

RELATIVE WATER CONTENT AND PROLINE ACCUMULATION IN COCONUT GENOTYPES UNDER MOISTURE STRESS

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ABSTRACT

Response of two tall (West Coast Tall, WCT, and Laccadive Ordinary, LO) two dwarfs (Chowghat Orange Dwarf, COD and Gangabondam, GB) and four hybrids *viz.*, WCT × COD, COD × WCT, LO × GB and LO × COD of coconut to moisture stress simulated with an osmoticum of 30% polyethylene glycol (PEG - 6000 mol. wt) and air desiccation for similar period of 24 h was investigated. The two methods of simulation had differential effect on the genotypes. In general the relative water content (RWC) of leaves of all the genotypes was reduced more due to air desiccation than the osmotic stress. However the accumulation of proline did not differ much between the two types of stress simulation, except in the dwarfs which exhibited higher proline content in PEG - stressed leaves than in air desiccated ones. There was inverse relationship between RWC and proline accumulation. The data revealed that proline content is not associated with drought tolerance in coconut.

INTRODUCTION

The coconut palm (*Cocos nucifera* L) experiences, moisture stress for four to six months in an year especially in Northern parts of Kerala due to the prevailing weather conditions. Recent studies have shown the influence of the atmospheric and soil drought on coconut genotypes (Kasturi Bai, Voleti and Rajagopal, 1988 and Voleti et al. 1990). As such the concept of drought with different classification (Levitt, 1980) based on annual crops of short duration may not actually apply to crops like coconut, wherein the indeterminate flowering habit influencing yield variations interact with environmental variables over the years. As the time

lag between the initiation of inflorescence primordium and the maturity of coconut is about 44 months, with longer prefertilization (32 months) than post-fertilization (12 months) period, the impact of drought occurring during any of the critical stages of the development of the inflorescence like ovary development, fertilization etc, persist for considerable time. Thus the terminologies drought escape or drought avoidance do not strictly apply to coconut. Hence the term drought tolerance is used in the case of coconut. Rajagopal, Kasturi Bai and Voleti (1990) screened the coconut genotypes for drought tolerance by using the sensitive parameters like stomatal resistance, leaf water potential

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and epicuticular wax content. But not much report has been available on the influence of moisture stress on RWC and proline accumulation in perennial plantation crops except in cocoa (Balasimha et al. 1988). In order to know the influence of moisture stress on RWC and proline accumulation in coconut, the present work was undertaken by inducing artificial stress to the leaflets.

MATERIALS AND METHODS

Eighteen year old coconut palms belonging to talls (WCT and LO) dwarfs (GB and COD) and their hybrids (LO × GB, LO × COD, COD × WCT and WCT × COD) planted with a spacing of 7.5m × 7.5m in a randomized block design were selected for the study. The palms were well maintained under rainfed condition with normal cultural and agronomic practices. Four palms per genotype were selected and from each palm 10–12 leaflets from 14th leaf (Physiologically mature leaf) were collected for the experiment. The leaflets were divided into 4 sets of 12 leaflets each and given the following treatments. One set was kept in 150 ml of 30% PEG (6000 mw) solution corresponding to an osmotic potential of -11 bars for 24 h with occasional stirring, the second set was air desiccated in diffuse day light in the open condition (550 to 600 μ E.m⁻².S⁻¹) with temperature and relative humidity ranging between 27.0°C–29.0°C and 35%–38% respectively as per the procedure of Rajagopal et al (1988). The third set was kept in 150 ml distilled water and maintained as experimental control. The fourth set was immediately processed to determine the initial RWC and proline levels ('0' h). At the

end of the 24 hours samples were analysed for RWC and proline content.

For RWC, ten leaf discs of 1.3 cm diameter were punched throughout the leaflet at random excluding the midrib portion, weighed and floated in deionised water. After 24 hrs the turgid weight was determined and dried in an hot air oven at 80°C for 48 hrs and determined the weight. From this RWC was determined following the method of Barrs and Weatherly (1962). From the remaining part of the leaflet one gram material was taken for the estimation of proline content following the method of Bates, Waldren and Teare (1973).

