.08d
GIV 2.5	16.59g	15.63h	44.51k
GVI 68	11.45kl	22.91ef	13.99ij
M 13.12	9.45m	51.51h	11.88kl

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level .T_1- No irrigation; T_2- Life saving irrigation (once in five days); T_3- Daily irrigation

4.6 FIELD MONITORING OF COCOA TREES

Transpiration rate, stomatal conductance, leaf water potential, stomatal frequency, leaf thickness, bark thickness and soil moisture content of ten varieties included in the secondary screening (sixteen year old cocoa trees) were measured during April, 2004.

Field performance of varieties included in the secondary screening of seedling and budlings were presented separately (Table 49, and Table 50).

4.6.1 Seedlings

Transpiration rate

In seedlings, transpiration rate differed significantly between varieties. It was the highest with the variety of GII 20.4(49.10 $\mu g\ cm^{-2}s^{-1}$) followed by GI 5.9 (48.51 $\mu g\ cm^{-2}s^{-1}$) and GVI 55 (48.06$\mu g\ cm^{-2}s^{-1}$) and was the lowest in M13.12 (3.19 $\mu g\ cm^{-2}s^{-1}$).

Stomatal conductance

The stomatal conductance differed between varieties. It was the highest in GI 5.9(15.07 m mol $m^{-2}s^{-1}$) followed by GVI 50 (14.29 m mol $m^{-2}s^{-1}$), G VI 51(13.69 m mol $m^{-2}s^{-1}$) and the lowest in M 13.12 (0.47 m mol $m^{-2}s^{-1}$).

Leaf water potential

The leaf water potential differed considerably between varieties. It was the highest in GVI 51(-0.81 bars) and the lowest in M 13.12 (-1.73 bars).

Stomatal frequency

The stomatal frequency differed considerably between varieties. It was the highest in GVI 61 (32.00) followed by GI 4.8 (31.67) and the lowest in GII 20.4 (19.67).

Leaf thickness

It differed between varieties. It was the highest in GVI 61(0.24mm) followed by GVI 50 (0.19 mm), GI 4.8 (0.19 mm) and the lowest in GII 19.5 (0.13 mm).

Bark thickness

Bark thickness differed considerably between varieties. GVI 61 and GII 19.5 had the maximum bark thickness (3.00 mm) and minimum values were observed in GII 20.4 and GVI 51 (1.5 mm).

Soil moisture content

Soil moisture content was the highest in GII 20.4 (17.38%), GVI 61 (15.80%) and GI 5.9 (15.58%) and the lowest in GVI 51 (4.21%).

Low transpiration rate, low stomatal conductance, high leaf water potential, high stomatal frequency, high leaf thickness, high bark thickness and high soil moisture content under water stress are the features of drought tolerant plants and with opposite characters as drought sensitive. Based on these features, the varieties, GVI 61, M 13.12 and GI 4.8 seems to be drought tolerant and GVI 51 drought sensitive.

4.6.2 Budlings

Transpiration rate

In budlings, transpiration rate differed significantly between varieties. It was the highest with the variety of GIII 4.1 (49.75 $\mu g\ cm^{-2}s^{-1}$) followed by M 16.9 (45.95 $\mu g\ cm^{-2}s^{-1}$) and GVI 51 (45.61 $\mu g\ cm^{-2}s^{-1}$) and was the lowest in M13.12 (3.19 $\mu g\ cm^{-2}s^{-1}$).

Table 49. Field performance of the cocoa varieties/accessions included in the secondary screening (Sixteen year old cocoa trees during April 2004)

Varieties/ accessions	Transpiration rate ($\mu g\ cm^{-2}s^{-1}$)	Stomatal conductance ($m\ mol\ m^{-2}s^{-1}$)	Leaf water potential (bars)	Stomatal frequency (cm^2)	Leaf thickness (mm)	Bark thickness (mm)	Soil moisture content (%)
GI 5.9	48.51^{ab}	15.87^{a}	-1.00^{b}	27.67^{bc}	0.13^{f}	2.50^{b}	15.58^{ab}
GI 10.3	41.74^{d}	10.37^{c}	-1.06^{c}	25.33^{cd}	0.14^{ef}	2.16^{cd}	13.85^{ab}
GVI 50	44.98^{c}	14.29^{b}	-1.00^{b}	24.67^{d}	0.19^{b}	2.50^{b}	12.53^{b}
GII 20.4	49.10^{a}	10.74^{c}	-0.81^{a}	19.67^{e}	0.15^{de}	1.50^{e}	17.38^{a}
GVI 51	45.61^{c}	13.69^{b}	-1.00^{b}	28.33^{b}	0.17^{cd}	1.50^{e}	4.21^{c}
GI 4.8	18.97^{f}	3.41^{d}	-1.73^{d}	31.67^{a}	0.19^{b}	2.00^{d}	14.92^{ab}
M 13.12	3.19^{b}	0.47^{e}	-1.00^{b}	25.33^{cd}	0.18^{bc}	2.50^{b}	15.41^{ab}
GVI 55	48.06^{b}	10.00^{c}	-1.00^{b}	25.67^{cd}	0.18^{bc}	2.33^{bc}	14.41^{ab}
GVI 61	5.66^{g}	0.67^{e}	-1.00^{b}	32.00^{a}	0.24^{a}	3.00^{a}	15.80^{ab}
GII 19.5	37.91	9.69^{c}	-1.00^{b}	27.33^{bc}	0.13^{ef}	3.00^{a}	11.51^{b}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level

Stomatal conductance

The stomatal conductance differed between varieties. It was the highest in GIII 4.1(14.29 m mol $m^{-2}s^{-1}$) G VI 50(14.29 m mol $m^{-2}s^{-1}$) and the lowest in M 13.12 (0.47 m mol $m^{-2}s^{-1}$).

