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STOMATAL STUDIES OF COCONUT(*COCOS NUCIFERA* L.) VARIETIES AT COASTAL AREA OF PAKISTAN

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Abstract

Leaf samples of six coconut varieties were collected to determine stomatal studies. Scanning electron micrograph showed that stomata, found only on the abaxial epidermis, were arranged in parallel rows and sunken below the epidermis. The stomatal complex is characterized by the presence of two guard cells and four subsidiary cells, two of which are roundish and the other two are lateral to the guard cells. The values of stomatal features were statistically significant for some Tall, Dwarf and Hybrid coconuts. The mean number of the stomates per mm^2 of leaf surface were higher in Tall and Hybrid compared to the Dwarf varieties of coconut. The mean length of the varieties ranged from 31.66µm to 39.06µm and the width 9.46µm to 12.8µm was noted. Stomatal densities of the Sri Lanka Green Dwarf and Ranthambly have 215.18mm^2 and 189.87mm^2 respectively.

Introduction

The coconut tree (Cocos *nucifera* L.) is known as the tree of life because of its range of environmentally sustainable uses (Foal, 2003). Among the plantation crops of tropical humid areas, coconut occupies an important position in view of the commercial value of its oil on the international market. This monocot tree crop grows well in warm humid weather with an estimated 120 sunshine hours monthly (Murray, 1977).The ideal temperature is around 27°C with a diurnal variation of 5°C to 10°C. Coconut is found in places where the annual precipitation is between 1300 and 2500 mm or above. An average monthly rainfall of 150 mm is ideal for good growth and high nut yield. A prolonged dry spell lasting from 5 to 7 months adversely affects the palms (Rajagopal *et al*., 1990). The stomata allow gases exchange. In green leaves they occur either on both surfaces (amphistomatic leaf) or only one, either the upper (epistomatic leaf) or more commonly on lower that is hypostomatic leaf and in dicotyledonous flora of Karachi 6 major type of stomata are recognized such as anomocytic, paracytic, diacytic, anisocytic, parallelocytic and cyclocytic types were noted (Perveen *et al*., 2007). According to (Abid *et al*., 2007) monocotyledonous flora of Karachi clearly reveals the presence of three major types of stomata such as paracytic, tetracytic and anomocytic type and Gramineae, Cyperaceae, Palmae and Juncaceae are characterized by paracytic type. The stomates are arranged in parallel rows as noted in leaves with parallel veins such as those of monocotyledons and some dicotyledons (Esau, 1977; Cutter, 1969).

Menon & Pandalai, (1958) observed to occur of nearly 200 stomata in each square millimeter of leaf surface of the coconut palm, each stoma being approximately 38u x 40u including the subsidiary cells. Ghose & Davis, (1973) noted that the size of the stomata either increases (*Cocos nucifera* L.) or remains more or less the same in young and adult palms. Similarly, the number of rows of inter stomatal cells and their width either increases or decreases slightly in adult. A low stomatal conductance is of consequence in the evaluation and screening of plant type for drought resistance. Higher resistance indicate reduced water loss, hence it's important in the maintenance of water status. The resistance to transpiration helps to maintained higher water potential of plants that imports turgidity. It was observed whether the determinations were made at different times of the day (6–18 h) or under irrigated and unirrigated conditions or in different seasons ('dry' and 'wet'). Thus, the stomatal regulation was significantly impaired in the diseased palms resulting in excessive water loss compared to the healthy palms (Rajagopal, 2008).

Nainanayake & Morison, (2007) reported that the drop in coconut production when soil water is deficient is mainly due to limitations at the stomatal level rather than at the non-stomatal (biochemical) level of the assimilation process, especially in mild to moderate drought conditions. The effects of drought on stomatal conductance and water potential of four coconut genotypes were measured to develop an index for stomatal performance, Camron red dwarfwas identified as a drought sensitive genotype compared to the rest (Dewwanthi Karunarathna, 2008). Therefore stomatal resistance is considered as a parameter for screening cultivars for drought tolerance in coconut. The objective of the present studies was to differentiate the coconut type and screen coconut variety for their tolerance to drought using plant water relationship and stomatal study.

Materials and Methods

The coconut leaves for study were collected from Costal Agricultural Research Station (CARS), Pakistan Agricultural Research Council (PARC), Saleh Muhammed Goth, Karachi in June, 2009. The palm was identified with the help of Bioversity/ COGENT, descriptor, 2008. The Tall varieties consisted of Sri Lanka Tall (SLT), Ranthambly (RAN) and Gonthambly (GON). The Dwarf and Hybrid type of coconut varieties sampled were Sri Lanka Green Dwarf (SGD), Malayan Red Dwarf (MRD) and Hybrid (TXT) varieties (Anon., 2008).

