# Comparative drought tolerance of cacao accessions

D. Balasimha, V. Rajagopal*, E.V. Daniel, R.V. Nair and S. Bhagavan

*Central Plantation Crops Research Institute, Regional Station, Vittal 574 243, Karnataka, India*

Received April 1987; revised December 1987

**The water relation components, stomatal behaviour and biochemical parameters were determined in 14 cacao accessions. Based on the parametric relationships with drought tolerance, the rank sums of these accessions lead to a selection of five of them, NC 23, NC 29, NC 31, NC 39 and NC 42. These drought tolerant accessions had effective stomatal regulation resulting in decreased transpiration under water stress, which seems to be an important strategy for adaptation in cacao. Although a decrease in osmotic potential and nitrate reductase activity and an increase in soluble sugar were noticed in response to stress, there was no relationship with respect to drought tolerance. The stomatal diffusive resistance was negatively correlated with transpiration. The cacao accessions with the higher drought tolerance characteristics and higher yield potential under drought stress should be useful basal material for selective breeding.**

Keywords: Cacao; Drought tolerance; Water relations; Stomatal regulation

Various physiological and morphological parameters are affected by drought conditions (Balasimha, 1983, 1984) and adaptation to such stress is often expressed in changes in these parameters (Jones *et al.*, 1981; Hanson and Hitz, 1982). Cacao (*Theobroma cacao* L.), widely cultivated as a mixed crop mainly in coconut and arecanut gardens in southern India, often experiences prolonged drought resulting in considerable yield losses. In an earlier study on screening for drought tolerance it was shown that it is possible to discard 80% of test material based on leaf morphological characteristics (Balasimha *et al.*, 1985).

The present study aims at gaining information on the relative importance of several attributes of drought tolerance in cacao accessions, in terms of crop adaptation and yield stability. This is desired because cocoa trees are very sensitive to water stress and are cultivated largely as a rain-fed crop.

## Materials and methods

### Plant material

The location, soil characteristics and meteorological data for the Regional Station have been detailed in an earlier paper (Balasimha *et al.*, 1985). The cacao accessions used in the present study were obtained from Nigeria and planted in 1975 at a spacing of 2.3 × 5.0 m under the shade of coconut palms. Each tree was fertilized annually with 100 g N, 40 g $P_2O_5$ and 140 g $K_2O$. 14 accessions were screened, NC 9, NC 23, NC 24, NC 26, NC 29, NC 30, NC 31, NC 38, NC 39, NC 40, NC 42, NC 49, NC 52 and NC 55. The measurements on physiological parameters in 1984, 1985 and 1986 were carried out at three stages: (a) immediately after a normal level of irrigation (pre-stress), (b) 20 days after withholding irrigation (stress) and (c) seven days after irrigating the plants following the 20-day stress treatment (post-stress or recovery). Since similar results were obtained over the three years, only data from 1986 measurements are presented in the Tables.

### Soil moisture content

The soil samples (0–50 and 50–100 cm depth) were collected using a soil auger at the three stages and the percentage water content was determined gravimetrically.

### Plant water relations

5–10 leaves from all parts of the outer canopy were measured. The sampling was done between 1000–1200 h with three replicates distributed over the period. Using a Li-Cor 1600 steady state porometer (Lambda Instruments, Nebraska, USA), leaf temperature, diffusive resistance and transpiration rate were measured. Five measurements were made on each leaf. Simultaneously, the air temperature, quantum flux and relative humidity were also recorded for each set of determinations. Leaf water potential was determined using Scholander's pressure chamber (Soil Moisture Equipments Corp., USA). The leaves taken for water potential measurements were pooled for osmotic potential and relative water content (RWC) determinations. Leaves were cut into 1 $cm^2$ pieces, frozen in liquid nitrogen, squeezed through a sap extractor into a vial and stored in a deep freeze for later analysis. The osmotic potential of the sap was measured on a Vapour Pressure Osmometer (Wescor model 5100c) with NaCl as a standard. The RWC was estimated by floating 0.5 cm leaf discs on distilled water for 4 hr (Weathereley, 1960). Turgor potential was calculated by the difference in water and osmotic potentials (Slavik, 1974).

### Biochemical analysis

The pooled leaf sample from each tree was used for chemical analysis and enzyme assay. Leaf tissue (0.5 g) was extracted in 3% sulphosalicylic acid,

Contribution No. 575
* Present address: Central Plantation Crops Research Institute, Kasaragod 670 124, Kerala, India.

