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THERMAL STABILITY OF NITRATE REDUCTASE IN RELATION TO DROUGHT TOLERANCE IN COCONUT*

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ABSTRACT

During the development of drought stress in the field, the leaf Relative Water Content (RWC) and Nitrate Reductase (NR) activity of a few coconut genotypes showed considerable reduction. The changes in NR activity were found to be positively correlated with the changes in RWC. However, when the effect due to temperature (Tmax) was eliminated, the correlation between NR activity and RWC low. On the contrary, the maximum temperature (Tmax) was found to have a strongly negative correlation with NR activity. Based on this relationship, the effect of pretreating the leaves at various temperatures on NR activities of several genotypes was studied. The results indicated differences in the thermal stability of NR *in vivo*, which could be employed as an additional parameter in screening coconut genotypes for drought tolerance.

INTRODUCTION

Drought stress brings about considerable reduction in nitrate reductase (NR) activity of several crop plants (Morilla *et al.*, 1973; Plaut, 1973; 1974; Teare *et al.*, 1974). The decrease in nitrate reductase activity has frequently been attributed to changes in leaf water potential (Huffaker *et al*, 1970; Morilla *et al*; 1973; Heuer *et al.*, 1979) and leaf relative water content (RWC) (Dusky, 1978; Sairam and Dube, 1984). Since drought is often associated with an increase in temperature, its effects on nitrate reductase activity cannot be overlooked. Earlier reports on heat tolerance of thermophiles have shown that their proteins and enzymes display remarkable stability at high temperatures (Militzer *et al.*, 1949: Koffler, 1957; Sanwal and Krishnan, 1961). However, there is very little information available on the thermal stability of proteins and enzymes from higher crop plants. It was therefore felt that useful information may be gained by studying the influence of temperature (Tmax) on the *in vivo* nitrate reductase activity. In view of this, investigations were carried out on a few selected coconut genotypes to explore the possibility of applying this knowledge to screen for drought tolerance in coconut.

MATERIALS AND METHODS

Leaf samples for the study were collected from rainfed adult coconut palms growing in the Institute campus under scientific management. Leaflets from the middle portion on either side of the rachis on the fourteenth leaf were used for the experiments. Sampling was done between 10.00 and 11.00 a.m.

Indication of activity

Nitrate reductase activity was induced by placing the cut ends of excised leaves under nitrate solution in daylight. Measurement of activity was made after induction for four hours.

Temperature pre-treatment

One gram leaf discs (6 mm dia) were incubated in a buffered medium at various temperatures for 30 minutes. Activity was determined after washing the discs with excess buffer.

Measurement of relative water content

Leaf discs (10 mm dia) were weighed and floated on distilled water for 24 hours at 25°C in the dark. RWC has calculated by the following equation according to Barrs and Weatherly (1962).

$$\text{RWC\%} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

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Determination of nitrate reductase

Nitrate reductase activity *in vivo* was determined as described earlier (Shivashankar and Ramadasan, 1983).

Data on the maximum temperature recorded on the day of measurement of nitrate reductase activity was obtained from the Institute's climatological station. Statistical correlations among the various parameters were worked out by applying standard methods.

RESULTS AND DISCUSSION

The changes in nitrate reductase activity, and relative water content of four coconut genotypes during the period September 1986 upto May 1987 are presented in Table I. The NR activity of all genotypes showed a rapid decline from September to November followed by a more gradual fall upto May. Leaf RWC also exhibited a decreasing trend during this period indicating that NR activity could be related to the changes in leaf water status.

A closer look at Table I showed that a small reduction in RWC (about 2%) caused a rapid decline in NR activity initially while further reduction in RWC did not bring about a proportional decrease of NR activity. However, when NR activity was transformed to logarithmic scale, it showed a good linear relationship with RWC and maximum temperature (Tmax) (Fig.1). The results of the correlation among NR activity (transformed to logarithmic scale), RWC and temperature are presented in Table II. From this, it was seen that NR activity exhibited a significantly positive correlation with RWC in all the genotypes (r_{32} = 0.6774 to 0.8431). However, when the effect due to temperature was eliminated, it was found that the NR activity was poorly correlated with RWC ($r_{32.1}$ = 0.1708 to 0.5667) showing thereby that the decline in NR activity was

Table I. Changes in Relative Water Content and Nitrate Reductase activity during the development of drought stress