Leaf water potential

The leaf water potential differed considerably between varieties. It was the highest in GVI 51 (-0.81 bars) and the lowest in GVI 68 (-1.8 bars).

Stomatal frequency

The stomatal frequency differed considerably between varieties. It was the highest in GI 4.8 (31.67) followed by GVI 51 and GII 12.3 (31.67) and the lowest in GVI 50 (19.67).

Leaf thickness

It differed between varieties. It was the highest in GIV2.5 (0.24mm) followed by GI 4.8 and GIII 4.1 (0.19mm) and the lowest in M 16.9 (0.14mm).

Bark thickness

Bark thickness differed considerably between varieties. GVI 68 had the maximum bark thickness (3.00mm) and the minimum in GVI 50 and GVI 51 (1.5mm).

Soil moisture content

Soil moisture content was the highest in GIII 4.1(18.26%) and the lowest in GVI 68(5.22%).

Low transpiration rate, low stomatal conductance, high leaf water potential, high stomatal frequency, high leaf thickness, high bark thickness and high soil moisture content under water stress are the features of drought tolerant plants and with opposite characters as drought sensitive. Based on these, the variety M 13.12 and GI 4.8 seems to be drought tolerant and GVI 51 and GIII 4.1 drought sensitive.

Table 50. Field performance of cocoa budlings included in the secondary screening (Sixteen year old cocoa trees, during April 2004)

Varieties/ accessions	Transpiration rate (µg cm^{-2} s^{-1})	Stomatal conductance (m mol m^{-2} s^{-1})	Leaf water potential (bars)	Stomatal frequency (cm^{2})	Leaf thickness (mm)	Bark thickness (mm)	Soil moisture content (%)
GI 4.8	18.97^{g}	3.41^{e}	-1.00^{b}	31.67^{a}	0.19^{d}	2.00^{d}	14.92^{bcd}
M 16.9	45.95^{b}	10.37^{c}	-1.06^{c}	25.33^{c}	0.14^{c}	2.33^{c}	14.01^{cde}
GIII 4.1	49.75^{a}	14.29^{a}	-1.00^{b}	24.67^{d}	0.19^{b}	2.50^{b}	18.26^{a}
GVI 50	44.98^{d}	14.29^{a}	-1.00^{b}	19.67^{e}	0.15^{d}	1.50^{e}	12.53^{dc}
GVI 51	4561^{c}	13.69^{d}	-0.81^{a}	28.33^{b}	0.17^{cd}	1.50^{e}	4.216^{f}
GII 12.3	44.65^{e}	3.41^{e}	-1.73^{d}	31.67^{a}	0.18^{c}	2.00^{d}	12.04^{e}
GIV 32.5	41.17^{f}	0.47^{d}	-1.00^{b}	25.33^{c}	0.18^{c}	2.5^{b}	17.18^{abc}
GIV 2.5	6.03^{h}	10.00^{c}	-1.00^{b}	25.67^{c}	0.24^{a}	2.33^{c}	16.42^{abc}
GVI 68	3.54^{i}	0.67^{f}	-1.8^{e}	27.33^{bc}	0.15^{d}	3.00^{a}	5.22^{f}
M 13.12	3.19^{j}	0.47^{g}	-1.73^{d}	25.33^{c}	0.18^{c}	2.50^{b}	15.41^{bc}

The values in a column followed by a common letter do not differ significantly in DMRT at 5% level.

Discussion

5. DISCUSSION

In Kerala, cocoa is usually grown under irrigation as an intercrop in coconut gardens. The vegetative and reproductive growth of cocoa is influenced by a complexity of environmental factors like rainfall, temperature and water stress. Rainfed cocoa is always at the risk of moisture stress. The moister stress is more pronounced in northern regions of Kerala and Coastal Karnataka, as the northeast monsoon is weak in these regions. Rainfed cocoa in these regions usually suffers because of moisture stress. Non-availability of water towards the end of summer exposes the plant to stress. In the present investigation, an attempt was made to identify the drought tolerant cocoa varieties suitable for drought prone areas, so that the crop can be introduced in more rainfed areas without sacrificing yield.

Six-month old seedlings and budlings of ten cocoa varieties and twenty promising accessions (Table 2) were subjected to a two-stage screening (preliminary and secondary screening) by imposing soil moisture stress. Field grown cocoa plants of the varieties/accessions selected for secondary screening were also monitored for important physiological characters. The results obtained from the investigation are discussed in this chapter.

5.1 PRELIMINARY SCREENING

Both seedlings and budlings of the selected 30-varieties/accessions were subjected to a preliminary screening. Observations on relative water content (RWC), dry weight fraction (DWF), percentage of dried leaves at 15 days after withholding water, and number of days took for complete drying were recorded. In general, varieties having higher RWC and DWF, lower percentage of dried leaves and longer duration of life during moisture stress could be treated as apparently tolerant varieties. From the preliminary screening, seven apparently tolerant and three apparently sensitive varieties were selected for secondary screening. Apparently sensitive varieties were included in the study for comparison.