0041-3216/88/030271-04 $03.00

centrifuged and the supernatant used for proline estimation (Bates *et al.*, 1973). For sugars the air-dried leaf material was ground, passed through a fine sieve and extracted in 80% ethanol. The total soluble sugar content was determined by the phenol-sulphuric acid method, using glucose as a standard (Dubois *et al.*, 1956). The *in vivo* assay procedures were employed for nitrate reductase (NR) activity with 0.2 g tissue in 0.1 M $KNO_3$ and 20 mM potassium-phosphate buffer and a total volume of 5 ml (Jaworski, 1971).

## Statistical analysis

Means and standard errors were calculated for all study variables. Analysis of variance was performed on each variable, classified into the effects of treatment (pre-stress, stress and recovery) and group (tolerant and susceptible) using a two-factor completely randomized design model. Correlations were calculated among all variables to assess the extent of possible relationship.

## Results and discussion

During the experimental period (April–May), dry conditions prevailed with high evaporative demand, as indicated by high light intensity and temperature and low relative humidity (Table 1). This resulted in reduction of the soil moisture content by 32%, as compared with pre-stress values, when irrigation was stopped for 20 days. Thus, the plants were exposed to both soil and atmospheric droughts, and the extent of stress experienced by the crop was indicated by various parameters – leaf temperature, RWC, diffusive resistance, transpiration, water potential components and certain biochemical reactions. At this level of stress a RWC decrease of 5–10% significantly changed other water relation components and a comparison among accessions with respect to their drought tolerance was possible.

The ten plant variables studied are shown in Table 1. The correlation studies among the variables revealed the following facts: leaf temperature was positively correlated with transpiration during pre-stress ($r = 0.69$, $P > 0.05$) and recovery ($r = 0.56$, $P > 0.05$); diffusive resistance was negatively correlated with transpiration during pre-stress ($r = -0.82$, $P > 0.01$) and under stress ($r = -0.81$, $P > 0.01$); soluble sugar content was positively correlated with osmotic potential under stress ($r = 0.61$, $P > 0.05$). The accessions were ranked separately for each of the important parameters. Considering the parametric relationships with drought tolerance, the rank sums of these accessions were calculated. The first five accessions, NC 23, NC 29, NC 31, NC 39 and NC 42, were selected, these accessions showing 54–59% decrease in transpiration under stress as compared with pre-stress levels. The mean values and their comparison with nine susceptible accessions are presented in Table 2. A significant difference in the stomatal regulation was discernible between the two groups; the tolerant accessions exhibited 56.6% higher diffusive resistance under stress as against 31% in those classified as susceptible; this resulted in less transpirational loss in tolerant accessions than in susceptible ones.

In cacao, therefore, an important strategy for adaptation to drought appears to be through effective stomatal regulation. In tea, a significant reduction in transpirational water loss by higher stomatal resistance was reported (Saikia and Dey, 1984). The relationship between transpiration and diffusive resistance in pre-stress and stress conditions is given in Figure 1. From this relationship it is possible to draw a threshold level of diffusive resistance in relation to drought tolerance. A stomatal resistance of 10 s $cm^{-1}$ or above which resulted in less than 2 $\mu g\ cm^{-2}\ s^{-1}$ transpiration rate under stress could be considered as a drought tolerant characteristic. All five accessions mentioned above fall into this category.

Variations in osmotic adjustment by accumulation of solutes have been reported in several crop plants

**Table 1** Means of variables measured in cacao accessions

| Variable | Units | Pre-stress | Stress | Recovery |
|---|---|---|---|---|
| Climatic factors | | | | |
| Temperature | °C | 33.6 ± 0.27 | 35.1 ± 0.24 | 31.0 ± 0.4 |
| Photosynthetically active radiation | $\mu E\ m^{-2}\ s^{-1}$ | 1444 ±29.6 | 1319 ±62.2 | 1200 ±104.1 |
| Relative humidity | % | 49.32± 0.74 | 44.89± 1.11 | 65.3 ± 1.3 |
| Soil moisture | | | | |
| 0–50 cm | % | 17.48± 1.12 | 12.54± 1.55 | 16.47± 1.27 |
| 50–100 cm | % | 20.78± 1.90 | 13.10± 2.53 | 18.58± 0.54 |
| Plant characters | | | | |
| Relative water content | % | 86.63± 1.06 | 81.39± 0.76** | 83.38± 1.21 |
| Leaf temperature | °C | 33.8 ± 0.30 | 35.07± 0.22** | 31.09± 0.43 |
| Stomatal diffusive resistance | $s\ cm^{-1}$ | 4.74± 0.22 | 8.78± 0.49** | 3.70± 0.23 |
| Transpiration | $\mu g\ cm^{-2}\ s^{-1}$ | 4.31± 0.29 | 2.77± 0.16** | 3.06± 0.16 |
| Leaf water potential | bars | −5.33± 0.21 | −9.19± 0.53** | −5.58± 0.22 |
| Osmotic potential | bars | −8.04± 0.15 | −9.37± 0.20 | −9.35± 0.21 |
| Turgor potential | bars | 2.71 | 0.18 | 3.77 |
| Proline content | $\mu g\ g^{-1}$ FW | 34.17 | 20.02 | 21.52 |
| Soluble sugars | $mg\ g^{-1}$ DW | 22.93± 0.98 | 28.23± 1.05** | 23.98± 1.90 |
| NR activity | nmol g FW $h^{-1}$ | 399.3 ±32.09 | 281.5 ±33.8* | 436.2 ± 25.7 |