RESULTS

The RWC did not show any variation initially among the genotype (Table I). It was in the range of 90.0 to 93.9%. In the control set there was no significant change in RWC between '0' and '24' hrs, whereas exposure of leaflets to osmotic stress resulted in the reduction of RWC to varying degree among the genotypes. The difference in RWC between control and stressed was only 6.4% in LO × GB and 8.9% in both LO × COD and WCT, while it was as high as 20.7% in GB. When subjected to air desiccation there was greater reduction in RWC of all the genotype ranging from 61.3% in COD × WCT to 72.9% in COD.

The proline content ranged from 3.6 to 6.6 μ g g⁻¹ dwt initially among the genotypes, the highest content being in GB (Table II). After 24 hrs the control leaves showed a marginal increase from

Table I. *Changes in relative water content (%) of coconut genotypes*

Genotypes	Initial ('O' h)	After 24 hours			Remarks	
		Control	Osmotic stress	Air desiccation		
WCT	90.8	90.4	81.5	68.1	S. E. Plot	2.71
LO	91.1	91.1	79.8	68.0	Gen. Mean	86.40
GB	93.9	88.5	67.8	63.8	C. V. %	3.93
					<i>CD values</i>	
COD	90.4	91.5	78.3	72.9	Genotype	2.21
LO × GB	91.0	87.1	80.7	67.4	Treatment	1.35
LO × COD	91.6	89.4	80.5	64.6	G × T	3.83
					<i>'F' test</i>	
WCT × COD	91.6	92.6	78.6	67.5	Genotype	NS
COD × WCT	91.9	88.9	75.4	61.3	Treatment	160.14**
					G × T	2.80*

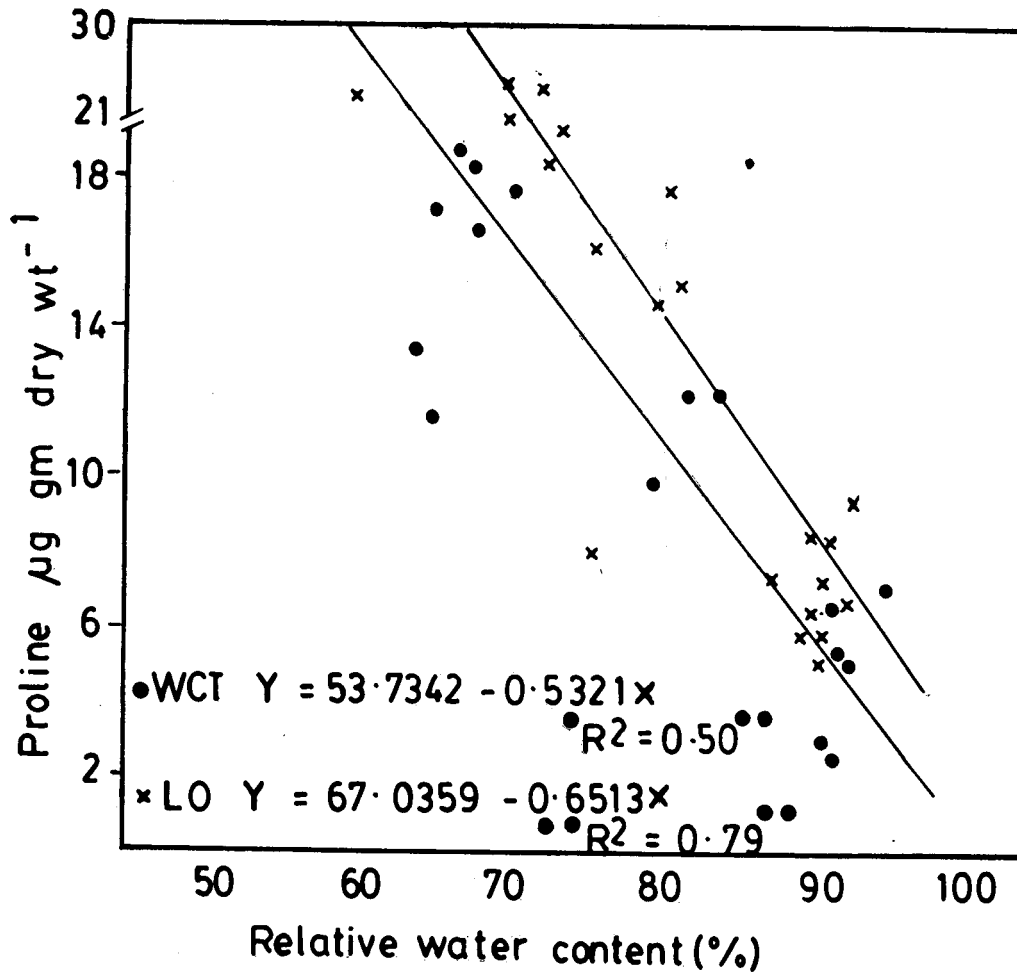
'O' h in all the genotypes except GB and COD which exhibited more than two fold increase. The percentage increase in proline accumulation on 'O' h control to 24 hrs ranged from 14.5 in LO × COD and COD × WCT to 138 in dwarfs. This shows that the two dwarf genotypes had a tendency to accumulate proline. The increase in proline accumulation due to stress stimulation ranged from 277% in WCT × COD to 951% in COD. Figures 1, 2 and 3 show the relationship between