Relative water content is the ratio of fresh weight of leaves to oven dry weight expressed as a percentage. RWC is an alternative measure of plant water status (Sinclair and Ludlow, 1985). Of the 30 varieties/accessions tested, the seedlings of M 13.12, GI 5.9, GII 19.5, GI 4.8, GI 10.3, GII 2.4 and GVI 50 had high RWC (above 80%) (Table 3), high DWF (above 0.5) (Table.4), low leaf drying percentage (less than 30%) and longer duration of life under water stress (more than 30 days). Therefore, they were treated as apparently tolerant varieties and those varieties having opposite characters were treated as apparently sensitive varieties. The varieties GVI 55, GVI 51 and GVI 61 were selected as apparently sensitive varieties. The varieties were selected as apparently tolerant and sensitive after doing DMRT (Duncan's Multiple Range Test) and cluster analysis.

The preliminary screening of budlings was done as in the case of seedlings. The apparently tolerant varieties were GII 12.3, GIII 4.1, GIV 2.5, GIV 32.5, GVI 50, GVI 51 and GVI 68. The apparently sensitive varieties were M 16.9, GI 4.8, and M 13.12.

The varieties selected under seedlings and budlings evaluation were different. As cocoa is a predominantly cross-pollinated crop, seedlings may not be expressing their parents' characters. This is evident from the contrasting results obtained here. The accessions, M 13.12 and GI 4.8, selected as apparently tolerant under seedlings found a place in the list of apparently sensitive varieties under budlings. Similarly, in the case of seedlings, the accession GVI 51 is sensitive to drought. However, in the case of budlings, it is tolerant. Therefore, secondary screening was conducted separately for seedlings and budlings.

5.2 SECONDARY SCREENING

Seedlings of seven apparently tolerant accessions (M 13.12, GI 5.9, GII 19.5, GI 4.8, GI 10.3, GII 20.4, GVI 50) and three apparently sensitive accessions (G VI 55, GVI 51,GVI 61) identified in the preliminary screening were subjected to a secondary screening under three moisture regimes of no irrigation (T_1), watering once in five days i.e. life saving irrigation (T_2), and regular watering (T_3). Observations on physiological parameters such as relative leaf water content, dry weight fraction, leaf drying percentage, number of days took for complete drying, stomatal conductance, transpiration rate, leaf water potential, chlorophyll 'a', 'b' and total chlorophyll, chlorophyll stability index, leaf area per seedling, membrane stability, relative injury; growth characters like plant height, collar girth, number of leaves and total biomass; biochemical characters such as proline and nitrate reductase activity were recorded.

The methodology of secondary screening was repeated in the case of budlings of seven apparent tolerant (GII 12.3, GIII 4.1, GIV 2.5, GIV 32.5, GVI 50, GVI 51 and GVI 68) and three sensitive varieties (M 16.9, GI 4.8, and M 13.12). The results of the experiments showed considerable differences between drought tolerant and sensitive varieties.

5.2.1 Physiological parameters

Relative leaf water content and dry weight fraction

High relative water content and dry weight fraction under water stress is a feature of drought-adapted plants. Helkvis *et al* (1974) related the dry weight ratio of leaf lamina to its turgid weight and drought tolerance and indicated the ability of the plant to tolerate drought. In cocoa seedlings, the RWC was high in the varieties GVI 61(87.52%), G II 20.4(85.62%) and GI 4.8 (75.08%). The variety, GI 5.9 showed the lowest RWC (43.45%) under stress. In GII 20.4, DWF was the highest (0.387).

In budlings, the variety GVI 68 showed the highest RLWC (26.98%) and DWF (0.3%) where as the variety GI 4.8 showed the lowest RLWC (14.25%) and DWF (0.31%).

Leaf drying percentage and number of days took for complete drying

In seedlings, leaf-drying percentage increased with increase in levels of moisture stress. However, in the case of budlings, there was no significant relation between moisture regimes and varieties in leaf drying percentage. The varieties having low leaf drying percentage may have tolerance to drought. Although the number of days took for complete drying was worked out in no irrigation treatments, there was no variation between varieties.

Transpiration rate

Water stress, in general, reduces transpiration rate of plants. In seedlings, at 11 hrs, transpiration rate was the highest in regular irrigation (47.05 $\mu g\ cm^{-2}s^{-1}$) and the lowest in no irrigation (20.16 $\mu g\ cm^{-2}s^{-1}$) (Fig 1). At 14 hrs also, the same trend was prevailing.

In the case of budlings too, similar results were observed. Drought tolerant varieties, in general, exhibit low transpiration rates. The variety GI 4.8 showed the lowest transpiration rate (37.33 $\mu g\ cm^{-2}s^{-1}$) and M 13.12 the highest transpiration rate (40.75 $\mu g\ cm^{-2}s^{-1}$). Balasimha *et al.,* (1988) reported 54 to 59 per cent decrease in transpiration under stress in drought tolerant accessions compared to plants under irrigation. As the severity of water stress increased, a decrease in transpiration rate was observed in all the varieties except M 13.12 (36.17 $\mu g\ cm^{-2}s^{-1}$) in regular irrigation where as in life saving irrigation, it was 38.08 $\mu g\ cm^{-2}s^{-1}$. It indicates the drought sensitive nature of M 13.12.