The fresh leaves were collected from mid region of the leaf No.7. All leaflets were washed with tap water and air dried. The stomata were examined using scanning electron microscope (SEM, Model JEOL, JSM 6380A) made in Japan at the Centralized Laboratory University of Karachi. Before analyzing the sample on SEM, they were coated with gold up to 300 A0. Model no. of the coater used is JFC-1500 made in Japan. The scanning electron micrographs of the stomates were also taken. More over, the length of the stomatal pore was measured from tip to tip using a calibrated eyepiece micrometer. Width of the stomatal pore was measured from the middle portion of the stomata. Arrangement of stomata in the epidermal layer was also noted.

A thin layer of natural nail polish was applied to a $1cm^2$ strips approximately at the middle portion of the lower epidermis of leaflets. When the nail polish dried up, the impression of the stomata was pulled away slowly with forceps and mounted on glass slides. Stomatal density was determined using a light microscope at 40x magnification (Olher, 1984; Solangi, 2001).

The data recorded for all the traits were also categorized into different varieties. The statistical evaluation of the stomata included mean, standard deviation and difference of standard error (Choudhary & Kamal, 2004).

Table 1. **Length and width of Stomates, Stomatal density and total number of Stomates /leaf of Tall, Dwarf and Hybrid coconut varieties.**

Coconut varieties	Stomatal length (um) Mean ± SE	Stomatal width (um) Mean ± SE	Stomatal density (No./mm^2) Mean ± SE	Total number of stomata/leaflet (x104) Mean ± SE
Sri Lanka Tall (SLT)	36.3 ± 1.06	12.5 ± 1.06	185.65 ± 2.10	98055.49 ± 1074.43
Gonthambly (GON)	38.7 ± 0.80	12.33 ±0.95	183.54 ± 9.67	58070.96 ± 5040.30
Ranthambly (RAN)	33.1 ± 0.84	9.46 ± 0.06	189.87 ± 7.31	73523.79 ± 9789.62
Sri Lanka Green Dwarf (SGD)	31.66 ± 0.04	10.33 ± 0.28	215.18 ± 14.63	55584.96 ± 3247.88
Malyan red Dwarf (MRD)	39.06 ± 1.74	12.8 ± 1.21	229.06 ± 5.82	58410.81± 2270.51
Hybrid (T&T)	33.36 ± 1.71	11.3 ± 0.35	194.09 ± 11.75	82960.56 ± 4823.09

± S.E = Standard error
Each figure represents the mean of three leaves
Mean ± SE Compared using paired T-test
Mean significant at p= 0.05

Results and Discussion

The data presented in Table 1 indicates the stomatal characteristic such as length width and density per unit leaf area. The highest stomatal length was 39.06 and 38.7µm MRD and GON respectively followed by 36.3µm in SLT. The rest of the varieties were in the range between 33.36 to 31.1µm. whereas the highest stomates width was found to be 12.8µm, 12.5µm and 12.33µm respectively in MRD, SLT and GON. While the rest of the varieties ranged between 11.3 µm to 9.46 µm. Similarly the highest stomatal density per mm of leaf surface was 229.06 and 215.18 respectively in MRD and SGD. The rest of the varieties ranged between 194 to 183 stomates per mm of leaf surface.

Statistical analysis done on the stomatal length of six coconut varieties revealed significant differences among them as shown in Table 1. The average stomatal length of the Tall varieties was slightly higher than of the Dwarf and hybrid varieties. Tall coconut varieties SLT had the height stomatal length and SGD the lowest. The value obtained from RAN was significantly different from that of the Tall variety GON and that of SLT, MRD and SGD among the dwarf varieties. Among the tall varieties the longest length of stomata was measured from SLT. No significant difference was observed between SLT and the rest varieties GON and RAN. These results are in agreement with to report of Manthriratna & Sambasivam (1974) on typical, Nana and Aurantiaca Varieties. Scanning electron micrographs showed that stomata, found only on the abaxial epidermis were arranged in parallel rows and sunken below the epidermis. The values of stomatal features were statistically significant for some tall and Dwarf coconut; however they can not be used reliably to establish differences between the two coconut types (Solangi, 2001).

Stomatal width among the tall, dwarf and hybrid varieties was studied, the maximum width of the stomata was measured in SLT and the minimum was recorded in SGD (Table 1). The stomata width of RAN was similar to that of GON among the tall varieties. SLT on the other hand, had wider stomata than RAN and GON among the tall varieties. The value obtained from SLT was no different from the Dwarf varieties SGD and MRD. The average stomatal density was relatively lower in tall compared to the dwarf coconut varieties (Table 1). The dwarf varieties SGD had greater number of per unit area than rest of the varieties except hybrid and MRD.