the proline content and RWC for tall, dwarfs and hybrids respectively. Linear regression equations were worked out for individual genotypes and were given in figures. The regression co-efficients were found to be low for tolerant genotypes *viz.*, LO × COD and LO × GB (Fig. 3) as compared to the most susceptible genotypes *viz.*, COD × WCT and GB (Figs. 3 and 2). From the differences in the R² values it is very clear that the variation exist in the accumulation of proline in

Table II. *Proline content ($\mu g g^{-1}$ dry wt.) in the leaf tissues of coconut genotypes*

Genotype	Initial ('O' h)	After 24 hrs.			Remarks	
		Control	Osmotic stress	Air desiccation		
WCT	4.0	5.4	12.0	11.6	S. E. plot	2.32
LO	6.5	8.2	15.8	15.1	Gen. mean	10.97
GB	6.6	15.3	31.8	19.6	CV (%)	21.68
					<i>CD values</i>	
COD	5.6	13.2	36.1	12.0	Genotype	1.93
LO × GB	3.6	5.3	15.1	16.9	Treatment	1.18
LO × COD	4.0	4.5	15.3	13.8	G × T	3.35
WCT × COD	4.5	8.5	17.2	17.8	<i>'F' test</i>	
					Genotype	43.55**
COD × WCT	4.1	4.1	16.4	21.7	Treatment	358.67**
					G × T	12.62**