Year/Month		Maximum air temp °C (Tmax)	Relative water content %				Nitrate Reductase Activity (n mol) NO 2 formed/g leaf/h			
			WCT	WCT x COD	COD x WCT	COD	WCT	WCT x COD	COD x WCT	COD
1986	Sep.	30.0	93.8	94.4	94.4	92.6	331.3	348.0	361.3	341.0
	Oct.	31.0	92.7	93.9	93.5	92.0	236.0	222.3	315.3	267.0
	Nov.	31.9	91.3	91.6	92.1	92.0	197.0	176.1	253.0	232.0
	Dec.	33.1	91.5	91.6	92.5	91.6	162.0	158.2	153.0	144.0
1987	Jan	32.9	92.0	91.1	92.5	91.8	121.0	143.3	89.7	89.0
	Feb	32.7	92.1	91.0	91.8	90.0	112.0	104.0	77.6	75.3
	Mar	32.9	90.8	90.1	89.9	88.7	96.4	89.7	61.7	59.0
	Apr.	33.9	89.9	89.4	87.1	86.3	88.6	83.4	53.0	50.8
	May	34.9	88.0	87.0	86.1	85.1	82.3	74.0	48.7	42.3
S.E./Plot					1.79			12.31		
Gen. mean					90.90			153.83		
C.V. (%)					1.97			8.00		
C.D (P = 0.05)										
Genotype (G)					0.971			6.685		
Month (M)					1.457			10.028		
Genotype x month (GxM)					NS			20.055		

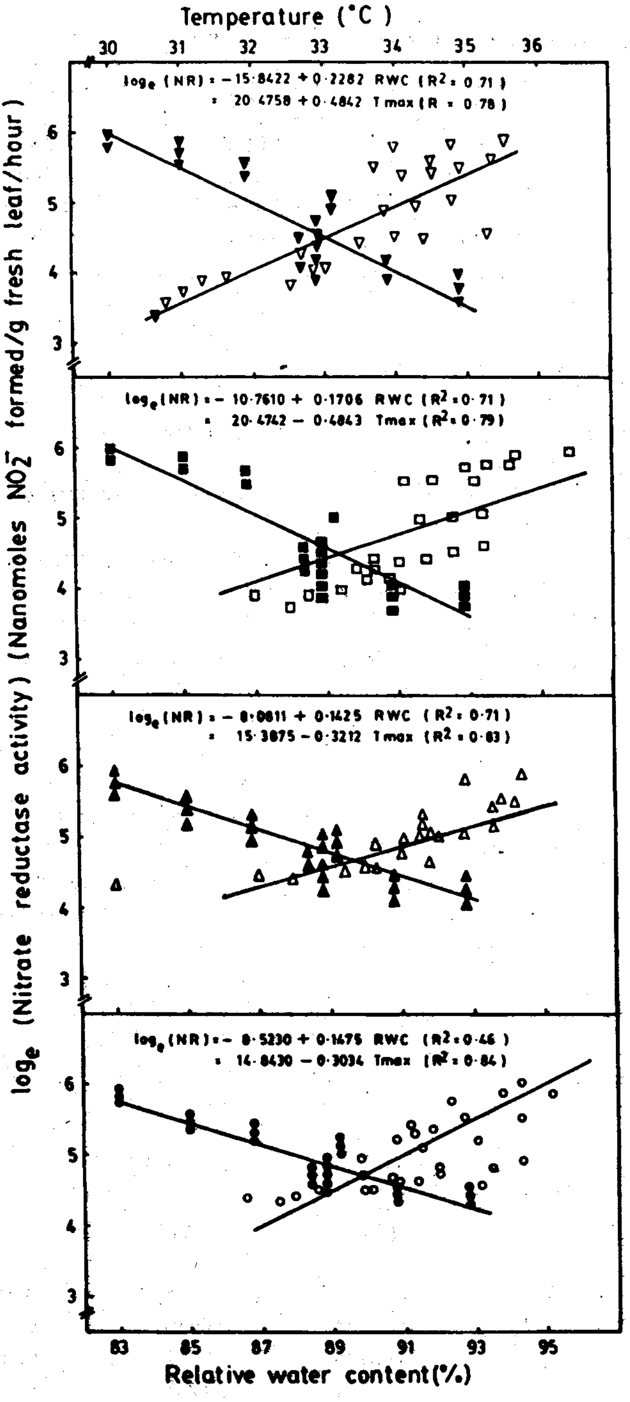

Fig 1. Relationship of $\log_e$ (NR activity) with temperature (Solid symbols) and RWC (open symbols).
O-O WCT Δ-Δ WCT X COD □-□ COD X WCT
∇-∇ COD

Table II **Table of total and partial correlation Co-efficients**

	WCT	WCTxCOD	CODxWCT	COD
r_{12}	-0.9178	-0.9088	-0.8873	-0.8859
$r_{12.3}$	-0.8469	-0.7882	-0.7641	-0.7035
r_{23}	0.6744	0.7466	0.7396	0.8431
$r_{23.1}$	0.1708	0.1924	0.3373	0.5667

1 = Tmax 2 = $\log_e$ (NR activity)
3 = RWC
$r_{12.3}$ means correlation between 1 and 2 eliminating the effect of 3

not wholly due to the reduction in RWC. In other words, the changes in NR activity during the development of stress could not be fully explained by the changes in leaf RWC.