Stomatal conductance

Stomatal regulation is considered to be an important physiological function deciding the water stress tolerance of crop plants. Stomatal opening and closing is controlled by the turgor of both guard cells of stomata and other epidermal cells which in turn depends on the water stress tolerance of the species (Smart and Binghma, 1974). Stomatal conductance dropped with the increase in the levels of water stress in both seedlings and budlings.

In seedlings, there was no significant variation between varieties; whereas in budlings, GIV 2.5 showed low stomatal conductance (3.48 m mol $m^{-2}s^{-1}$) during stress. Stomatal conductance decreased as stress increased in GIV 2.5. It was 4.21 m mol $m^{-2}s^{-1}$ in regular irrigation and decreased to 2.99 m mol $m^{-2}s^{-1}$ in no irrigation; indicating its drought tolerant feature. In GVI 68, high stomatal conductance was observed in no irrigation indicating sensitivity to drought. Balasimha *et al* (1988) reported effective stomatal regulation in cacao clones NC-23, NC-29 and NC-31 resulting in decreased loss of transpiration water. Latha (1998) reported decreased stomatal conductance with the increase in reduction of water stress in cashew seedlings.

Leaf water potential

Leaf water potential is an important quantitative character used to assess water status of plants. Balasimha and Daniel (1988) identified leaf water potential as a rapid screening technique for drought tolerance in coconut. In the present study on cocoa seedlings, leaf water potential decreased with the increase in moisture stress, and it was the highest in plants under regular irrigation and the lowest in plants raised under life saving irrigation. The varieties G II 19.5 (-2.91 bars), G I 4.8 (-2.93 bars) and GVI 50 (-3.25 bars) showed high leaf water potential during water stress and GI 5.9 (-5.83 bars) showed low leaf water potential under water stress. Latha (1998) observed a decrease in water potential of cashew seedlings with increase in duration of water stress.

In the budlings of GVI 50, high leaf water potential was observed (-3.35 bars) followed by GII 12.3 (-3.66 bars). It showed their drought tolerant nature. In regularly irrigated plants, the leaf water potential was -3.78 bars where as in no irrigation, it was -4.67 bars.

Leaf area per plant

In cocoa seedlings, there were no significant differences in leaf area per plant due to moisture regimes. Seedlings of GI 4.8 had the highest leaf area (4224 cm^2) and the lowest with variety GVI 51 (4118 cm^2). However, in cocoa budlings, leaf area decreased as stress increased. The variety, GI 4.8 had the highest leaf area (794 cm^2) and the lowest by GVI 50 (738 cm^2). Shedding of leaves is a common phenomenon in plants to reduce transpiration rate to tide over drought. Reduction in leaf area under drought stress will reduce further transpiration losses, thus enhancing the survival ability of the plants (Subbarao *et al.*, 1995). Variation in total leaf area may result from changes in leaf number or in leaf size. Leaf number depends on the number of growing points, the length of time during which leaves are produced, the rate of leaf production during the period and the length of life of the leaves. Leaf size is determined by the number and size of the cells of which the leaf is built and is influenced by light, moisture regimes and the supply of nutrients (Arnon, 1975). Low leaf water potential also causes the loss of existing leaf area (Arnon, 1975; Begg and Turner, 1976).

Chlorophyll content

High chlorophyll content during water stress is a characteristic of drought tolerant plants. In seedlings, GI 4.8 (0.568 mg g^{-1} leaf tissue) and GVI 61 (0.545 mg g^{-1} leaf tissue) had high chlorophyll 'a'. Chlorophyll 'b' content was high in varieties GII 19.5 (0.657 mg g^{-1} leaf tissue) and GI 5.9 (0.655 mg g^{-1} leaf tissue) and the lowest in GII 20.4 (0.419 mg g^{-1} leaf tissue). Total chlorophyll was also high in GI 5.9 (1.329 mg g^{-1} leaf tissue). GII 19.5 (1.305 mg g^{-1} leaf tissue). The chlorophyll 'b' content decreased with increase in moisture stress in GII 20.4 from 0.531 mg g^{-1} leaf tissue to 0.515 mg g^{-1} leaf tissue and the lowest under life saving irrigation (0.212 mg g^{-1} leaf tissue). Balasimha *et al.*, (1998) reported that the chlorophyll content of cocoa accessions was low in plants under water stress when compared to irrigated plants.

Chlorophyll 'b' content decreased with the increase in levels of water stress in pepper (Thankamony, 2000). In the present study with cocoa budlings, GIV 32.5 showed high chlorophyll 'b' content (0.37 mg g^{-1} leaf tissue) followed by M 16.9 (0.31 mg g^{-1} leaf tissue) indicating their drought tolerant nature. The accession GII 12.3 had the lowest chlorophyll 'b' content (0.12 mg g^{-1} leaf tissue) indicating its sensitivity to drought.

Chlorophyll stability index

Chlorophyll stability index is a measure of the integrity of leaf membrane under stress condition. Chlorophyll stability under stress is one of the factors, which contribute drought tolerance in plants. In seedlings, CSI had no significant effects whereas in budlings, the accession MI 16.9 (55.84%) and GIV 32.5 (54.57%) had high CSI and M 13.12 had low CSI (33.79%).