Differences between control and stress means are significant at $P > 0.05$ (*) or $P > 0.01$ (**)

**Table 2** Differences in plant variables between drought tolerant and susceptible cacao accessions

| Variable | Accession type | Treatment | | | Mean | LSD* | | |
|---|---|---|---|---|---|---|---|---|
| | | Pre-stress | Stress | Recovery | | 1 | 2 | 3 |
| Relative water content (%) | Tolerant | 91.55 | 82.35 | 86.52 | 86.81 | 3.59 | 4.40 | 6.2 |
| | Susceptible | 89.37 | 79.43 | 83.69 | 84.17 | | | |
| | Mean | 90.46 | 80.89 | 85.10 | | | | |
| Stomatal diffusion resistance (s $cm^{-1}$) | Tolerant | 5.04 | 11.62 | 3.42 | 6.69 | 0.85 | 1.05 | 1.4 |
| | Susceptible | 4.83 | 7.00 | 4.08 | 5.30 | | | |
| | Mean | 4.93 | 9.31 | 3.75 | | | | |
| Transpiration ($\mu g\ cm^{-2}\ s^{-1}$) | Tolerant | 3.45 | 1.89 | 2.55 | 2.63 | 0.58 | 0.71 | 1.0 |
| | Susceptible | 4.58 | 3.30 | 3.56 | 3.81 | | | |
| | Mean | 4.02 | 2.59 | 3.06 | | | | |
| Turgor potential (bars) | Tolerant | 2.77 | 1.64 | 3.66 | 2.69 | 0.91 | 1.12 | 1.5 |
| | Susceptible | 2.84 | 1.69 | 4.39 | 2.97 | | | |
| | Mean | 2.80 | 1.66 | 4.02 | | | | |

* LSD ($P = 0.05$) 1, to compare tolerant *vs.* susceptible
2, to compare between treatments
3, interactions between variety and treatments

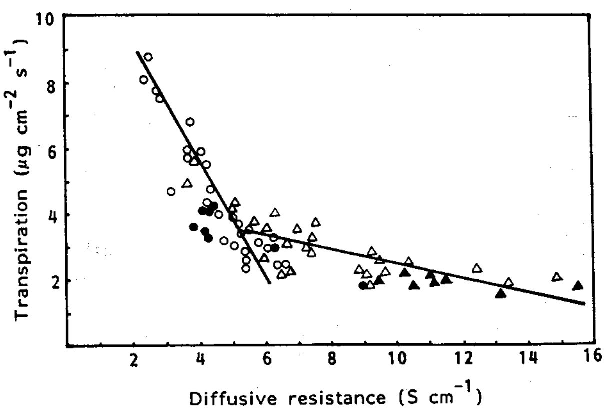


**Figure 1** Relationship between stomatal diffusive resistance and transpiration in pre-stress (○, ●) and stressed (△, ▲) plants; correlation coefficients were −0.82 and −0.81, respectively. Open and closed symbols represent susceptible and tolerant accessions respectively

**Table 3** Annual pod yield of drought tolerant and susceptible cacao accessions

| Yield (pods $tree^{-1}$) | Accession | |
|---|---|---|
| | Tolerant ($n = 5$) | Susceptible ($n = 23$) |
| 1982 | 65.2 ± 14.8 | 46.4 ± 4.2 |
| 1983 | 56.8 ± 11.1 | 45.3 ± 4.8 |
| 1984 | 81.0 ± 14.3 | 55.1 ± 4.5 |
| 1985 | 36.0 ± 4.5 | 26.0 ± 3.7 |
| 1986 | 72.6 ± 12.7 | 48.8 ± 6.5 |

Results are expressed as Mean ± standard error

(Davies and Lakso, 1979; Shackel and Hall, 1983; Morgan, 1984; Blum and Sullivan, 1986). Although soluble sugars accumulated with decrease in osmotic potential in cacao, no relationship with drought tolerance and susceptibility could be established. At this level of stress proline also did not accumulate; it has been reported that there is a threshold level of 70% RWC when proline accumulation in cacao begins (Balasimha, 1984). Osmotic adjustment did not occur in cacao, at least under the present stress condition. The turgor pressure was 30% higher in recovery compared with pre-stress conditions, because the osmotic potential did not recover as fast as water potential. Since turgor potential is calculated from the differences in these two parameters, higher values would obviously result. The NR activity was significantly inhibited under stress. The NR stability under stress was 0.59 and 0.53 in tolerant and susceptible groups, respectively, which was not significant.