When the influence of temperature (Tmax) was considered, it was found that NR activity was very closely related to changes in temperature (r_{12} = - 0.8859 to - 0.9178). Eliminating the effect due to RWC did not result in any appreciable change in their relationship. ($r_{12.3}$ = - 0.7035 to 0.8469). There was no significant variation among the genotypes in the relationship between NR activity and RWC, and between NR activity and temperature. These results clearly showed that temperature acts directly on the leaf NR activity. Thus, an increase on the atmospheric temperature seems to exert a very strong influence in the leaf NR activity rather than the reduction in leaf RWC *per se*. This was further supported from the results of path coefficient analysis in which it was seen that temperature had a high negative effect on NR activity whereas RWC showed a low direct effect (Table III).

Effect of temperature pre-treatment

The data presented in Table I further showed that the reduction in NR activity of WCT and WCT X COD during drought stress was much less as compared to COD X WCT and COD. In order to see whether the differential decline in the NR activity of genotypes under drought stress could be explained by their heat stability, an experiment was designed in which drought tolerant and susceptible genotypes were subjected to various temperature pre-treatments followed by assay of NR activity. Figure 2 shows the NR activity plotted as a function of temperature. From this it became apparent that the two genotypes which possessed similar activity initially began to show wide differences in activity after treatment at 45° and 50°C for 30 min. The tolerant variety (WCT) maintained 80% and 50% of its initial activity while the susceptible variety (MYD) retained 60% and 30% of the initial activity respectively at 45 and 50°C.

Table III. **Path analysis for NR activity using Tmax max and RWC**

Genotype		T max	RWC	r	Residual effect
WCT	T max	-0.8542	-0.0636	-0.9178	0.1530
	RWC	0.5844	0.0930	0.6774	
WCT x COD	T max	-0.8136	-0.0952	-0.9088	
	RWC	0.6221	0.1245	0.7466	0.1676
COD x WCT	T max	-0.7304	-0.1569	-0.8873	
	RWC	0.5187	0.2209	0.7396	0.1885
COD	T max	-0.5802	-0.3057	-0.8859	
	RWC	0.4399	0.4032	0.8431	0.1461

This observation supports the view that the NR enzyme from tolerant and susceptible genotypes exhibits differences in stability at higher temperatures, which means that the thermal stability of nitrate reductase could be realated to drought tolerance in coconut.

Thermal stability of nitrate reductase in coconut genotypes

Thermal stability NR expressed as the per cent of activity retained by the enzyme at 45°C and 50°C in relation to its activity at 30°C is represented in Fig.3. Accordingly, these genotypes could be classified into two groups, one retaining 40% or more of their original activity after treatment at 50°C and the other retaining less than 40%. Using such a classification, the former group consisting of Java giant, Philippines Ordinary, WCT x WCT, WCT, FMS, Fiji, Laccadive Micro and WCT x COD could be considered as relatively more drought tolerant than the latter group consisting of Gangabondam, Malayan Yellow Dwarf, Andaman Ordinary, Malayan Green Dwarf, SS Apricot, COD x WCT, Malayan Orange Dwarf and COD x COD. These genotypes have been ranked in the decreasing order of tolerance based upon the percentage of activity retained by these genotypes after treatment at 45 and 50°C and are shown in Figure 3. These results are in agreement with those of Rajagopal *et al.* (1990), thus confirming the contention that a higher thermal stability of nitrate reductase is a characteristic feature

