Is stress induced proline accumulation an indicator of leaf water status in Coconut?

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Abstract

Hybrid seedling combinations comprised of three Dwarfs (COD, CGD and MYD) each crossed with three Talls (WCT, FMS and Fiji) were evaluated based on. proline accumulation capacity under induced stress condition (by air desiccation) as well as recovery potential of the seedlings after relief from moisture stress under field condition. Leaf water potential ($Ø_{leaf}$) was determined during non-stress, stress and recovery periods. Seedling combinations did not show significant differences in *Øleaf* during both non-stress and stress periods. However, proline concentrations showed significant differences between the seedling combinations as well as between the treatments. The interaction between seedling combinations and the treatments was also found to be significant. Correlation worked out between the $Ø_{leaf}$ and the proline concentration did not show any relationship. Recovery of the seedlings after relief from stress indicated that among the seedling combinations CGD x WCT, CGD x FMS and MYD x WCT are comparatively more tolerant to water stress than the others. Proline accumulation capacity did not show any relationship with the recovery potential of the seedlings. This implies that leaf proline concentration is not a good indicator of leaf water status in coconut.

Key words: Coconut, proline, leaf water stress

Introduction

Accumulation of osmotic solutes-both sugars and amino acids in water stressed plants have been reported by several workers (Hsiao *et al.*, 1976; Munns and Wier, 1981; Kasturi Bai and Rajagopal, 2000). Proline is one of the important amino acids, which makes a substantial contribution towards osmotic adjustment and adaptation to stress (Aspinall and Paleg, 1981; Handa *et al.*, 1986; Rabe, 1990). The increase in proline is related to a decrease in leaf water potential (Blum and Ebercon, 1976). Genotypic differences in proline accumulation were shown in barley (Hanson *et al.*, 1977; Singh *et al.*, 1973) and Sorghum (Blum and Ebercon, 1976) and drought tolerant types were found to accumulate more proline (Singh *et al.*, 1973). In tomato, Thakur (1991) and in *Phaseolus*, Lazcano Ferrat and Lovatt (1999) reported that proline concentration in the leaf is an indicator of plant water status. The role of proline as a source of fixed nitrogen contributing to recovery of the plants after relief from stress has been reported (Blum and Ebercon, 1976). In Sorghum, Sivaramakrishnan *et al.* (1988) observed accumulation of proline takes place only under severe water deficit. In coconut Voleti *et al.*, 1990 observed no correlation between proline accumulation and drought tolerance. The objectives of the study are to understand the accumulation and role of leaf proline concentration in Dwarf x Tall (D x T) coconut seedlings and to evaluate D x T cross combinations for tolerance to drought.

Materials and Methods

Two year old seedlings of DxT cross combinations of three dwarfs (Chowghat Orange Dwarf - COD, Chowghat Green Dwarf - CGD and Malayan Yellow Dwarf- MYD) each crossed with three Talls (West Coast Tall- WCT, Federated Malayan States-FMST and Fiji-FIJT) were the material for the study. The seedlings were growing in the nursery as per standard conditions (Anonymous, 1985) in red sandy loam soil in a completely randomized design. Observations on leaf water potential and proline concentration were determined under induced stress condition in the leaflets sampled from 2nd leaf from the spindle. Stress was imposed by air desiccation of the detached leaflets for 24 hrs. Leaf water potential ($Ø_{leaf}$) was determined in the leaf lets before and after stress induction. To evaluate the seedlings for drought tolerance the seedlings were field stressed by withholding the irrigation for 22 days and recovery potential was determined after re-irrigating the seedlings for 10 days. From each combination six seedlings were randomly selected for the observations, each seedling was treated as a single replication. $\emptyset_{leaf}$ was determined during all the periods (non-stress, stress and recovery) using Scholanders' Pressure Chamber, as standardized for coconut by Milburn and Zimmerman (1977) and Rajagopal *et al.* (1987). Proline concentration was estimated as per the method of Bates *et al.* (1973). The data was analysed statistically as per standard methods (Panse and Sukhatme, 1967).

