

REVIEW ARTICLE

STRESS PHYSIOLOGY OF COCOA

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In India, plantation crops are grown in regions receiving high rainfall (3000-4000 mm/annum) during monsoon. However, this is followed by long periods of drought which varies in intensity at different regions. Soil and water deficits limit yield of crops in many regions. In Southern India, cocoa is cultivated as a mixed crop with arecanut and coconut. During the summer months the weather is dry for 3-5 months with high evaporative demand resulting in soil and atmospheric drought of varying degrees of severity. Arecanut to a considerable extent and coconut on a limited scale are raised as irrigated crops. However, non-availability of water towards late summer season makes it difficult to cultivate mixed crops. In order to develop drought tolerant lines in cocoa, detailed physiological studies to understand basic mechanisms of tolerance have been carried out at this Regional Station. The paper reviews the research work done here and elsewhere.

Effect of weather variables

An increase in evaporative demand owing to lowered humidity changes stomatal resistance. Besides, light and temperature also influence stomatal resistance. The stomatal diffusion resistance increased with decrease in

relative humidity which was magnified by soil moisture stress in field grown cocoa trees (Balasimha and Rajagopal, 1988). With higher relative humidity (RH) the leaf water potential was maintained at higher values. Stomata of intact leaves generally open in response to increase in light intensity. In cocoa leaves, however, the effects of RH and soil moisture stress decreased the effect of light. It indicates that RH has stronger influence than light. The correlation coefficients among various environmental factors and plant responses indicate that ambient temperature did not influence stomatal responses but showed highly significant correlation with leaf temperature. Stomatal regulation by internal (stress) and external factors (RH) lead to decrease in transpiration, which was a major adaptation for water conservation.

The climatic conditions in West African countries are similar to those prevailing in Southern India with two main rainy periods viz., March to July and September to December (Asomaning, 1976). In marked contrast to this, the rainfall in Brazil is very well distributed throughout the year. This difference in climate accounts for a bimodal crop pattern in Brazil in contrast to that of single main crop in

Africa (Alvim, 1976). The sustained water supply obviously helps in continuous flowering and fruiting throughout the year. Rainfall is also well distributed in other Latin American countries, Malaysia and Sri Lanka. High correlations between rainfall and yield have been reported (Gordon, 1976). Other climatic factors such as temperature, light intensity and day length normally are not limiting factors for cocoa yields except in Brazil which experiences nearly four months of low temperature during winter (Alvim, 1976).

The cocoa tree needs a high and well distributed rainfall, possibly with a short dry spell to stimulate flowering. Two dissimilar crop patterns are observed under rainfed and irrigated conditions in India. Correlations of yield of cocoa with various weather variables showed that number of rainy days of previous year, sunshine hour of current year, maximum temperature (4th fortnight and 21st fortnight) of current year were found significant (Vijayakumar *et al.* 1991).

Partitioning of dry matter is affected by environmental variables, especially rainfall. Variations in bearing trees from year to year can be explained from moisture availability (Glendinning, 1966; Hutcheon, 1977). Seasonal changes in stem diameter are useful indicators of internal stress (Hutcheon, 1977).

Water relations and stomatal regulation

Cocoa is very sensitive to water stress. Drought affects several physiological processes leading to a reduction in crop yield. Water potential (WP) of leaf is a major quantitative character used to assess water stress. Leaf water potential was measured

using Scholander's pressure chamber (Soil Moisture Equipments Corp., USA). Cocoa plants show changes in water relations when soil moisture drops to 60-70 per cent of available range. The onset of drought decreased water potential and relative water content (Balasimha 1982a, 1983, 1987). Diurnal variations exist in water potential, however, a plateau WP is reached once the stomata close beyond - 1.5 MPa (Balasimha *et al.*, 1990). This is the stage when turgor pressure falls and leaves start wilting. The changes in osmotic potential of cocoa during different months were not as marked as water potential (Balasimha, 1988). Leaf water potential was appreciably lower in exposed leaves as compared to shaded ones, presumably due to higher temperature and lower RH in exposed area.

The moisture stress results in accumulation of abscisic acid (ABA) in leaves (Alvim *et al.*, 1974). The accumulation of ABA was related to drought tolerance among cocoa accessions (Balasimha and Anil Kumar, 1998).

