Leaf Water Potential as an Index of Drought Tolerance in Coconut (*Cocos nucifera* L.)

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ABSTRACT

Leaf water potential as an index of drought tolerance in coconut (*Cocos nucifera* L.). Rajagopal V, Shivishankar S, Kasturibai K V, and Voleti S R. (Pl. Physiol. & Biochem. Divn., Central Plantation Crops Res. Inst., Kasaragod 670 124, India). Pl Physiol. & Biochem. 15(1) : 80–86, 1988—Coconut genotypes were screened for drought tolerance using the leaf water potential (ψ) measurements. Adult coconut palms comprising the talls (West Coast Talls, WCT and Laccadive Ordinary, LO), the dwarfs (Chowghat Dwarf Orange, CDO and Gangabondam, GB) and the hybrids (WCT × CDO, CDO × WCT, LO × GB and LO × CDO) were selected for the study. During the dry period, the ψ of the spindle leaves was measured in the field immediately after excision of leaflets and at three hour intervals until 9 h after subjecting them to dehydration. The ψ declined with time to different degrees among the genotypes. However, LO × CDO, WCT and LO & GB maintained higher ψ than the other genotypes, irrespective of the time of dehydration, indicating thereby the degree of tolerance. That the above three genotypes are more tolerant to desiccation was also revealed by relatively low activities of two sensitive enzymes viz. acid phosphatase (Aph) and glutamate oxalic acid transaminase (GOT), contrary to high activities shown by the susceptible genotypes. The field performance of the genotypes under drought conditions served as an evidence for their ability to withstand water deficit conditions. Thus, ψ measurement can serve as a rapid method of screening for drought tolerance in coconut plantations.

Key Words : *Acid phosphatse/*Cocos nucifera *L./Drought/Glutamate oxalic acid transaminase/Leaf water potential*

INTRODUCTION

It had long been recognized that certain physiological and biochemical attributes can be employed to screen crop plants for their tolerance to drought. Some of the well known tests were desiccation tolerance (1), chlorophyll stability index (2), heat tolerance (3), proline content (4), stomatal resistance (5) and abscisic acid levels (6). While some of these characters were successfully tested in annual crops and even the drought resistant genotypes were identified on the basis of yield performance as in sorghum (7, 8), barley (4) and wheat (9), there is hardly any report on tree crops. This perhaps is due to little knowledge on

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the impact of drought on perennial crops. Recently Balasimha *et al.* (10), reported the physiological traits of drought tolerance in cocoa.

In recent years the measurement of leaf water potential has gained wide acceptance in studies on water relations of crops. The relationship between the stomatal regulation and ψ has been well understood in many crops (5, 11). Genotypic variations on stomatal resistance, water potential (ψ) and epicuticular max content were noticed in adult coconut palms, which revealed drought tolerance in some genotypes (Rajagopal *et al.* unpublished). The present paper reports the determination of ψ on excised leaves subjected to dehydration as a rapid method for screening of large number of samples. Data on the activities of two sensitive enzymes (Aph and GOT) in relation to changes in ψ are presented as supporting evidence.

MATERIAL AND METHODS

Twenty year old coconut palms (*Cocos nucifera* L.) grown in the Institute Farm as monocrop with 7.5 × 7.5 m spacing under the normal cultural and agronomic practices in laterite soil were used in the experiments. The following genotypes were selected : Talls—WCT and LO, dwarfs—CDO and GB, and hybrids—WCT × CDO, CDO × WCT, LO × CDO and LO × GB. Six palms per genotype were taken up for the investigation, with each palm representing a replication.

Twenty tender leaflets from both sides of the midrib of the spindle leaves were collected and immediately the ψ was determined in 2 to 3 leaflets with the Scholander pressure chamber (Plant Water Console 3000, Soil Moisture Co, U.S.A), as described earlier (12). The remaining samples were brought to the laboratory, the surface cleaned and spread on the filter paper. They were then exposed to prevailing weather conditions (temperature ranging between 32 to 35 C, irradiation 850 to 1100 ηE. cm^{-2} s^{-1} and vapour pressure deficit 22 to 26 mbar), avoiding direct exposure to light. Samples (2 to 3 leaflets) from each genotype were collected at 3, 6 and 9 h after subjecting them to dehydration and ψ was determined. The same leaflets were then used for enzyme studies in all genotypes except LO and GB.