Fig. 1. Relationship between RWC vs proline for tall coconut genotypes

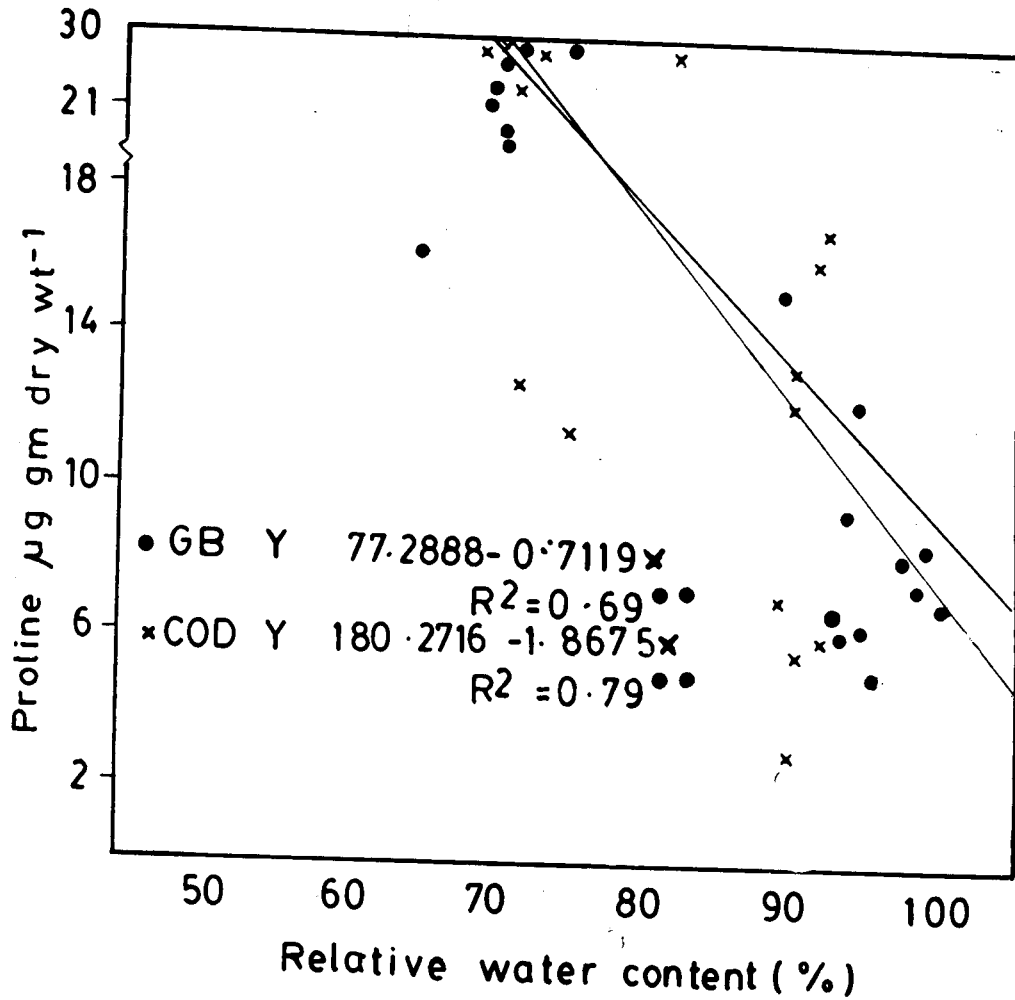


respect of the changes in the RWC among the hybrids, dwarfs and tall. From Fig. 3 it is very clear that the proline accumulation pattern in the tolerant genotypes LO × GB and LO × COD was not on par with the changes in RWC and are negatively correlated ($r^2 = 0.86$).

DISCUSSION

Effect of osmotic stress on RWC and its utility in screening for drought resistance has been reported in cereal crops like maize and oats (Downey, 1971, Sandhu and Horton, 1977 respectively). However coconut responded to higher concentration of osmoticum and also

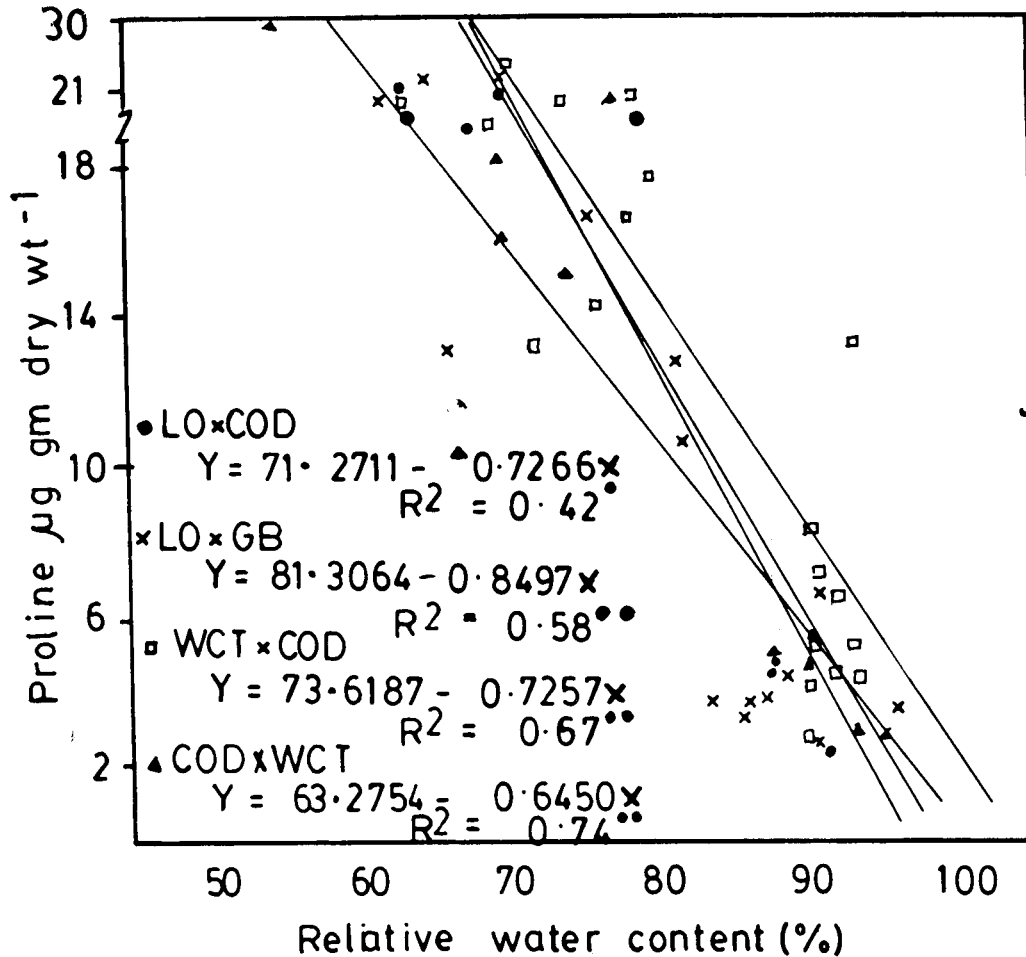
Fig. 2. Relationship between RWC vs proline for dwarf coconut genotypes



