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Laboratory screening of cocoa genotypes for drought tolerance*

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In this paper, results of screening of cocoa germplasm for drought tolerance/resistance using three tests are presented. The parameters used are heat stability of chlorophyll (CSI), proline accumulation ability and germination of seeds under low osmotic potential. The CSI is found to vary considerably among the cocoa genotypes. The highest CSI is in Criollo and the lowest in NC 34. In general the 'P' combinations are found to be more stable to heat degradation. Proline accumulation also is found to vary considerably among the cocoa genotypes investigated. The maximum proline accumulation is in NC 34 and the minimum in Criollo. Here also the 'P' combinations were found to accumulate more proline than the others. In the study of germination under low osmotic pressure conditions also, NC 34 fared much better than all the other types. The results indicate that certain genotypes are more tolerant to heat degradation of chlorophyll, accumulates more proline under induced stress and give higher germination percentage under low OP. Such types can be selected for further testing for field performance.

Cocoa *(Theobroma cacao)* is highly sensitive to water deficiency. Growth, photosynthesis and respiration in cocoa are markedly reduced when soil moisture drops below 60–70 per cent of the available range (Lemee, 1955). Necrotic areas appear on the leaves when about one sixth of its water content is lost and that it has also been shown that a five per cent dehydration of the leaf leads to complete cessation of photosynthesis (Alvim, 1965). A major constraint in growing cocoa in India is the prolonged summer drought period from about December to May, prevailing in the cocoa growing regions of Kerala and Karnataka. The immediate requirement of cocoa growing in India therefore, is to develop drought tolerant types. With this aim, the germplasm available at the Central Plantation Crops Research Institute, Regional Station at Vittal were subjected to preliminary laboratory screening to identify the tolerant types. The results are summarised here.

Three parameters were used for the screening work, *viz.*, heat stability of chlorophyll (chlorophyll stability index–CSI), proline accumulation in excised leaves under induced stress and seed germination under low osmotic potential. CSI has been defined as the difference between the light transmission of chlorophyll extracts from heated and unheated samples of leaves (Kaloyereas, 1958). The lesser the CSI, the greater the stability of chlorophyll and greater could be the heat tolerance. CSI has been used previously by many workers for detecting heat tolerance of plants (Murthi & Majumdar, 1962; Kilen & Andrews, 1969, Maillard, Daniel & Ochs, 1974; Kozhushko, 1974). Ravindran &

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Menon (1981) reported earlier the usefulness of this method in the screening of cocoa germplasm for drought tolerance.

Proline accumulates in excised or intact leaves in response to water stress and this has been suggested as a reliable laboratory screening test for drought tolerance (Barnett & Naylor, 1960; Singh, Aspinall & Paleg, 1972 and 1974; Waldren & Teare, 1974). This test has been used in field crops like rice, sorghum, and barley (Anon. 1975, Blum & Ebercom, 1978). It should also be mentioned that some workers failed to detect any simple positive correlation between field drought tolerance and proline accumulation (Hanson, Nelson & Eversen, 1977).

The capacity of a genotype to germinate and grow under conditions of low osmotic potential is probably an indication of its capacity to withstand drought conditions. Skylar (1976) is of the opinion that the most reliable method for testing drought tolerance in inbred maize is by germinating the seeds in a sucrose solution at an osmotic pressure of 20 atms. This method has been used earlier for screening drought tolerance in cereals (Vasudevan & Balasubramoniam, 1965; Williams, Snell & Ellis, 1967; Manohar Bhan & Prasad, 1968; Sharma, 1974; Singh & Singh, 1981).

In the present study all these three parameters were used, so that the results could be more reliable than those based on a single character.

MATERIALS AND METHODS

The cocoa genotypes available in the Central Plantation Crops Research Institute, Regional Station at Vittal were used for the present study. Chlorophyll stability index (CSI) was estimated, following the standard method (Murthy & Majumdar, 1962; Ravindran & Menon, 1981), using a leaf sample of 200 mg fresh leaves and replicated twice.