Relative injury

According to Silva *etal.* (1974), the leaf membrane stability is disturbed due to moisture stress and stability is indirectly measured by relative injury. Low RI during stress is a measure of drought tolerance. Relative injury was high in GI 5.9 (22.18%) and GVI 50 (17.84%). GVI 51 showed low RI during stress, indicating drought tolerance. However, in cocoa budlings, there were no significant effects on RI.

Membrane stability

The membrane damage and solute leakage under stress are measures of cell membrane stability (Blum and Ebercon, 1981). Clarke and Mc Craig (1982b) used membrane stability to evaluate water stress tolerance in wheat. In cocoa seedlings, membrane stability showed no significant relation between varieties, whereas in budlings GIV 32.5 had the highest membrane stability (0.56).

5.2.2 Growth characters

In both cocoa seedlings and budlings, the growth characters such as height, girth, number of leaves and total biomass decreased during water stress. Water deficit is likely to affect the two vital processes of growth, viz; cell division and cell enlargement, and according to Begg and Turner (1976) cell enlargement is more affected resulting in poor growth. According to Arnon (1975) growth is suspended during moisture stress and resumed upon its elimination. Several workers reported reduction in growth when there is a deficient supply in moisture.

5.2.3 Biochemical characters

Proline

Proline accumulation during water stress is a drought adaptive mechanism (Kramer, 1983). An increase in proline content by water stress has been suggested as a test of resistance to water stress (Gupta, 1997).

In seedlings, proline content was high in GVI 51 (369.7 $\mu g\ g^{-1}$ leaf) and M 13.12 (366 $\mu g\ g^{-1}$ leaf). In severe water stress, i.e., under no irrigation, GVI 51showed the highest proline accumulation (463$\mu g\ g^{-1}$ leaf). Plants accumulate osmotically active organic solute in free or combined form when exposed to environmental stress. According to Gardner *et al* (1985) under moisture stress, proline increases in concentration more than any other amino acid. Proline seems to act as a storage pool for nitrogen and or as solute molecule reducing the solute potential of the cytoplasm. Blum and Ebercon (1976) suggested that proline accumulation in water stressed leaves might provide a source of respiration energy to the recovering plant.

In budlings, proline accumulated under water stress. M 13.12 had the highest proline content under stress (516.86 $\mu g\ g^{-1}$ leaf). In cocoa, water stress increased the proline content.

Nitrate reductase activity

Nitrate reductase is the key enzyme, which catalyses the reduction of nitrate to nitrite, the first steps in nitrate assimilation by plants (Bhaskar, 1997). In cocoa seedlings, NR activity of leaves differed considerably between moisture regimes. The NRA was the highest in plants grown under regular watering (27.17 m mol NO^2 per gram per two hour at 30^0 C).

In budlings, NRA of leaves decreased under stress. The highest NRA under no irrigation was showed by GVI 50 (18.90 m mol NO^2 per gram per two hour at 30^0 C) and the lowest by GII 12.3 (8.75 m mol NO^2 per gram per two hour at 30^0 C). A decrease in NRA at stress condition was reported in many crops; rice (Sairam and Dube, 1984; Deka and Baruah (1994)); cluster beans (Garg *et al*, 1998); maize Foyer *et al* (1998), and wheat Yadav *et al* (1998).

5.3 FIELD MONITORING OF COCOA TREES.

Apart from observing various parameters on selected cocoa seedlings and budlings, attempts were also made to monitor field-grown plants of the above varieties/accessions selected from primary screening. Variation in important characters were monitored by recording the data on transpiration rate, stomatal conductance, leaf water potential, stomatal frequency, leaf thickness, bark thickness and soil moisture content during summer in 16-year old cocoa trees. Cocoa trees raised from both seedlings and budlings were monitored.

Low transpiration rate, low stomatal conductance, high leaf water potential, high stomatal frequency, high leaf thickness, high bark thickness and high soil moisture content are the features of drought tolerant plants. The varieties GVI 61, M 13.12 and GI 4.8 showed these features indicating their ability to tolerate drought. The variety GVI 51 was found to be sensitive to drought.

In the group of varieties selected under budlings, low transpiration rate, low stomatal conductance, high leaf water potential, high stomatal frequency, high leaf thickness, high bark thickness and high soil moisture content were observed in M 13.12 and GI 4.8, indicating their drought tolerant nature. The varieties, GVI 51 and GIII 4.1 showed sensitivity to moisture stress.

Two varieties showing tolerance to drought, M 13.12 and GI 4.8, were common to both seedlings and budlings. The variety GVI 51,included both in the group of seedlings and budlings, showed sensitivity to stress. However, the results of the secondary screening of accessions gave some contrasting results. The accessions, M 13.12 and GI 4.8, selected as apparently tolerant under seedlings were evaluated as apparently sensitive varieties under budlings. Similarly, in the case of seedlings, the accession GVI 51 is sensitive to drought. However, in the case of budlings, it is tolerant. The variation in the results might be due to the segregation in seedlings. Similarly, the effects of rootstock on the performance of budlings cannot be ruled out. Rootstocks are raised from seedlings and uniformity in genetic stock cannot be ensured.

