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Osmotic adjustment, proline accumulation and cell membrane stability in leaves of *Cocos nucifera* submitted to drought stress

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ABSTRACT

The role of drought-induced proline accumulation in coconut leaves is still unclear. With the objective of evaluating the impact of water shortage on leaf osmotic potential, proline accumulation and cell membrane stability in young plants of two Brazilian Green Dwarf coconut ecotypes from contrasting areas (Brazilian Green Dwarf from Una, Bahia, UGD, and from Jiqui, Rio Grande do Norte, JGD), a pot experiment was conducted under greenhouse conditions. Three drought cycles consisting of suspension of irrigation until the net photosynthetic rate (A) approached zero and rewatering until recovery of A to 85% of the irrigated control plants. Pre-dawn leaf water potential (Ψ_{PD}) reached −1.2 MPa at the point of maximum stress (PMS). Dry matter production and leaf area were severely reduced by drought treatment in the two ecotypes. Corrected values of osmotic potential were significantly reduced in stressed plants of the two ecotypes. Green dwarf coconut palm showed low osmotic adjustment (from 0.05 to 0.24 MPa) and significant accumulation of proline (from 1.5 to 2.1 times in relation to control) in leaflets in response to water deficit. Considering the growth reduction observed in both ecotypes, proline was not associated to osmoregulation. On the other hand, the absence of membrane damage, as indicated by electrolyte leakage method, suggests that the protective role of proline in this specie can be more important. The two ecotypes of Green dwarf coconut palm behaved similarly in the present experiment for most traits evaluated. Slight differences among the ecotypes were observed with respect to the response to treatments, such as higher proline accumulation in JGD.

1. Introduction

Coconut palm (*Cocos nucifera* L.) is a pan tropical species with great social and economic importance for the countries where it is cultivated. The effects of water deficit on the physiology, growth and productivity of coconut have been widely reported (Repellin et al., 1994, 1997a,b; Rajagopal and Kasturi Bai, 1999, 2002; Prado et al., 2001; Passos et al., 2009; Gomes et al., 2008, 2009; Gomes and Prado, 2010). These reports highlight some important traits such as stomatal control of water status in stressed and non-stressed plants, as well as the search and validation of morphological, physiological and biochemical indicators of drought tolerance, as potential criteria for breeding and selection of tolerant genotypes (Gomes and Prado, 2010).

Short-term responses of coconut to water stress such as low biomass accumulation and partitioning, reduced stomatal conductance to water vapor (gs) and leaf water potential (Ψ_w) which often impair photosynthesis (A) and transpiration (E) have been extensively documented (Gomes and Prado, 2010). Decreases in A in both tall and dwarf genotypes in response to atmospheric and soil water deficit have been reported (Prado et al., 2001; Gomes et al., 2008). Moreover, both stomatal and non-stomatal factors have been demonstrated to contribute to the reduction in A during a period of water deficit and during the recovery phase after resuming irrigation (Gomes et al., 2008). Besides photosynthesis, dry matter production and its partitioning are largely influenced by the water availability (Rajagopal et al., 1989).

It has been suggested that the accumulation of some compounds such as sugars, amino acids, alcohols and quaternary ammonium is an important feature of overcoming environmental stresses (Morgan, 1984). Those so-called compatible osmolytes lower water activity and reduce the osmotic potential of the cell so that turgor and turgor-related processes may be maintained during drought episodes. Moreover, the osmolyte may act as osmoprotectants, stabilizing macromolecular structures and/or subcellular membranes (Morgan, 1984). Proline accumulation in leaves of drought-stressed plants and its role as an osmolyte or osmoprotectant has been the theme of a long-standing debate (Seki et al., 2007; Szabados and Savoure, 2009). In *C. nucifera*, Jayasekara et al. (1993) found high levels of proline in leaves of tolerant genotypes during the

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dry season. However, the high contents of proline detected in coconut leaflets submitted to dry air (mainly in dwarf varieties) were unlikely to be directly associated with drought tolerance (Voleti et al., 1990; Kasturi Bai and Rajagopal, 2004).

Despite osmotic adjustment has been reported as an important component of drought tolerance mechanisms in various coconut genotypes (Kasturi Bai and Rajagopal, 2000), the importance of such strategy for coconut drought tolerance is still unclear (Gomes and Prado, 2010). As for proline, the results regarding the importance of osmotic adjustment for coconut drought tolerance are also contentious.

Cell membrane stability as measured by the electrolyte leakage technique has been used as a tolerance index for abiotic stresses, such as salinity (Ghoulam et al., 2002), drought (Sayar et al., 2008; Yang et al., 2009), air pollution (Neves et al., 2009), cold and heat (Arvin and Donnelly, 2008). The degree of cell membrane injury caused by stress can be assessed using this technique. It is relatively simple, repeatable, and rapid and requires inexpensive equipment.

This study aimed to evaluate the impact of repeated cycles of water stress on osmotic potential, proline accumulation and cell membrane stability in leaflets of two Brazilian Green Dwarf coconut ecotypes from contrasting areas in relation to climate. The hypotheses tested: (1) Dwarf coconut use the osmotic adjustment as a strategy for growth maintenance under water deficit and (2) such an adjustment is dependent on proline accumulation.