Proline accumulation was studied using excised cocoa leaves. Cocoa twigs were collected early in the morning, and kept in a bucket of water and the foliage completely enclosed in polythene bag and left for one hour for equilibration of moisture content. Three leaves were then removed and immediately transferred to 40 per cent polyethylene glycol (PEG) solution (−22.3 bars) and kept for one hour. The leaves kept in water formed the control. After one hour, the leaves were washed rapidly in water, blotted dry, and 20 mg of leaf tissue processed for extraction of proline as per method of Bates, Waldren & Teare (1973). The data presented here are the mean values of two estimations.

For the third test, germination ability under low osmotic potential seeds from cocoa genotypes were germinated giving 10–20 per cent PEG solution (−1.9 and −6.7 bars respectively). Seeds were immersed in the above solutions for three hours and then sown in neutral vermiculite irrigated with PEG solutions of the same concentration. Seeds soaked and irrigated with water formed the control. Germination counts were taken as and when the seeds germinated. Two replications of 100 seeds each were maintained per treatment and the mean values are presented here.

RESULTS AND DISCUSSION

Heat stability of chlorophyll

Table 1 gives the CSI values obtained

from 20 cocoa genotypes. There is considerable differences among the genotypes studied. The highest CSI was recorded for Criollo variety and the lowest for NC34. Among the Forastero (Upper Amazon) types, the highest CSI was recorded for IMC 67.

TABLE 1. CSI OF 20 COCOA GENOTYPES*

*Genotype/parentage***	*CSI*
NC 34 (P 9 x P 1)	0.0
NC 38 (P 10 x P 1)	0.5
NC 30 (P 3 x P 4)	2.0
NC 9 (P 1 x P 7)	2.4
NC 32 (P 10 x P 4)	1.0
NC 35 (P 5 x P 4)	1.8
NC 37 (P 7 x P 5)	2.5
NC 12 (P 9 x P 5)	5.7
NC 14 (C 77 x C 67)	2.5
NC 50 (C 44)	16.5
Red axil	1.5
ICS 1	14.0
ICS 6	6.0
SCA 6	4.0
Na 33	4.5
Na 32	10.0
IMC 67	20.0
Amel x Na 32	9.0
PA 7 x Na 32	12.0
Criollo	25.0

* Mean of two readings.
** The NC types are Nigerian clones (parentage given in parenthesis). 'P' indicates Pound's collections. SCA 6, Na 32, Na 33 & IMC 67 are well known Upper Amazon Foresteros originally obtained from Kew Gardens. ICS 1 and 6 are Trinitarios received from Kew. Red axil is an Amelnado type with purple pods and coloured petioles, received from Malaysia. Amel x Na 32 and PA 7 x Na 32 are hybrid progenies received from Malaysia.

Of the three commercial cocoa varieties (Forastero, Criollo and Trinitario), Criollo is the most delicate and sensitive to adverse growth conditions. The cultivation of this variety is in fact discontinued in most of the cocoa growing countries because of its delicate nature and poor yield, though in quality it is superior to all other types. The high CSI value obtained indicates high heat sensitivity of chlorophyll and thereby its susceptibility to moisture stress.

In general, the hybrid combinations involving 'P' types (Pound collections) are known to be more tolerant to drought. These are also known to give better survival when grown in marginal or semi-marginal areas. This greater heat tolerance are evident by their low CSI values, and they may prove more successful under the prevailing growth conditions of Kerala and Karnataka.