Seasonal changes in nitrate reductase and other indicators of water stress

The relative water content (RWC) of leaves of rainfed cocoa plants was lower than those of irrigated plants. This tended to decrease in both conditions with progress of summer months. The content of proline in leaves was maintained at similar levels in irrigated plants, while there was considerable accumulation in rainfed plants during the dry period. With the onset of rains proline decreased. The nitrate reductase (NR) activity was high during February-April in irrigated plants and low during rainy season,

whereas, in rainfed plants, the activity was low during the dry period. The decrease in NR in rainfed plants is an adaptation to stress conditions. Since NR is light inducible, the reduction during monsoon is due mainly to low light levels. When seasonal changes in NR were studied among tolerant and susceptible trees, the activity was higher in the former during summer months. However, the activities did not vary appreciably among the accession types (Balasimha, 1982a, 1982b).

Morphological adaptation

The reduction of cell growth is one of the earliest manifestation of water deficits. This is considered to be more sensitive to water stress than stomatal conductance and CO₂ assimilation. A regression model has been developed to predict leaf expansion rates in cocoa leaves for predicting the effect of water stress (Joly and Hahn, 1989a). The reduction in leaf area helps plants to adopt to periods of drought. A reduction in leaf area might have an advantage in reducing transpiration. However, once leaf expansion is complete, an increase in epicuticular waxes (EW) of leaves which can reduce cuticular transpiration is beneficial.

In cocoa accessions there was no significant differences in leaf area under watered conditions (Balasimha *et al.*, 1985). However, under water stress, the accessions showed variations in leaf expansion rates (Balasimha, 1982b).

The specific leaf weight and EW content were significantly different among cocoa accessions. Accessions having high specific leaf weight (SLW) and higher EW were found to be drought tolerant (Balasimha, 1987, Balasimha, *et al.*, 1985). Leaf anatomical characters of some cocoa accessions have also been

studied. The SLW can be a good indicator of leaf anatomy and positive relation between leaf thickness and palisade layer thickness were obtained. Using some of the morphological attributes it was possible to screen out 80 per cent of test material in the initial stages for drought tolerance.

Comparative drought tolerance

The water relation components and stomatal behaviour were determined in 14 accessions of cocoa for detailed analysis after a preliminary screening. Stomatal resistance and transpiration were measured using LI-1600 steady state porometer (Li-COR INC., USA). Differences in plant variables between drought tolerant and susceptible accessions is presented (Balasimha *et al.*, 1988). Based on parametric relationships with drought tolerance, the rank sums of these accessions led to a selection of NC23, NC29, NC31, NC39 and NC42. These drought tolerant accessions had effective stomatal regulation resulting in decreased transpiration water loss. There was a decrease in osmotic potential and NR and an increase in soluble sugars was noticed in respect to stress. The stability of NR during summer season was higher in tolerant than susceptible trees both in leaf and root. The r_s was negatively correlated with transpiration. The cocoa accessions with higher drought tolerance characteristics and yield potential under drought should be useful material for selective breeding.

Screening germplasm holding and hybridization

After evaluating the mechanism of drought tolerance in cocoa it was found that as a result of reduced transpiration, leaf turgidity was maintained thereby giving higher water potential. A rapid

screening method for drought tolerance in cocoa has been described which eliminated difficulty of using field plants. This method utilizes measurements of water potential in excised leaves. The decrease in water potential was more pronounced in susceptible as compared to tolerant accessions under induced stress (Balasimha and Daniel, 1988).

Sixty five cocoa accessions have been so far screened for drought tolerance. Five have been found to be drought tolerant as mentioned above. The dendrogram of some of these accessions has been constructed (Anil Kumar and Balasimha, unpublished).

Four high yielding trees and three drought tolerant trees were used for crossing to combine these characters in the hybrids. High yielding trees viz., I-14 (Red axil), I-21, I-29 (Amel x Na33 and II-67 (Landas 363) were used as female parents, while NC23/43 (P3 x P), NC 29/66 (P6 x P4) and NC42/94 (T86/2) were used as pollen parents. Nine F₁ hybrids raised from these cross combinations were screened for drought tolerance by rapid screening technique. The parents were also screened for comparison. The seedlings were planted in the field on a replicated randomized block design. Measurements of leaf water potential during dry periods was made every year starting from 1992. The seedling screening and field evaluation showed that hybrids I-21 x NC 42/94 and I-29 x NC 23/43 retained higher water potential and stomatal resistance indicating their drought tolerance (Balasimha *et al.*, unpublished). Photosynthetic parameters were also measured in these hybrids and parents in the field. During stress period stomatal conductance was lowered while transpiration was

reduced (Anil Kumar and Balasimha, 1997).