While the lack of osmotic adjustment may have an advantage to cacao because it does not involve a 'cost' to the plant (Turner and Jones, 1980), the efficient stomatal closure might hamper carbon assimilation (Jones, 1979). It was therefore necessary to study the yield performance of tolerant accessions *vis-à-vis* susceptible ones. Annual yield was higher in tolerant than in susceptible accessions (Table 3). The mean annual dry crop was 2.31 and 1.64 kg $tree^{-1}$, and single bean weight was 1.12 g and 0.80 g in tolerant and susceptible accessions, respectively. Since the stomata resume their original open condition immediately after stress is relieved (Tables 1 and 2), it is reasonable to assume that stomatal closure has little affect on productivity. This is corroborated by the lack of correlation between yield and diffusive resistance due to stress ($r = 0.04$). It is also of interest to note that there was a positive correlation to yield with diffusive resistance at recovery ($r = 0.52$, $P > 0.05$).

The five accessions with high drought tolerance characteristics, high productivity and good bean weight under stress have been shown also to differ from accessions with less drought tolerance in a number of physiological parameters, indicating the genetic nature of this drought tolerance. Therefore, accessions which display these specific favourable attributes can be used as a source for breeding to bring desirable characters into a single ideotype with a good expectation of increasing drought tolerance.

## References

Balasimha, D. (1983) Water relations and physiological responses to water stress in cacao, *Plant Physiol Biochem.* (New Delhi) **10** 65–71

Balasimha, D. (1984) Leaf growth and associated physiological changes in six cacao accessions under water stress, *Proc. Fifth Annual Symposium on Plantation Crops*, 1982, Kasaragod, CPCRI, pp 224–230

Balasimha, D., Subramonian, N. and Subbaiah, C.C. (1985) Leaf characteristics in cocoa (*Theobroma cacao* L.) accessions, *Café Cacao Thé* **29** 95–98

Bates, L.S., Waldren, R.P. and Teare, I.D. (1973) Rapid determination of free proline for water stress studies, *Plant Soil* **39** 205–207

Blum, A. and Sullivan, C.Y. (1986) The comparative drought resistance of landraces of sorghum and millet from dry and humid regions, *Ann. Bot.* **57** 835–846

Davies, F.S. and Lakso, A.N. (1979) Diurnal and seasonal changes in leaf water potential components and elastic properties in response to water stress in apple trees, *Physiol. Plant.* **46** 109–114

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. Determination of sugars and related substances. Determination of concentration of pure sugar solution, *Anal. Chem.* **28** 350–356

Hanson, A.D. and Hitz, W.D. (1982) Metabolic responses of mesophytes to plant water deficits, *Annu. Rev. Plant Physiol.* **33** 163–203

Jaworski, E. (1971) Nitrate reductase assay in intact plant tissues, *Biochem. Biophys. Res. Commun.* **43** 1274–1279

Jones, H.G. (1979) Stomatal behaviour and breeding for drought resistance, in: *Stress Physiology in Crop Plants* (Eds Mussel, H. and Staples, R.C.), New York, Wiley Interscience, pp. 408–428

Jones, M.M., Turner, N.C. and Osmond, C.B. (1981) Mechanisms of drought resistance, in: *Physiology and Biochemistry of Drought Resistance in Plants* (Eds Paleg, L.G. and Aspinall, D.), Sydney, Academic Press, pp. 15–37

Morgan, J.M. (1984) Osmoregulation and water stress in higher plants, *Annu. Rev. Plant Physiol.* **35** 299–319

Saikia, D.N. and Dey, S.K. (1984) Leaf water potential and stomatal resistance of the leaf as influenced by soil moisture and potash application, *Ind. J. Agric. Sci.* **54** 727–732

Shackel, K.A. and Hall, A.E. (1983) Comparison of water relations and osmotic adjustment in sorghum and cowpea under field conditions, *Aust. J. Plant Physiol.* **10** 423–435

Slavik, B. (1974) *Methods of Studying Plant Water Relations*, Berlin and New York, Springer-Verlag

Turner, N.C. and Jones, M.M. (1980) Tugor maintenance by osmotic adjustment: a review and evaluation, in: *Adaptation of Plants to Water and High Temperature Stress* (Eds Turner, N.C. and Kramer, P.J.), New York, Wiley Interscience, pp. 87–103

Weatherely, P.E. (1960) Studies in the water relations of cotton I. The field measurement of water deficits in leaves, *New Phytol.* **49** 81–97