5.4 CLUSTER ANALYSIS

In general, the varieties with high RWC, DWF, total chlorophyll content, leaf water potential and proline content; low stomatal conductance, transpiration rate and leaf area during water stress can be considered as drought tolerant and with opposite characters as drought sensitive. However, the superiority or inferiority of a variety in one character alone will not qualify it to be classified as a drought tolerant one. To arrive at meaningful conclusions, ranking based on all the relevant characters has to be conducted. Therefore, from the various drought tolerant characters observed, Non-hierarchical Euclidean Cluster Analysis (Chat field, and Collins,1980)) was done for selection of drought tolerant and sensitive varieties.

The parameters selected for clustering were relative leaf water content, dry weight fraction, stomatal conductance, transpiration rate, leaf area per seedlings, total chlorophyll content, leaf water potential and proline content. The varieties with high RWC, DWF, total chlorophyll content, leaf water potential and proline content; low stomatal conductance, transpiration rate and leaf area during water stress is considered as drought tolerant and with opposite characters as drought sensitive.

Based on these criteria, in seedlings; the varieties M 13.12, GII 19.5 and GVI 55 were ranked as moisture stress tolerant and GVI 61, GVI 50 and GI 4.8 as sensitive under no irrigation (Plate 3 and 4).

In budlings, the varieties GI 4.8 and GII 19.5 were selected as drought tolerant and GIII 4.1, GVI 51 and GII 12.3 as drought sensitive under no irrigation (T_1).

The variety M13.12 showed drought tolerant features in secondary screening of seedlings and field performance. The variety M 13.12 (CCRP-2) is a promising variety released from Kerala Agricultural University. The variety GVI 51, which showed sensitivity to moisture stress in seedlings (but tolerant in budlings) under secondary screening was showing similar behavior of sensitivity in field grown plants. However, final conclusions are difficult to be drawn as only 10 accessions were subjected to secondary screening and field monitoring. Moreover, the accessions, M 13.12 and GI 4.8, selected as apparently tolerant under seedlings found a place in the list of apparently sensitive varieties under budlings. Similarly, in the case of seedlings, the accession GVI 51 is sensitive to drought. However, in the case of budlings, it is tolerant. The variation in the results might be due to the segregation in seedlings. As cocoa is a predominantly cross-pollinated crop, seedlings may not be expressing their parents' characters. Similarly, the effects of rootstock on the performance of budlings cannot be ruled out. Rootstocks are raised from seedlings and uniformity in genetic stock cannot be ensured. In the light of the results obtained, screening trails involving more number of varieties/accessions has to be conducted and monitored for more number of years to get consistent values.

T_1 - No irrigation

Plate 3.effect of water stress on 10 cocoa varieties at 30 days after withholding water [secondary screening of seedlings]

Note the potential of G II 19.5 to tolerate drought

T_2 - Life saving irrigation.

Plate 4. Effect of water stress on 10 cocoa varieties at 30 days after withholding water [secondary screening of seedlings] M 13.12 and G VI 61 shows better growth.

Summary

6. SUMMARY

Experiments were conducted at the College of Horticulture, Vellanikkara to investigate the drought tolerant characteristics of the most promising cocoa varieties and accessions. It is hoped that by identifying cocoa cultivars/accessions that are able to tide over the drought period, the crop can be introduced into more rainfed areas without sacrificing yield. The investigation involved a two-stage screening of cocoa seedlings and budlings and monitoring of their field grown plants. As the first step, a preliminary screening was done on ten cocoa cultivars released from Kerala Agricultural University and twenty promising accessions A secondary screening of cocoa cultivars/accessions selected from the preliminary screening was then conducted. Simultaneously, field monitoring of cocoa trees of the varieties selected from the primary screening was also done.

In the primary screening, both seedlings and budlings of the selected 30-varieties/ accessions were evaluated after withholding water for relative leaf water content (RLWC), dry weight fraction (DWF), percentage of dried leaves and duration of life. Those varieties having higher RLWC and DWF, lower percentage of dried leaves and longer duration of life is treated as apparently tolerant and with opposite characters as apparently sensitive varieties to moisture stress. Among the seedlings, the varieties, M 13.12, GI 5.9, GII 19.5, GI 4.8, GI 10.3, GII 2.4 and GVI 50, were selected after conducting a cluster analysis as apparently tolerant and GVI 55, GVI 51 and GVI 61 as apparently sensitive varieties. Among the group of cocoa budlings, the varieties, GII 12.3, GII 4.1, GIV 2.5, GIV 32.5, GVI 50, GVI 51 and GVI 68, were selected as apparently tolerant and M 16.9, GI 4.8, and M 13.12 apparently sensitive to moisture stress.

The apparently tolerant and sensitive varieties/accessions identified in the preliminary screening were subjected to a secondary screening under three moisture regimes of no irrigation, watering once in five days (life saving irrigation), and regular watering. Observations on physiological parameters such as relative leaf water content, dry weight fraction, leaf drying percentage, number of days took for complete drying, stomatal conductance, transpiration rate, leaf water potential, chlorophyll 'a', 'b' and total chlorophyll, chlorophyll stability index, leaf area per seedling, membrane stability, relative injury; growth characters like plant height, collar girth, number of leaves and total biomass; biochemical characters such as proline content and nitrate reductase activity were recorded.

In both cocoa seedlings and budlings, RLWC and DWF decreased under stress. In seedlings, RLWC was high in GVI 61 (87.52%), G II 20.4 (85.62%) and GI 4.8 (75.08%). The variety GII 20.4 showed the highest DWF (0.387). In budlings, the variety GVI 68 showed the highest RLWC (26.98%) and DWF (0.3%).