The total number of stomata per leaflets of the six coconut varieties is presented in Table1. In general, the tall varieties had more number of stomates per leaflet compared to the dwarf varieties. The highest value for this parameter was recorded in tall SLT which was about 70% higher than that of the Dwarf variety SGD.

The number of the stomata per leaflets of RAN was comparable with the rest of the tall varieties except GON. On the other hand, it was observed that Hybrid had similar statistical values as the Tall varieties SLT, RAN and GON. The stomatal Figures (1-6) also showed the variation in length and width of the stomata of each variety.

Stomatal assessment and characterization was carried out using the lower epidermis. The stomata were total absent on the adaxial (Upper) epidermis of the coconut (Ghose & Davis, 1973). Based on scanning electron micrographs (Figs. 1-6), the stomates of different coconut varieties did not vary widely in appearance.

According to Nainanayake *et al.*, 2008, coconut yield is greatly influenced by environmental factors with rainfall playing a major role in replenishing the soil moisture reservoir within the root zone. Soil water deficits reduce transpiration from the palm, leading to an increase in canopy temperature which in turn could reduce photosynthesis and yield.

The irrigation lowered the temperature of the canopy microclimate and the nut surface temperature and thereby reduced the possibility of empty nut formation during dry spells. Further, irrigation reduced stomatal resistance and thereby probably nullified the effects of drought on photosynthesis. Irrigation increased the female flower production and reduced immature nut fall during droughts, thus improving the yield. Application of 80 litres of water per palm per day lowered the canopy temperature more than 40 litres of water and significantly improved female flower production and nut setting (Nainanayake, *et al*., 2008).

The stomates were arranged in parallel rows as noted in leaves with parallel veins such as those of monocotyledons and some dicotyledons (Esau, 1977). They, however, were not evenly distributed. They were generally sunken below the epidermis. They have a stomatal complex characterized by the presence of two guard cells and four subsidiary cells, two of which are roundish and the other two are lateral to the guard cells as observed in Cocos and other palm species (Stebbins & Khush, 1961). The guard cells had more or less uniform width. The narrowing of middle portion of the guard cells (Dumbble-shaped) was not noted among the stomata examined. The mean value of stomatal features was higher for SLT and RAN except for stomatal width and density (Table 1). The mean stomatal density per mm^2 among the six coconut varieties examined in this study were 185.65mm^2, 183.54mm^2, 189.87mm^2, 215.18mm^2, 229.06mm^2 and 194.09mm^2. The above findings are in accordance with work of (Alfenso, 1974) who reported that the mean number of stomates per mm^2 of leaf surface ranged from 2549.0 to 4808.0. Stomatal density and length of green and purple SanRamon coconut variety did not vary significantly.

Result of the present study on stomatal features suggest that despite the significant differences noted among the six varieties, they cannot be used to establish differences between the three coconut types. Manthriratna & Sambasivam (1974) also reported that stomatal densities of varieties and forms of the coconut palms could be varietals characteristic and it could be used as genetic marker to identify typical x pumila F1 hybrids. Of all coconut varieties examined in the present study SLT was found to be relatively resistant to drought stress compared to the dwarf and Hybrid coconut. Despite the significant differences noted among the six varieties, the stomatal features cannot be used to differentiate the three coconut types.

Fig. 1. Scanning electronic micrograph of the stomata of Sri Lanka Tall coconut variety.

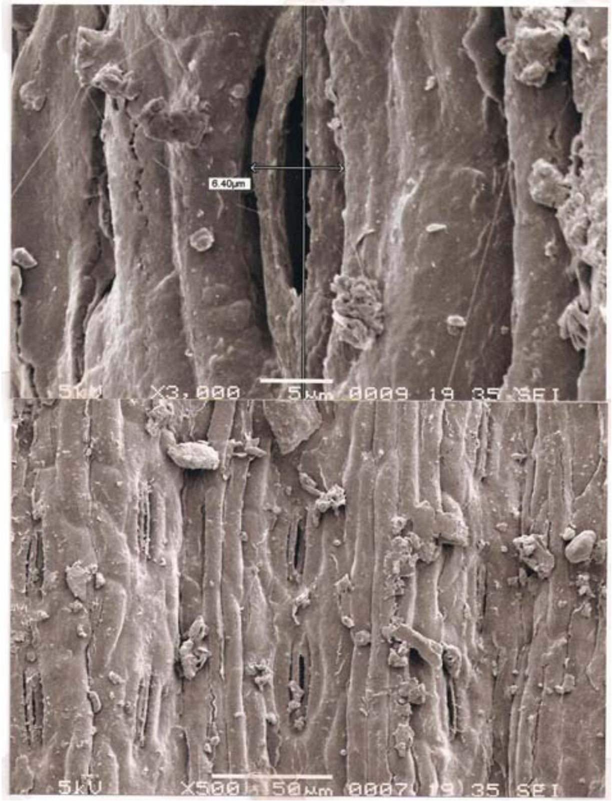

Fig. 2. Scanning electronic micrograph of the stomata of Ranthambly Tall coconut variety.