Photosynthesis

The efficient stomatal closure might hamper carbon assimilation. It was therefore, necessary to study photosynthesis and yield performance of tolerant accessions vis-a-vis susceptible ones. Annual pod yield was higher in tolerant accessions than in susceptible ones studied over a period of five years. Since the stomata resume their original open condition immediately after stress is relieved, it is reasonable to assume that stomatal closure did not affect productivity. This is corroborated by the lack of correlation between yield and r_s due to stress ($r=0.04$). It is also of interest to note that there was a positive correlation to yield with r_s at recovery ($r = 0.52$, $P 0.05$). Therefore, accessions which display these specific favourable attributes can be used as source for breeding to bring desirable characters into a single ideotype with a good expectation of increasing drought tolerance (Balasimha *et al.*, 1988). Thus, the stomatal responses are primary events in affecting the photosynthesis. Influence of environmental factors on photosynthesis in cocoa accessions were studied (Balasimha *et al.*, 1991). Measurements of microclimatic and photosynthetic variables were made using LI-6200 portable photosynthesis system (LICOR Inc, USA). Net photosynthesis (P_N), transpiration and stomatal conductance showed significant seasonal variations. During periods of low vapour pressure deficit (VPD), P_N was highest. The drought tolerant accessions maintained higher leaf WP. There was no significant difference in P_N between accession types. The depression in P_N was

principally due to lowered stomatal conductance. Because of such linear relationships of P_N to g_s , ratio of CO_2 internal/ CO_2 ambient remained relatively constant despite seasonal and accession type variations (0.80 to 0.88). This type of relationship is rarely measured in the field. There was also no significant difference in internal CO_2 showing that it did not limit net assimilation rate. The P_N/g_s ratio increased during dry months accompanied by a decrease in internal CO_2 . Similar decrease in CO_2 assimilation in seedlings have been reported (Deng *et al.*, 1990; Joly and Hahn, 1989b). Daily carbon balances of individual leaves of cocoa seedlings were decreased with reduced leaf water potentials (Deng *et al.*, 1990). The instantaneous water use efficiency (P_N/E) was positively correlated with P_N and it was higher in drought tolerant trees. The P_N/C_i ratio decreased in dry season and was positively correlated with P_N/E . Thus, high P_N/C_i with low g_s and E during stress period may be an important contributing factor to drought tolerance in cocoa.

Diurnal patterns of P_N and related parameters were studied in the months of December and April. P_N and g_s were highest at 10:30 h and declined towards midday and afternoon. The stressed plants had lower P_N and g_s values. The lowest value of WP was recorded at midday. At midday highest levels of PAR and VPD were recorded. The decrease in g_s was more pronounced in April when both soil and atmospheric moisture was low and demand for water was high. Thus, there was depression in P_N under transient water stress.

Stomata normally close in response to increasing VPD. The responses of P_N become even clearer when it is plotted

against VPD. Maximum P_N was recorded upto a VPD of 1.8 kPa, thereafter P_N declined. Temperature also had an effect on P_N , being optimum at 31-33°C which is characteristic of tropical crops grown in warm, less arid tropics. Light saturation of P_N occurred at a PAR of 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$. However, high light intensity decreased P_N under water stress conditions. The relationship of environmental variable and P_N in cocoa clearly demonstrate that the latter is influenced by both soil and atmospheric droughts.

Chlorophyll fluorescence

Chlorophyll fluorescence parameters were studied in cocoa accessions belonging to drought tolerant and susceptible types. The fluorescence parameters were measured in the plant efficiency analyser (Hansatech, UK). Chlorophyll fluorescence showed significant differences at seasons and between accession types and leaf ontogeny (Balasimha, 1992; Balasimha and Daniel, 1994, 1995). The F_v values were lower during drier months (March to May) as compared to other months. The F_o was significantly higher in susceptible accessions showing that PSII was affected to a greater extent. However, F_M and F_v were lower in tolerant types. Correlations between P_N and fluorescence quenching parameters were done to know the nature of relationships. The fluorescence parameter viz., F/F_M can be used to estimate quantum yield of non-cyclic electron transport and derived as $F_M - F_S/F_M$. These values were found to be directly proportional to fluorescence quenching (qQ) in cocoa. There was also a linear correlation of P_N with PQ. Thus, the data demonstrate that the P_N shows linear relations with F_o/F_M and qQ.

These values can be used for routine measurement of photosynthetic performance of leaves.