2. Materials and methods

2.1. Plant material and growing conditions

A pot experiment was conducted in the Plant Growth Unit of the Universidade Federal de Viçosa, Brazil (20°45′S, 42°52′W, 648 masl) with two ecotypes of the Brazilian Green Dwarf cultivar from contrasting regions of the Northeastern Brazil: Brazilian Green Dwarf from Una (UGD) is cultivated in the municipality of Una at the southeast region of Bahia State (15°15′S, 39°05′W, 105 masl) and Brazilian Green Dwarf from Jiqui (JGD) is cultivated in the Rio Grande do Norte State (05°47′S, 35°12′W, 30 masl) (Gomes et al., 2008).

The plants were grown in containers of 150 L filled with a soil/sand mixture (1:2), under greenhouse conditions and were irrigated daily to maintain the soil water content close to field capacity. All the essential nutrients were supplied, following the leaf and soil analyzes and the requirements of the species (Magat, 2003).

When the plants were 12 months-old, the drought treatment was initiated by suspension of irrigation until the net photosynthetic rate (A) approached zero, which was considered the point of maximum stress (PMS). Then, those so-called water stressed (WS) plants were rewatered until recovery of A to values >85% in relation to the well-watered (WW) control plants. This procedure was repeated two times, giving a total of 3 consecutive drying/recovery cycles. A Portable Photosynthesis System LI-6400 (LI-COR Biosciences Inc., Nebraska, USA) was used for measurements of A weekly, according to the procedure described by Gomes et al. (2009). The measurements were also performed in the fourth and eighth days after rewatering the plants.

2.2. Leaflet water potential

Pre-dawn leaflet water potential (Ψ_{PD}) was measured weekly at 4:30 am in one leaflet of four randomly selected plants per treatment, with a pressure chamber Skye SKPM 1400 (Skye Instruments LTD., UK). All measurements were done on leaflets opposite to those used for gas exchange measurements, following the methodology described by Milburn and Zimmermann (1977) with modifications. Briefly after detaching the leaflet, the base of the lamina was trimmed with a sharp knife to free the cylindrical midrib the leaflet was rolled (keeping the adaxial surface outermost to avoid breaking the rib) and sealed into the pressure chamber. The pressure was increased slowly (0.02 MPa s^{-1}) with compressed nitrogen until the sap was visible at the exposed base of the midrib, characterizing the balancing pressure.

2.3. Osmotic potential and osmotic adjustment

Osmotic potential was measured at the points of maximum stress and after eight days of recovery in each cycle. Samples of mature leaflet blades stored in plastic bags at −20 °C were thawed and pressed using a hand press. Then the liquid obtained was centrifuged at 2500 × g for 20 min and the supernatant used for the determination of osmotic potential (Ψ_{S}) in four randomly selected replicates per treatment of each ecotype. Determination of Ψ_{S} was performed using a microosmometer (Osmette 2007, Precision System, Inc., USA). To avoid the effects of passive concentration associated to water loss (Babu et al., 1999), the values of Ψ_{S} (stressed and control) were multiplied by a factor based on the tissue dry weight, which referenced osmotic potential to a common water content of 70% of fresh weight (Alves and Setter, 2004). Osmotic adjustment (OA) was calculated as the difference in corrected osmotic potential (Ψ_{SC}) between control and stressed plants.

2.4. Proline concentration

Samples of leaflet tissue were collected simultaneously with gas exchange measurements and immediately frozen in liquid nitrogen. All the samples were stored at −80 °C until the analyses. Proline concentration was determined in 80% ethanol extracts of fresh leaflet tissue using the nyhnidrin method (Bates et al., 1973).

2.5. Growth measurements

At the end of experiment four plants of each ecotype in each treatment were harvested and separated into roots, shoots and leaves. Total leaf area was measured using a Li-3000 (LiCor Biosciences, INC., USA) leaf area meter. Dry mass weight was obtained after drying the plant parts in a forced air circulating oven at 70 °C for 72 h. Specific leaf area was obtained from the ratio between leaf area and leaf dry mass.

2.6. Electrolyte leakage measurements

Electrolyte leakage was determined on leaflet disks removed from the material used for osmotic potential measurements as described by Ghoulam et al. (2002) with some modifications. Thirty leaflet disks (5 mm diameter) were thoroughly rinsed in deionized water and immersed in a 15 mL of deionized water for 60 min. Percent electrolyte leakage of the sample was estimated by measuring the electrical conductivity (EC) of the water after 60 min (EC60) and after disrupting cell membranes by heating the samples up to 100 °C for 30 min (ECboil). Membrane stability was estimated as [Electrolyte leakage % = (EC30/ECboil) × 100].

2.7. Statistics

A completely randomized design was used with two ecotypes × two irrigation regimes × three drying/recovery cycles and four replicates. The data were submitted to factorial analyze of variance (ANOVA), with treatment (drought and control), ecotype (UGD and JGD) and cycle as main factors. The Tukey's test at prob-