Fig. 3. Scanning electronic micrograph of the stomata of Gonthambly Tall coconut variety.

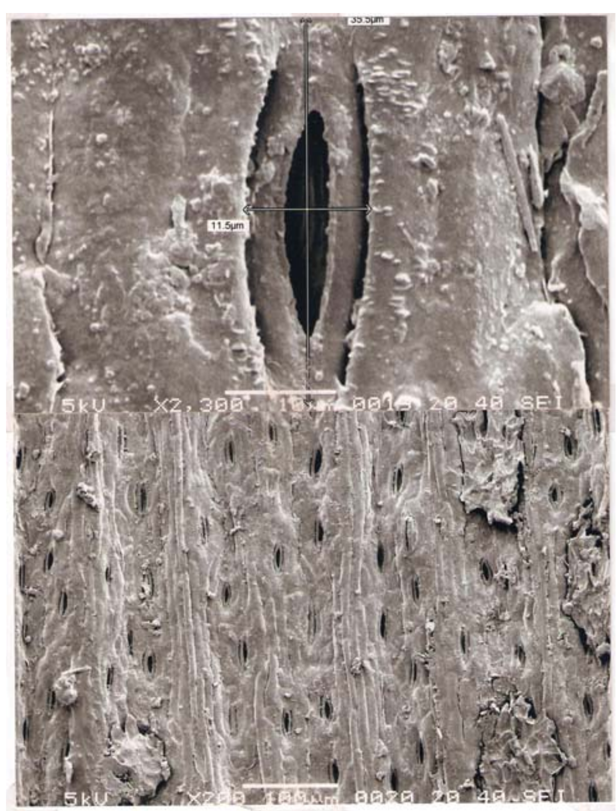

Fig. 4. Scanning electronic micrograph of the stomata of Sri Lanka Green Dwarf coconut variety.

Fig. 5. Scanning electronic micrograph of the stomata of Malayan Red dwarf coconut variety.

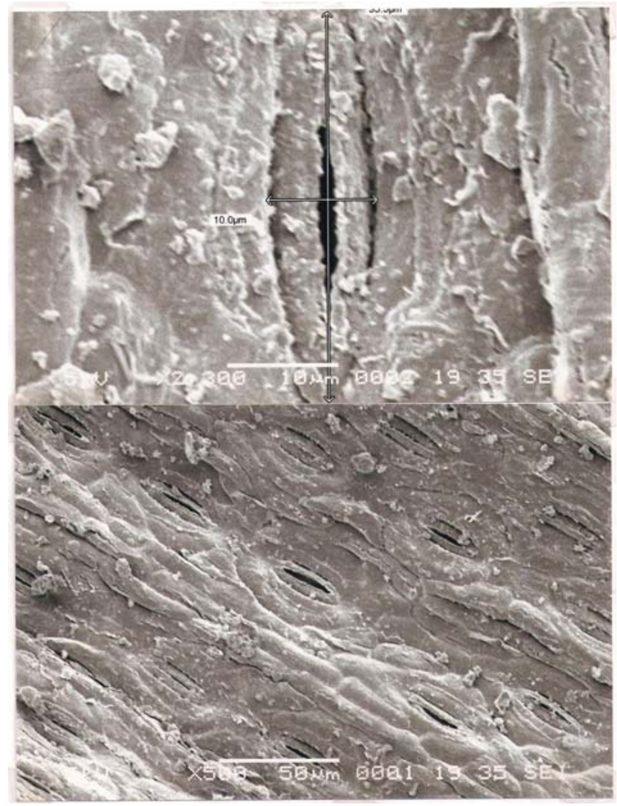

Fig. 6. Scanning electronic micrograph of the stomata of Hybrid (txt) coconut variety.

Conclusions

Leaf samples of six SLT, RAN, GON, SGD, MRD and Hybrid (txt) was not significantly different in stomatal length. No parameter eg., Stomatal length, width and density vary significantly between Tall and Hybrid (txt). It was concluded that stomatal length, width and density were not suitable genetic marker of coconut. Thus it is inferred from the results that less stomatal may be used as a parameter to screen the variety for drought tolerance in coconut. Scanning electron micrograph showed that stomata, found only on the abaxial epidermis, were arranged in parallel rows and below the epidermis. The values of stomatal features were statistically significant for some Tall, Dwarf and Hybrid coconuts. The mean number of the stomates per mm²of leaf surface ranted higher in Tall and Hybrid compared to the Dwarf varieties of coconut.

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