Results and Discussion

During the experimental period photosynthetically active radiation ranged from 1000–1300 $\mu E\ m^{-2}s^{-1}$, air temperature 32-34 °C and VPD 2.5 – 2.8 Kpa. Soil moisture content, which was 13% under irrigation, reduced to 5-6% (up to 100cm depth) with a soil water deficit of 54 mm after with holding irrigation for 22 days.

Under induced stress condition (by air desiccation), $\emptyset_{leaf}$ did not show significant differences between the seedling combinations during unstressed or stressed periods. However, between the two periods, the differences were found to be significant (Table 1). Among the seedling combinations comparatively lower water potential was observed in COD x FMST than the others. On the other hand, proline concentration showed significant differences between the seedling combinations as well as between the two periods. The interaction was also found to be significant (Table 1).

Table 1. Variations in leaf water potential (LWP) and proline concentration in coconut seedlings during non- stress (NS) and stress (S) periods.

Seedling combinations	Leaf water potential (- bars) NS	S	Mean	Proline ($\mu g\ g^{-1}$ d.wt) NS	S	Mean
COD x WCT	-6.87	-21.40	-14.14	38.55	119.10	78.83
COD x FMST	-8.23	-26.33	-17.28	37.37	114.51	75.94
COD x FIJT	-7.53	-20.00	-13.77	40.06	105.34	73.20
CGD x WCT	-6.71	-21.80	-14.26	36.79	73.82	55.30
CGD x FMST	-6.70	-22.07	-14.39	42.69	75.99	59.34
CGD x FIJT	-7.13	-22.20	-14.67	48.33	71.59	59.96
MYD x WCT	-5.80	-23.67	-14.74	39.66	65.43	52.55
MYD x FMST	-6.10	-21.20	-13.65	33.58	60.49	47.04
MYD x FIJT	-7.33	-21.87	-14.60	35.33	54.42	44.88
Mean	-6.93	-22.28		39.15	82.41	
CD: P=0.05						
Seedlings:	NIL				17.294	
Treatment:	1.299				8.152	
Interaction:	NIL				24.457	

Among the seedling combination, COD crosses accumulated more proline (range 106.3 to 119.1 $\mu g\ g^{-1}$ d.wt) than the other crosses and lower accumulation was observed in MYD crosses (range-54.4 to 65.4 $\mu g\ g^{-1}$ d.wt) under stress. Correlation worked out between the leaf water potential and proline accumulation did not give any correlation between the two. Naidu *et al.* (1992) reported that in barley leaves proline is more closely co-related with decreasing relative water content (RWC) than with $\emptyset_{leaf}$. Relationship between RWC and proline concentration has been reported in coconut (Voleti *et al.*, 1990). Critical examination of the values for $\emptyset_{leaf}$ as well as proline concentration among the combinations revealed that the accumulation of proline did not commensurate with the $\emptyset_{leaf}$ (Fig. 1). This confirms that the extent of accumulation of proline is not an indicator of leaf water status in coconut contrary to that reported in *Phaseolus* (Lazcano Ferrat and Loratt, 1999).

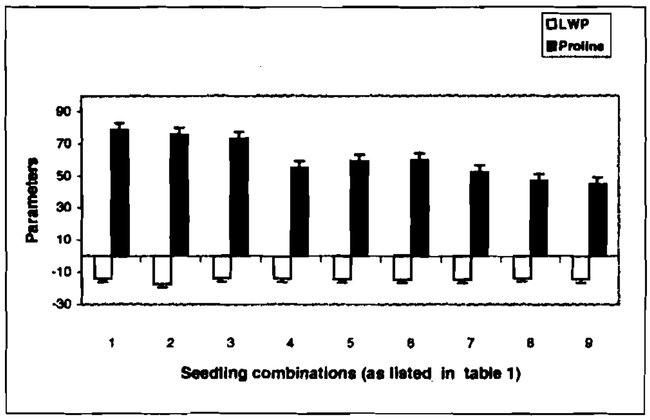

Fig. 1. Relationship between leaf water potential (LWP- bars) and proline concentration ($\mu g\ g^{-1}$ d.wt) in coconut seedlings