The application of chlorophyll fluorescence as a tool to screen cocoa for drought tolerance was investigated. Excised leaves were incubated in PEG 4000 (-1, 0 Mpa) solution either in low or high light conditions for 2 h. The incubation in low light ($80 \mu\text{mol m}^{-2}\text{s}^{-1}$) did not change F_o/F_M ratios in accession types. Under high light incubation ($1690 \mu\text{mol m}^{-2}\text{s}^{-1}$), F_o value increased in susceptible leaves. There were also changes in F_V/F_M ratios which decreased when incubated with either PEG or air drying. These changes were more pronounced in susceptible leaves than in tolerant ones. Under PEG stress WP was higher in tolerant as compared to susceptible leaves. Due to heat stress (47°C) treatments in excised leaves F_o values increased, while F_M , F_V and F_V/F_M decreased significantly. This increase was more in susceptible trees. The higher F_V/F_M ratio in tolerant cocoa under heat stressed conditions indicate that reduction of quantum yield of PSII was to a lesser extent. The Hill activity in isolated chloroplasts showed a similar trend. Thus the over all efficiency of PSII was affected more in susceptible leaves. The results suggest that relative chlorophyll fluorescence can be used for screening cocoa drought tolerance (Balasimha and Namboothiri, 1996).

Epicuticular waxes, lipids and membrane stability

Epicuticular wax was extracted in chloroform and determined spectrophotometrically. Wax components were separated on TLC using suitable solvent systems. Lipids were extracted and separated by column chromatography.

Epicuticular wax increases due to stress and helps the plants to withstand drought. Waxes accounted for 0.4-6.8 per cent as total leaf dry weight. The EW increased due to stress and tolerant accessions showed higher levels. Waxes contained hydrocarbons, esters, ketones, alcohols, free fatty acids and two unidentified components. No qualitative differences were observed between accession types. Lipids constituted 0.62 to 1.04 per cent of total leaf fresh weight. Water stress in April increased total lipid content to an extent of 20 per cent in tolerant and 30 per cent in susceptible accessions. Polar lipids fraction decreased while neutral lipids increased due to stress. The main fatty acids of lipid fractions were myristic, palmitoleic, oleic, linoleic and linolenic acids (Bhat *et al.*, 1990).

The activities of peroxidase and superoxide dismutase were studied in cocoa accession types. The peroxidase activity remained almost constant with decreasing WP in tolerant while it increased in susceptible trees. There was also a higher stability of SOD in tolerant trees. The lipid peroxidation was lower in tolerant trees both during pre-stress and stress conditions. These changes, along with lipid fraction ratios recorded and lower electrolytic leaching demonstrate the increased membrane stability.

Effect of stress on seedlings

Cocoa seedlings of drought tolerant and susceptible accessions were subjected to water stress. Drought caused decrease in water potential to a greater extent in susceptible as compared to tolerant accessions. This was accompanied by increased activities of phosphatase and amylase. Starch, sugar, phenol and protein did not show any significant differences. The total

soluble protein content also remained unchanged, but amino acid content increased in tolerant trees. There was no change in neutral and glycolipids, while phospholipids increased, the change being significant in tolerant accessions. The changes in EW, lipids and electrolytic leaching in seedlings revealed that tolerant ones had higher ECW and membrane stability (Bhat, 1988).

The early growth of seedlings is very important for better establishment and higher yield potential at maturity. Progenies which are more vigorous have capacity for high yield but it depends on effective dry matter partitioning between pod production and vegetative growth. Antitranspirants can reduce the transplant shock during field planting of seedlings from nursery. The application of cycocel and abscisic acid imparted tolerance under drought in seedlings as shown by growth and metabolic amelioration (Balasimha and Subramonian, 1984). The application of potassium and proline externally also ameliorated nitrate metabolism in cocoa seedlings under water stress (Rajagopal and Balasimha, 1994). Besides improving the survival rate and growth after recovery, both potassium and proline maintained leaf area expansion.

The accumulation of proline in mesophytic plants under water stress is well known. Proline accumulated in cocoa seedlings in response to water stress. In cocoa the proline accumulation

was negatively correlated with RWC. Thus, in drought tolerant accessions when RWC was higher under stress, the proline accumulated to a lesser degree (Balasimha, 1982a). Similar results were obtained when drought ameliorative sprays of cycocel, ABA, potassium or proline was given to cocoa seedlings (Balasimha, 1983; Balasimha and Subramonian, 1984). The accumulation of proline was to a lesser extent, whenever the leaf turgidity was maintained. It seems reasonable to assume that as the metabolic rates are higher because of high leaf turgor, proline might be utilized efficiently.

CONCLUSIONS

In cocoa, an effective stomatal regulation facilitate the trees to withstand moisture stress during summer months. Morphological and anatomical adaptations also favoured the accessions during drought conditions. Development of rapid screening methods for stress tolerance and identification of promising accessions to cope up with drought are the highlights of the investigations. Biochemical parameters like waxes that impart stress tolerance in some of the accessions have been well delineated. A crossing programme was undertaken to combine the characters of drought tolerance and high yield. Evaluation of seedlings raised thus has resulted in identifying the desirable hybrids for further studies on molecular basis of drought tolerance in cocoa.

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