took longer duration for stress effects than the cereals obviously due to the thick and fibrous nature of coconut leaf tissues. The maintenance of higher RWC by LO×GB, LO×COD and WCT than the other genotypes indicates the degree of stress tolerance while the low RWC in the dwarf genotype (GB) the susceptibility. This is in support of the data obtained with stomatal resis-

tance, leaf water potential and epicuticular wax content which also showed that the hybrids LO×GB and LO×COD and the tall WCT to be more tolerant to drought than the other genotypes (Rajagopal et al. 1990). This indicates that RWC determination can be reliably used for screening large number of samples for drought tolerance in coconut.

Fig. 3. Relationship between RWC vs proline for hybrid coconut genotypes



The relationship between proline accumulation and drought resistance is well established in literature with annual crops (Aspinall and Paleg, 1981). In response to osmotic stress proline content was enhanced significantly in all the genotypes, with both GB and COD maintaining distinctly higher levels than the others. Stress induced by air desiccation also resulted in high content of proline, but the level was less than that

in PEG-stressed leaves in all except LO×GB and COD×WCT hybrids which had a higher content with desiccation. Even the water control leaves after 24 hrs showed a marginal increase from 'O' h in all the genotypes except GB and COD, which exhibited more than two fold increase. The accumulation of proline in the detached leaves not subjected to any water stress has been reported by Singh, Aspinall and Paleg

(1973). While proline accumulation was positively correlated with drought resistance in barley, sorghum and bean (Singh, Paleg and Aspinall, 1973; Blum, 1974; Stewart and Boggers, 1977); there was no such relationship in wheat and soyabean (Sinha and Rajagopal, 1977; Guo, Liu and Zhang, 1988). In barley proline accumulation was shown to be a heritable trait (Stewart and Hanson, 1980). In soyabean (Guo et al., 1988) high leaf water holding capacity and low proline content has been taken as criteria in the selection for drought resistance. In the present study the drought tolerant genotypes LO × COD and LO × GB failed to accumulate proline, while the susceptible ones *viz.*, dwarfs tended to show higher proline content. This corroborate with the result of soyabean that the tolerant genotypes responded to water stress with low proline content and high water holding capacity.

The maintenance of higher RWC and

lower proline accumulation under osmotic stress in the tolerant genotype indicates its suitability in the screening work. Since the accumulation of proline as a stress indicator is a controversial aspect, RWC can be taken as a positive measure of drought tolerance in coconut palm than the proline content. The usefulness of RWC as a better parameter than water potential, to explain the physiological changes in leaf has been reported by Schulze (1986). The present study also indicates the importance of RWC in the screening of drought tolerant genotypes in coconut.

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