Leaf drying percentage increased with the increase in moisture stress. However, in budlings, no significant effects could be noticed on leaf drying percentage due to moisture regimes or varieties. Although the number of days taken for complete drying was recorded under no irrigation, there were no significant differences between varieties.

Water stress, in general, reduced the transpiration rate of plants. In both seedlings and budlings, transpiration rate was the highest in regular irrigation. Varietal variation was also found to be significant.

Leaf water potential decreased with the increase in moisture stress in seedlings. The varieties G II 19.5 (-2.91 bars), G I 4.8 (-2.93 bars) and G VI 50 (-3.25 bars) showed high leaf water potential during water stress. In the group of budlings, GVI 50 showed high leaf water potential (-3.35 bars).

With respect to leaf area per plant, there were no significant differences due to moisture regimes in cocoa seedlings. Seedlings of GI 4.8 had the highest leaf area (4224 cm^2). However, in cocoa budlings, leaf area decreased as stress increased. The variety, GI 4.8 had the highest leaf area (794 cm^2).

High leaf chlorophyll content was observed under water stress. In seedlings, GI 4.8 (0.568 mg g^{-1} leaf tissue) and GVI 61 (0.545 mg g^{-1} leaf tissue) had high chlorophyll 'a'. Chlorophyll 'b' content was high in varieties GII 19.5 (0.657 mg g^{-1} leaf tissue) and GI 5.9 (0.655 mg g^{-1} leaf tissue) and the lowest in GII 20.4 (0.419 mg g^{-1} leaf tissue). Total chlorophyll was also high in GI 5.9 (1.329 mg g^{-1} leaf tissue), GII 19.5 (1.305 mg g^{-1} leaf tissue). In budlings, GIV 32.5 showed high chlorophyll 'b' content (0.37 mg g^{-1} leaf tissue) followed by M 16.9 (0.31 mg g^{-1} leaf tissue). The accession GII 12.3 had the lowest chlorophyll 'b' content (0.12 mg g^{-1} leaf tissue). Although chlorophyll stability index, another factor that contribute drought tolerance in plants, did not show significant effects in seedlings, in budlings, the accession MI 16.9 (55.84%) and GIV 32.5 (54.57%) showed high CSI and M 13.12 low CSI (33.79%).

Low relative injury (RI) during stress is also noticed. Relative injury was high in GI 5.9 (22.18%) and GVI 50 (17.84%). GVI 51 showed low RI during stress. However, in budlings, there were not much effects. In cocoa seedlings, membrane stability showed no significant relation between varieties, whereas in budlings, GIV 32.5 had the highest membrane stability.

In both cocoa seedlings and budlings, the growth characters such as height, girth, number of leaves and total biomass decreased during water stress.

The amino acid, proline accumulated under water stress in both seedlings and budlings. In seedlings, proline content was high in GVI 51 (369.7 µg g^{-1} leaf) and M 13.12 (366 µg g^{-1} leaf). Under severe water stress, i.e., under no irrigation, GVI 51showed the highest proline accumulation (463µg g^{-1} leaf). In budlings, M 13.12 had the highest proline content under stress (516.86 µg g^{-1} leaf).

In cocoa seedlings, nitrate reductase activity of leaves differed considerably between moisture regimes .The NRA was the highest in plants grown under regular watering (27.17 m mol NO^2 per gram per two hour at 30^0 C). In budlings, NRA of leaves decreased under stress. The highest NRA under no irrigation was shown by GVI 50 (18.90 m mol NO^2 per gram per two hour at 30^0 C) and the lowest by GII 12.3 (8.75 m mol NO^2 per gram per two hour at 30^0 C).

In general, the varieties with high RLWC, DWF, total chlorophyll content, leaf water potential and proline content, low stomatal conductance, transpiration rate and leaf area during water stress can be considered as drought tolerant and with opposite characters as drought sensitive. Based on these criteria, in seedlings; the varieties M 13.12, GII 19.5 and GVI 55 were ranked as moisture stress tolerant and GVI 61, GVI50 and GI 4.8 as sensitive under no irrigation. In budlings, the varieties GI 4.8 and GII 19.5 showed drought tolerant features and GIII 4.1, GVI 51 and GII 12.3 drought sensitive features.

Attempts were also made to monitor field grown plants of the varieties/accessions selected from primary screening. Variation in important characters were monitored by recording the data on transpiration rate, stomatal conductance, leaf water potential, stomatal frequency, leaf thickness, bark thickness and soil moisture content during summer in 16-year old cocoa trees. Low transpiration rate, low stomatal conductance, high leaf water potential, high stomatal frequency, high leaf thickness, and high bark thickness are the features of drought tolerant plants. The varieties GVI 61, M 13.12 and GI 4.8 showed these features indicating their ability to tolerate drought. According to this criterion, the variety GVI 51 was found to be sensitive to drought. In the group of varieties selected under budlings, low transpiration rate, low stomatal conductance, high leaf water potential, high stomatal frequency, high leaf thickness, and high bark thickness were observed in M 13.12 and GI 4.8, indicating their drought tolerant nature. The varieties, GVI 51 and GIII 4.1 showed sensitivity to moisture stress.