The enzymes, Aph (EC 3.1.3.2) and (GOT (EC 2.6.1.1), were extracted by grinding the leaf tissues in cold 0.1 M Na-phosphate buffer, pH 7.6 containing 0, 5 per cent 2-mercaptoethanol and 10 per cent PVP. The homogenate was strained through two layers of cheese cloth and centrifuged at 8,000 × g for 10 min. The clear supernatant was used for enzyme assays. All operations were carried out below 5°C. Aph was assayed using *p*-nitrophenylphosphate as substarte (13) and GOT according to Bergmeyer (14). Soluble protein was estimated following Lowry *et al.* (15). Results are expressed as percentage increase or decrease over the initial activity.

RESULTS

Changes in ψ with time in different genotypes are represented in Figure 1. The genotypes are arranged (1 to 8) in the decreasing order of ψ recorded immediately after excision of leaflets. Initially (O h) the ψ varied from —0.49 MPa to —1.10 MPa among the genotypes. LO × CDO, followed by WCT and LO × CB had higher ψ than the others; LO had the lowest. Subjecting the leaflets to dehydration resulted in lowering the ψ in all the genotypes and with time the effect was marked. However, irrespective of the time of dehydration, the ψ in the leaves of LO × CDO, WCT and LO × GB remained higher than the other genotypes, which tended to show rapid decrease with increase in time. For instance, the ψ in LO × CDO declined from —0.49 MPa to —1.0 MPa during 3 h treat

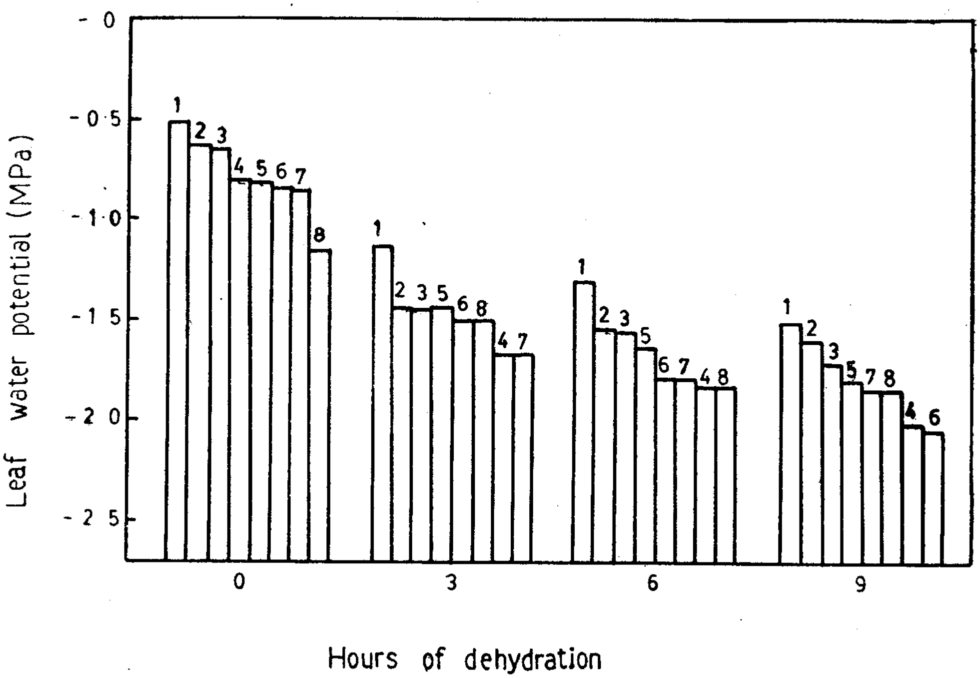

Fig. 1.

ment, whereas in CDO × WCT it dropped from —0.68 MPa to —1.71 MPa during the same time.

The activity of Aph at O h ranged from 209.4 to 283.1 ηmoles PNP (g dwt)$^{-1}$h^{-1}, the maximum being in CDO (Table 1a). The enzyme was not affected or only marginally affected in WCT, LO × GB and LO × CDO when the leaves were dehydrated upto 6h; WCT × CDO also remained unaffected at 3h, but there was an increase at 6h by 11.5 per cent over O h activity. Exposure of leaves even for 3h to dehydration enhanced the enzyme activity by 16.1 per cent and 31.3 per cent over O h in CDO × WCT and CDO. This increase was marked in CDO by 6h. At the termination of the experiment (9h) Aph activity was less