Recovery potential of the seedlings after relief from stress is an important trait for drought adaptation (Miah *et al.*, 1988). The seedlings were further evaluated under field condition during March/April months consecutively for two years. $\emptyset_{leaf}$ was determined during non-stress (under full irrigation), stress (withholding irrigation for 22 days) and recovery (after irrigating the seedlings for 10 days). Although, reduction in leaf water potential to the tune of 26 to 50% was observed among the seedlings (except in COD combinations - 8%) after withholding irrigation for 22 days with a soil water deficit of 54 mm, actual leaf water potential values were in the range of -7.5 to – 9.5 bars (Table 2). This indicates that seedlings were not severely stressed even after withholding irrigation for 22 days. The reason for this can be attributed to conservation of moisture by the husk of seed nuts that persists in the seedlings even after two years of growth. This implies that the schedule of irrigation of the coconut nursery once in a week

(Anonymous, 1985) can be re-scheduled to once in 15 / 20 days depending on the severity of the meteorological condition existing during that period. Although $\emptyset_{leaf}$ did not return to the original levels, the seedlings recovered to different degrees on re-watering. Based on the $\emptyset_{leaf}$ during non-stress, stress and recovery periods, the seedlings were ranked, indicating that among the seedling combinations, CGD x WCT, CGD x FMS and MYD x WCT are more adapted to water stress than the others. These combinations maintained higher $\emptyset_{leaf}$ during both stress and recovery periods (Table 2). Further observations are needed to confirm this. In contrast to the report in sorghum (Blum and Ebercon, 1976), proline accumulation capacity of these seedlings did not show any relationship with the recovery potential (Table 3). For example CGD x FMST has higher recovery potential even with less accumulation of proline (78% increase in proline during stress over control condition). On the other hand, the recovery potential of CGD x WCT is less than that of CGD x FMST, even though it exhibited greater accumulation of proline (100.7% over control) during stress period. Similar trends can be observed in other combinations as well.

Table 2. Variations in leaf water potential (- bars) in coconut seedlings during non stress (NS) stress (S)and recovery (R) periods in field condition.

Seedling combinations	NS	S	R
COX x WCT	-6.27	-8.67	-7.92
COD x FMST	-7.35	-8.63	-9.48
COD x FIJT	-6.71	-9.33	-8.68
CGD x WCT	-5.14	-7.01	-6.75
CGD x FMST	-5.43	-7.57	-6.80
CGD x FIJT	-5.46	-8.19	-8.59
MYD x WCT	-5.96	-7.53	-7.27
MYD x FMST	-7.17	-9.53	-8.22
MYD x FIJT	-6.18	-8.66	-8.06
Mean	-6.17	-8.43	-8.01

CD: P = 0.05: Seedlings: 0.879: Treatment: 0.465

Table 3. Proline accumulation (over NS) and increase (+) / decrease (-) in leaf water potential (LWP) during stress (over NS) and recovery (over stress) periods in seedling combinations.

Seedling combinations	Proline (%)	Leaf water potential	
		Stress (%)	Recovery (%)
COD x WCT	208.95	-38.3	+8.7
COD x FMST	206.42	-8.5	-18.8
COD x FIJT	165.45	-38.9	+6.9
CGD x WCT	100.65	-42.5	+4.9
CGD x FMST	78.00	-65.7	+24.4
CGD x FIJT	48.13	-50.1	-4.9
MYD x WCT	64.98	-26.2	+3.5
MYD x FMST	80.14	-33.0	+13.8
MYD x FIJT	54.03	-40.1	+6.9

From the observations it is clear that as in other crop plants in coconut also proline accumulates with the development of stress. However, this accumulation of proline neither reveal the actual water status of the leaf nor stress intensity. The observation further confirms that accumulation of proline in coconut under stress is not a good trait for screening for drought tolerance. In an earlier study Kasturi Bai and Rajagopal (2000) reported higher accumulation of free amino acids in coconut under severe stress conditions, where the soil moisture content ranged between 3 -4%. Thus, it can be presumed that higher accumulation of proline during stress period may improve the pool size of the free amino acid concentration which contributes to the osmotic adjustment of the seedlings under severe stress.

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