Proline accumulation

Table 2 gives the proline accumula-

TABLE 2. PROLINE ACCUMULATION IN 16 COCOA GENOTYPES FOLLOWING ONE HOUR INDUCED STRESS IN PEG

Genotype	*Proline accumulation/g/leaf tissue (μg)*		
	A control	*B stressed*	*B – A (μg)*
Na 33	13.00	17.50	4.50
IMC 67	8.35	11.35	3.00
ICS 6	8.25	8.55	0.30
Amel x Na 32	18.00	25.40	7.40
PA 7 x Na 32	8.75	14.50	5.75
Red axil	16.30	21.35	5.05
NC 9	14.50	17.55	3.05
NC 12	18.00	22.50	4.50
NC 34	11.75	23.85	12.10
NC 36	20.05	26.25	6.20
NC 37	17.50	22.65	5.15
NC 38	13.75	20.00	6.25
SCA 6	13.70	16.30	2.60
ICS 1	7.90	10.75	2.85
Na 31	16.15	12.04	0.19

tion in 16 cocoa genotypes following one hour stress. The results indicate that cocoa is a proline accumulating species. The rate of accumulation was found to vary much among the genotypes, which may be indicative of genetic differences among the genotypes. The low values of proline accumulation observed in the present study is due to the short stress treatment given. The maximum proline accumulation was in NC 34, and the minimum in Criollo. In general the NC types derived from the 'P' combinations were found to accumulate more proline which may be indicative a genetic difference in the proline accumulating ability. This observation further supplements the earlier data on the heat stability of chlorophyll.

Seed germination under low osmotic potential

Data on germination under low external water potential is presented in *Table 3*. An osmotic potential of −1.3

TABLE 3. GERMINATION OF TEN COCOA GENOTYPES UNDER LOW OSMOTIC PRESSURE

	Polyethylene glycol solution		
Genotype	*At 10% (−1.9 bars)*	*At 20% (−6.7 bars)*	*Control (water)*
ICS 6	95	62	98
Na 33	91	71	98
IMC 67	95	59	98
SCA 6	90	74	100
Na 31	91	70	97
Red axil	86	54	99
Tr. 27*	90	68	98
NC 34	94	79	100
NC 36	92	77	99
NC 37	91	73	99

*A promising tree – parentage unknown.

bars (10 per cent PEG) reduced the germination in all the genotypes tested, though the reduction was not very significant. But at −6.7 bars (20 per cent PEG) the reduction in seed germination was very marked and there were much differences among the genotypes. IMC 67 gave only 59 per cent germination as against 98 per cent in the control and 95 per cent at −1.9 bars. The reduction in germination percentage was least in NC 34 followed by NC 36, SCA 6 and NC 37. The differences in germination among the genotypes might be indicative of an inherent ability of the genotypes to germinate at very low water potentials. This observation provides additional evidence to their tolerance to stress conditions.

All the three parameters studied here were used in predicting drought tolerance in plants, though proline accumulation has been studied more widely and critically. The cause of accumulation of proline by plants under moisture stress is not well understood, though it has been suggested to be due to blockage in the metabolic pathway by which amino acids are synthesised or due to stress induced protein breakdown (Parker, 1968; Naylor, 1972).

Data from a variety of tests such as proline accumulation, heat stability of chlorophyll, germination of seeds under low osmotic potential conditions, nitrate reductase activity and electrolytic leakage have been in use for assessing the overall drought tolerance capacity of a cultivar. Williams *et al.*, (1967) is of opinion that germination against a 15 atms mannitol solution is a useful criterion for screening for drought resistance in maize. Similarly Skylar (1976) reported 'osmotic screening' as the most useful method for detecting drought tolerance in inbred maize. Similar results were also mentioned by Vasudevan & Balasubramoniam (1965), Hurd (1975), Sharma (1974) and Singh & Singh (1981).

CONCLUSION

In the present study all the three tests employed seem to indicate that certain genotypes withstand heat stress and external water stress to a greater extent than others. Probably these genotypes may have the genetic superiority to withstand stress conditions. Though the ultimate evidence should come from field performance, the results from the laboratory tests indicate the general superiority of certain types over others. These apparently superior types can be put in field evaluation to establish their superiority. Thus the preliminary laboratory screening trials can be of considerable use in selecting the apparent superior types before they go into the field.

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