TABLE 1

Changes in the activities of Aph and GOT and in the level of soluble protein in coconut genotypes during stress development. Values are means of six palms

Genotypes	Activity at	% increase at the end of		
	0h	3h	6h	9h
	(a) Acid phosphatase activity (μmoles PNP liberated g^{-1} dry wt h^{-1})			
WCT	253.32	0	0	7.1
LO × GB	278.39	0	0	18.1
LO × CDO	269.84	0.8	1.5	22.8
CDO	283.12	31.3	56.1	130.4
WCT × CDO	209.45	0	11.5	143.8
CDO × WCT	224.63	16.1	20.6	86.2
	(b) Glutamate oxalic acid transaminase (μg pyruvate mg^{-1} protein)			
WCT	3.056	5.4	5.4	18.1
LO × GB	3.620	0	3.1	12.4
LO × CDO	3.813	1.4	2.9	7.6
CDO	4.102	16.6	19.1	104.2
WCT × CDO	3.755	10.8	17.2	115.3
CDO × WCT	3.522	4.5	8.8	86.2
	(c) Soluble protein [mg (g dry wt)$^{-1}$]			
WCT	2.42	2.1	2.8	13.2
LO × GB	2.68	7.4	11.1	11.2
LO × CDO	2.71	0.7	7.9	16.6
CDO	2.38	11.7	11.7	42.5
WCT × CDO	2.69	7.1	14.2	32.6
CDO × WCT	2.76	7.2	22.6	53.2

than 23 per cent in LO × CDO, LO × GB and WCT, while the increase was maximum (143.8 per cent) in WCT × CDO.

The initial activity of GOT ranged from 3.05 to 4.10 μg pyruvate mg^{-1} protein h^{-1} among the genotypes (Table 1b). LO × GB, WCT and LO × CDO had a marginal increase in the enzyme activity at 3 and 6h over Oh, while the increase was significant with 19.1 per cent in CDO. With 9h dehydration, GOT activity showed an increase of less than 20 per cent in the first three genotypes, wherease the increase ranged from 86.2 per cent to 115.3 per cent in CDO × WCT, CDO and WCT × CDO.

Soluble protein levels showed little difference initially among the genotypes, but with the development of stress there was gradual increase to different degrees (Table 1c). Dehydration for 9h resulted in enhancing the level of soluble proteins by 32.6 per cent, 42.5 per cent and 53.2 per cent over O h in WCT × CDO, CDO and CDO × WCT, whereas the increase ranged only between 11.2 per cent to 16.6 per cent in the other three genotypes.

DISCUSSION

The maintenance of high ψ under stress conditions is a desirable trait, as that would enable the tissues to maintain favourable metabolic activities to withstand desiccation (16, 17). Relatively high ψ in the tissues of LO × GB, WCT and LO × CDO, as comapred to others, subjected to dehydration reveals the extent of drought tolerance among the genotypes. Recent studies also showed high stomatal resistance and high epicuticular wax content, both indicative of adaptation to stress conditions, in the above three genotypes (Rajagopal *et al.*, unpublished). Conversely, the dwarfs like CDO and the hybrid CDO × WCT had low diffusive resistance and low wax content. These reflected on the transpirational loss of water from the leaf surface, which was less in LO × CDO, LO × GB and WCT. The relationship between the ψ and stomatal resistance has been reported in several crops (11, 18).

To what extent the changes in ψ affected the metabolic status of leaf tissues was assessed by determining the activities of Aph and GOT, which are known to be indicators of stress in plants (19). A significant increase in the activities of these enzymes with progressive development of stress under field conditions between December and May was observed in coconut (Shivishankar *et al.* unpublished). It is clear from the data that the enzyme activities increased considerably only in those genotypes (CDO, CDO × WCT and WCT × CDO) which exhibited greater decline in ψ with the development of stress; conversely, higher stability in the enzymes correlated with high ψ in the other three genotypes.

Enhanced enzyme activities during stress development is a result of increased soluble protein levels. The specific activity of both the enzymes remained steady during stress, which indicates that the increase might be due to solubilization of more enzyme protein or to additional synthesis in response to stress. Vieira da Silva (20) reported that the Aph increased in the soluble fraction of stressed cotton plants due to increased solubilization of the enzyme from the chloroplasts. The drought resistant species *Gossypium anomalum* was found to be slow in solubilizing Aph; a similar situation occurred in LO × GB, LO × CDO and WCT. The present study also reveals that higher activity was associated with decrease in ψ as it happened in the other three genotypes. This might indicate their susceptibility to drought. The increase in Aph activtiy during stress was shown to be associated with rapid destruction of choloroplast organization within the cell (21). The increase in soluble protein levels in finger millet during water stress has been reported (22, 23). The authors ascribed this to the synthesis of certain 'stress proteins' with adaptive consequences. That such proteins are present in cononut is a good indication, which if exploited might reveal the range of drought tolerance among the genotypes.