The accession M13.12 (CCRP2) showed drought tolerant features in secondary screening of seedlings and field performance. The accession GVI 51, which showed sensitivity to moisture stress in seedlings (but tolerant in budlings) under secondary screening, was showing similar behavior of sensitivity in field grown plants.

The study shows the possibility of exploiting drought tolerant features of cocoa varieties/accessions. However, final conclusions are difficult to be drawn as only 10 accessions were subjected to secondary screening and field monitoring, and data for only one year were collected. In the light of the promising results obtained, screening trails involving more number of varieties/accessions have to be conducted and monitored for more number of years to get consistent values.

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* Originals not seen

Appendix

APPENDIX 1

Weather data during the period of experiment

YEAR	MONTH	AIR TEMPERATURE		RAINFALL	EVAPORATION	SUNSHINE HOURS	MEAN RH(%)
		MAX	MIN	(M.M)	(M.M)	PER DAY	
2003	January	33.9	22.6	23.6	5.5	8.7	39
	February	33.8	23.3	70.0	4.7	8.7	91
	March	35.1	24.9	1.0	4.7	8.1	59
	April	33.7	24.7	8.0	4.9	5.9	58
	May	34.7	25.7	0.2	5.9	7.2	50
	June	29.7	22.7	46.9	3.2	2.9	75
	July	31.1	23.6	25.0	4.4	4.0	69
	August	29.8	22.7	144.4	25.4	4.5	75
	September	32.0	22.9	2.8	5.1	2.2	62
	October	32.2	23.4	142	4.6	8.1	60
	November	31.1	23.4	0.0	5.5	2.2	58
	December	32.9	22.9	0.0	6.2	9.3	48
2004	January	34.5	22.8	0.0	5.7	9.4	41
	February	36.5	22.5	0.0	7.0	9.7	35
	March	39.1	24.5	8.6	5.7	7.6	50
	April	31.6	23.8	242.5	3.4	2.3	71
	May	30.3	24	79.4	3.5	3.7	24
	June	29.8	22.9	118.2	3.8	5.0	64
	July	28.6	22.7	242.0	3.0	1.6	80
	August	30.5	23.5	0.2	4.6	7.2	65
	September	31.9	23.4	51.8	3.6	5.0	70

IDENTIFICATION OF DROUGHT TOLERANT COCOA TYPES

By

BINIMOL B.

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Science in Agriculture

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ABSTRACT

Experiments were conducted at the college of Horticulture, Vellanikkara to investigate the drought tolerant characteristics of the most promising cocoa varieties and accessions. The investigation involved a two-stage screening of cocoa seedlings and budlings and monitoring of their field grown plants. As the first step, a preliminary screening was done on six month old seedlings and budlings of ten cocoa cultivars released from Kerala Agricultural University and twenty promising accessions. A secondary screening of cocoa cultivars/accessions selected from the preliminary screening was then conducted. Simultaneously, field monitoring of cocoa trees of the varieties selected from the primary screening was also done.

From the preliminary screening, seven apparently tolerant varieties were selected for secondary screening in both seedlings and budlings based on higher RWC and DWF, lower percentage of dried leaves and longer duration of life under during moisture stress. Three apparently sensitive verities were also selected based on opposite values of the above characters. Varieties identified in the preliminary screening were subjected to a secondary screening under three moisture regimes of no irrigation , watering once in five days (life saving irrigation), and regular watering. Observations on physiological parameters such as relative leaf water content, dry weight fraction, leaf drying percentage, number of days taken for complete drying, stomatal conductance, transpiration rate, leaf water potential, chlorophyll 'a', 'b' and total chlorophyll, chlorophyll stability index, leaf area per seedling, membrane stability, relative injury; growth characters like plant height, collar girth, number of leaves and total biomass; biochemical characters such as proline content and nitrate reductase activity were recorded.

After conducting a cluster analysis, the varieties with high RWC, DWF, total chlorophyll content, leaf water potential and proline content; low stomatal conductance, transpiration rate and leaf area during water stress were rated as drought tolerant and with opposite characters as drought sensitive. Based on these, in

seedlings; the varieties M 13.12, GII 19.5 and GVI 55 were ranked as moisture stress tolerant and GVI 61, GVI 50 and GI 4.8 as sensitive under no irrigation. In budlings, the varieties GI 4.8 and GII 19.5 were selected as drought tolerant and GIII 4.1, GVI 51 and GII 12.3 as drought sensitive under no irrigation

Important characters were also monitored in 16-year old clonal trees of the seedlings/budlings of the varieties selected from primary screening. Transpiration rate, stomatal conductance, leaf water potential, stomatal frequency, leaf thickness, bark thickness and soil moisture content were recorded during summer. The varieties GVI 61, M 13.12 and GI 4.8 showed drought tolerant features. The accession M13.12 (CCRP2) showed drought tolerant features in secondary screening of seedlings and field performance. The accession GVI 51, which showed sensitivity to moisture stress in seedlings (but tolerant in budlings) under secondary screening, was showing similar behavior of sensitivity in field grown plants.

The study shows the possibility of exploiting drought tolerant features of cocoa varieties/accessions. However, final conclusions are difficult to be drawn as only 10 accessions were subjected to secondary screening and field monitoring, and data for only one year were collected. In the light of the promising results obtained, screening trails involving more number of varieties/accessions have to be conducted and monitored for more number of years to get consistent values.