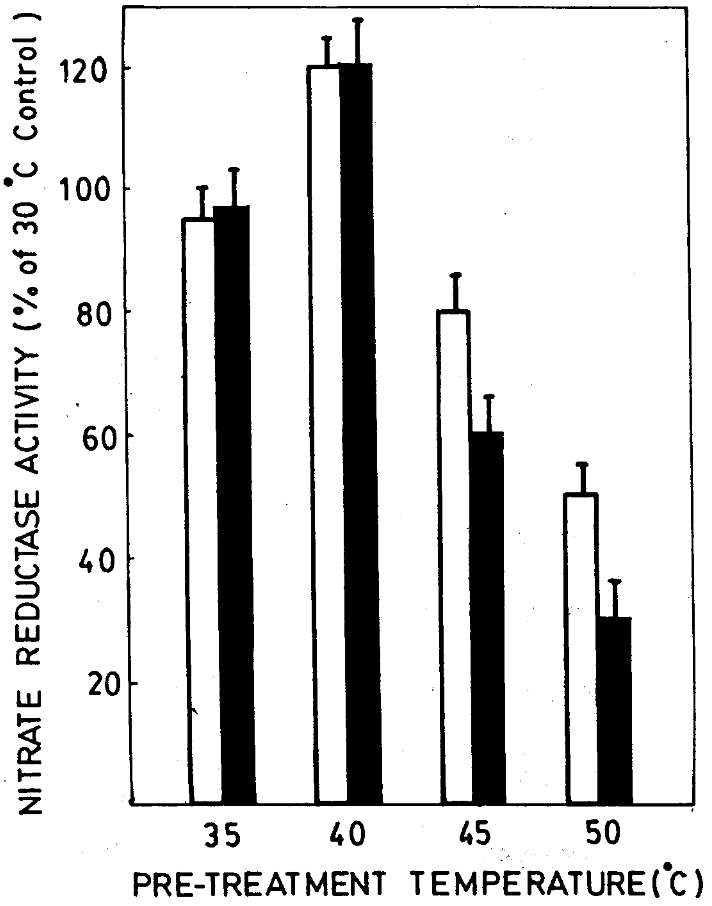

Fig. 2 Changes in nitrate reductase activity of tolerant (□) and susceptible (■) genotypes as a function of temperature

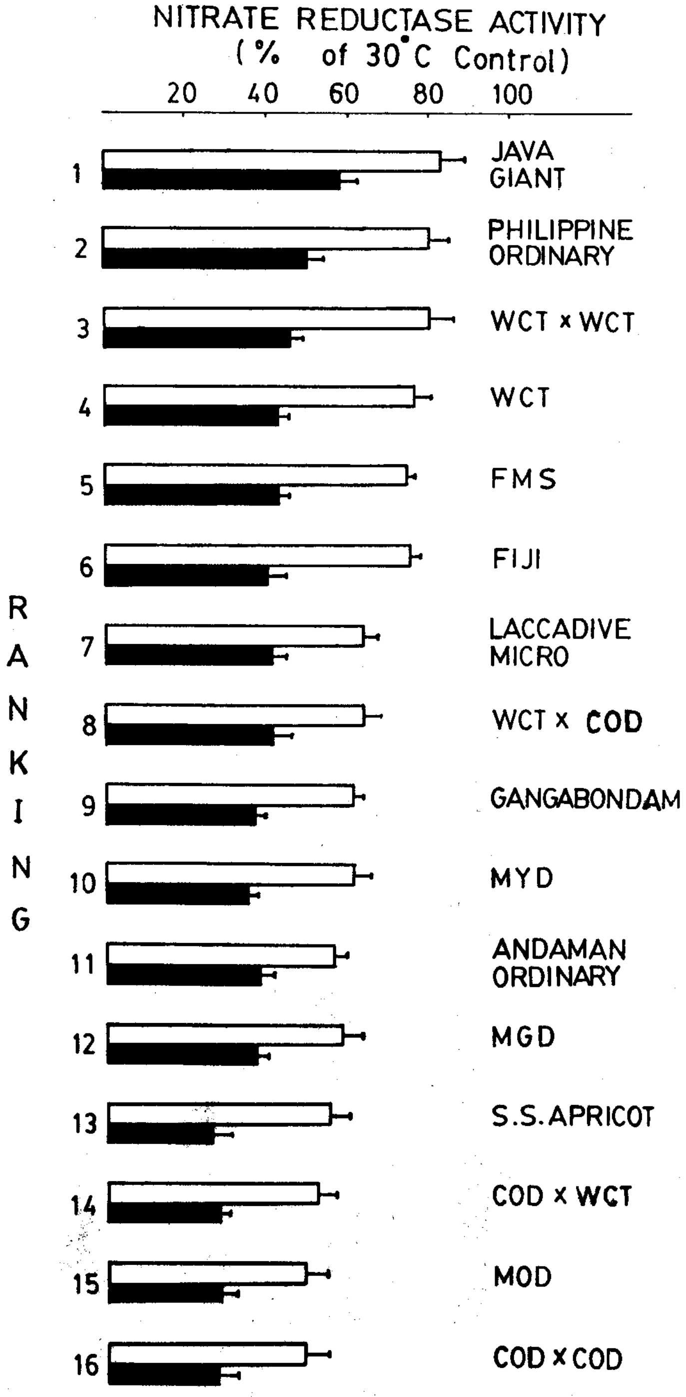

Fig. 3 Ranking of coconut genotypes based on the per cent of NR activity retained at 45°C (□) and 50°C (■)

of the drought tolerant coconut genotypes. It therefore follows that under the agroclimate of Kasaragod, temperatures exceeding 34°C, prevailing even for short durations in the field, could greatly affect the NR activity of the coconut genotypes. It is in this context that a higher thermal stability of nitrate reductase may be playing a vital role.

The present study has thus established the direct effect of increasing temperatures during drought, on the nitrate reduction potential of coconut. The differential thermal stability of NR displayed by the coconut genotypes could, therefore, be used as a valuable adjunct in screening of varieties for drought tolerance.

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