Earlier observations on the stomatal regulation in coconut genotypes (Rajagopal *et al.* unpublished) under field conditions and the present data on the changes in ψ and the enzyme activities support the contention that LO × CDO, LO × GB and WCT are relatively more tolerant to drought than the others. Yield performance of these palms over the years also indicate the same view (24). The present study highlights the relevance of using ψ determinations for rapid screening for drought tolerance in coconut, as has been done in cocoa. (Balasimha and EV Dainial, unpublished date).

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REFERENCES

1. Levitt, J, *In* Hardiness of Plants to Academic Press, New York. (1956).
2. Kaloyereas, SA, *Plant Physiol.*, 33, 232 (1958).
3. Sullivan, CY, *In* Drought Injury and Resistance in Crops. Crop Sci. Soc. of America, p. 1 (1971).
4. Singh, TN, Aspinall, D & Plaeg, LG, *Nature New Biology*, **236**, 188 (1972).

5. Jones, HG, *In* Stress Physiology of Crop Plants, (HG Mussel & RG Stapless eds). Wiley Publishers, New York, 407 pp. (1979).
6. Austan, RB, Henson, IE & Quarrie SA, *In* Droght Resistance in Crops with Emphasis on Rice. IRRI, Philippines, 171 pp. (1982).
7. Rao, NGP, Harinarayanl, G, *Curr. Sci.* **38**, 97 (1969).
8. Seetharama, N & Sivakumar, MVK, Bidinger, FR, Singh, S, Maiti, RK, Reddy, BVS, Peacock, JM, Reddy, SJ, Mahalakshmi, V, Sachan, RC, Raj, AS. Murthy, SPK, Narayanan, A, Kannangara, T, Durley, RC & Simpson, GM, *Proc. Indian Natl. Sci. Acad.*, **49** B, 498 (1983).
9. Chinoy, JJ, *Phyton*, **19**, 11 (1962).
10. Balasimha, D, Subramanian, N & Subbaiah, CC, *Cafe, Cocoa, Thea,* **29**, 95 (1985).
11. Turner, NC & Kramer, PJ, *In* Adaptation of Plants to Water and High Temperature Stress. John Wiley and Sons, New York (1980).
12. Rajagopal, V, Patil, KD & Sumathykuttyamma, A, *New Phytol.*, **105**, 298 (1987).
13. Linhardt, K & Walter, K, *In* Methods of Enzymatic Analysis. (HU Bergmeyer ed) *Verlag Chemie Gumbtl Weinheim* (1983).
14. Bergmerer, HU, Ibid, p. 779 (1963).
15. Lowry, OH, Rosebraugh, NJ, Fan, AL & Randal, RJ, *J. Biol. Chem.* **193**, 265, (1951).
16. Hsciao, TC, Acevedo, E, Fereres, E & Hendersen, DW, *Phil. Trans. Royal. Soc. London*, **273B**, 479 (1976).
17. Turner, NC, *Plant Physiol.*, **53**, 360 (1974).
18. McGree, KJ, & Richardson, SG, *Crop Sci.*, **27**, 539 (1987).
19. Levitt, J. *In* Response of Plants to Environmental Stresses. Vol. 2 Academic Press, New York (1980).
20. Vieira, DA, Sliva, J, Unesco Symp. "Plant Responses to Climatic Factors", Uppsaala, pp. 213, (1970).
21. Vieira DA, Silva, J. Naylor, WW & Kramer, PJ, *Proc. Natl. Acad. Sci. USA*, **71**, 3243 (1974).
22. Kandpal, RP, Vaidyanathan, KS, Udayakumar, M, Krishna Sastry, KS & Ppaji Rao, N, *J. Biosci.*, **3**, 361 (1981).
23. Kandpal, RP & Rppaji Rao, N, *Plant Sci.*, **40**, 73 (1985).
24. Anonymous, *Tech. Bull.* **17**, CPCRI, Kasaragod (1986).

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