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RESEARCH ARTICLE

Leaf anatomy and identification of drought-tolerant Coconut (*Cocos nucifera* L.) varieties in Kerala, India

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Abstract

Coconut (*Cocos nucifera* L.) is a tropical evergreen palm with high economic value. Drought stress, exacerbated by climate change, poses a significant threat to coconut production, prompting research to develop drought-tolerant varieties. In this study, eight hybrid combinations of Dwarf × Tall were evaluated for their drought tolerance. Among the varieties, the hybrid Chowghat Orange Dwarf (COD) × Laccadive Ordinary Tall (LCT) exhibited the greatest thickness of both upper and lower epidermal layers, as well as higher palisade and spongy mesophyll tissue thickness. Additionally, this hybrid recorded the largest xylem diameter. Malayan Yellow Dwarf (MYD) × West Coast Tall (WCT) showed the lowest stomatal density, whereas MYD × LCT recorded the highest trichome density. The combined analysis of the different parameters evaluated through PCA and thickness of the palisade mesophyll cells, width of the bundle sheath cells, number of nuts, stomatal length and width, thickness of spongy mesophyll cells and width of the xylem could distinguish the tolerant hybrids from others. Cluster analysis grouped the hybrids into four groups based on the drought tolerant nature. The identified drought-tolerant hybrids could undergo screening for additional crucial drought-resistant parameters, contributing to future research aimed at enhancing the drought resistance of hybrids.

Keywords

anatomical parameters; coconut; drought; stomata, trichome

Introduction

The coconut (*Cocos nucifera* Linnaeus) belongs to the Family Arecaceae and is a pantropical plant. Typically, coconut plantations are situated in lowland areas slightly above sea level. Recognized as 'one of Nature's greatest gifts to man' (1), every part of the coconut palm serves a purpose. Optimal growth and development of coconut thrive under specific weather conditions, including well-distributed rainfall ranging from 130 to 230 cm, an average annual temperature of 27°C, abundant sunlight ranging from 250 to 350 Wm^{-2} and a minimum of 120 hours of sunshine per month. Although coconut is primarily cultivated as a rainfed crop, its productivity can increase by up to 50% when grown under well-irrigated conditions (2).

Moisture stress in coconut palm is observed when it is exposed to above 265 Wm^{-2} of irradiation, 33°C of temperature and 26 m bar of vapour pressure deficit (3). Drought is a major environmental factor limiting coconut productivity. In coconut the time taken for the floral primordia initiation to the nut maturity is very long *i.e.* about 44 months. Again, pre and post fertilization periods are about 32 and 12 months respectively (4). Moisture stress in these critical stages of the inflorescence development stages affects the nut yield. The ill effects of drought in the current year will be reflected in next three years and in some worst affected situations, it takes four years to recover. Drought at early stages of the growth affects the seedlings growth and lead to seedling mortality (5). Growing of water efficient coconut cultivars is a key step in attaining sustainable coconut production in the areas affected by a long dry season. The impact of drought on coconut yield is well documented in literature (6). The effects of drought stress on the growth, physiology and productivity of coconut have been widely documented (7-9). Unlike the annuals, the adverse effect of drought, caused either by low rainfall or delayed onset of monsoon or both at any given time, persists for the subsequent two or three years in coconut and this was explained on the basis of the relationship between the degree and intensity of dry spell and the ontogeny of coconut inflorescence (10).

The extent of water stress on coconut varies depending on soil type and critical soil moisture levels. Recurring droughts in key coconut-growing regions in recent decades have resulted in significant yield losses and, in severely affected areas, the mortality of mature coconut palms.

During the mid-1990s, research on the creation of drought-tolerant coconut varieties gained recognition as both important and imperative. Given the escalating climatic challenges, the demand for drought-tolerant varieties has become crucial. Globally, numerous initiatives have been undertaken to develop drought-tolerant coconut varieties using inter and intra-varietal hybridization. Rajagopal *et al.* (11) had standardised the methodology of screening coconut varieties for drought tolerance using different traits such as, stomatal frequency and leaf water potential. His finding shows that, the West Coast Tall (WCT) and Federated Malaya States Tall (FMST), which are water stress tolerant with thick leaflets and cuticle on both surfaces and greater parenchyma and hypodermal cells compared to susceptible ones. Tall palms and tall hybrids exhibited relatively high stomatal resistance resulting in effective conservation of water in the tissues, whereas the dwarfs were sensitive to stress with a tendency to lose more water.

Plants respond to drought stress and adapt to such conditions through various morphological, physiological, biochemical and molecular changes. Plants also adopt various strategies to cope with drought stress and one such strategy is resistance mechanism (12-14). Swift, dependable and cost-effective techniques for identifying physiological and anatomical markers can be employed to characterize cultivars in germplasm banks. These markers play a crucial role in coconut breeding programs, aiding in the early stages of crop improvement, particularly in obtaining drought-tolerant cultivars. Some economical and effective markers utilized by previous researchers for screening drought-tolerant coconut cultivars include stomatal characteristics, leaf anatomical traits and leaf trichome parameters. Hybrids of coconut that demonstrate drought tolerance and maintain a higher and stable yield under stress conditions can be achieved through the strategic crossbreeding of carefully selected drought-tolerant tall and dwarf parent varieties. The developed hybrids could be screened with the drought tolerance parameters in field conditions for further selection. Compared to the Tall x Dwarf (T x D) hybrids, Dwarf x Tall (D x T) hybrids was superior in terms of yield of nuts, copra and oil outturn (15) and palm to palm variation was not as pronounced as T x D (16). Performance of D x T hybrids under sub optimal management practice is better than T x D hybrids (17). Hence in the present study, eight hybrid combinations of D x T were attempted in which the drought tolerant tall palms such as WCT, WAT, LCT and ADOT were used as a male parent and the resulting hybrids were screened for its efficiency against drought tolerance.

Materials and Methods

Experimental Site

The experiment was conducted at the Indian Council of Agricultural Research (ICAR) - Central Plantation Crops Research Institute (CPCRI), Kasaragod, Kerala, during the peak summer seasons from 2021 to 2023 (February to May). It is situated at approximately 12.31°N latitude and 74.51°E longitude, with an altitude varying between 15 and 17 meters above mean sea level. Details of the experimental site as well as the meteorological data are provided in Table 1.

Plant material

A total of one hundred and eight palms from the eight hybrid combinations (Chowghat Orange Dwarf (COD) × West Coast Tall (WCT), COD × West African Tall (WAT), COD × Laccadive Ordinary Tall (LCT), COD × Andaman Ordinary Tall (ADOT), Malayan Yellow Dwarf (MYD) × WCT, MYD × WAT, MYD × LCT and MYD × ADOT) were developed and evaluated in the study and the hybrids were maintained at Germplasm Block, ICAR-CPCRI, Kasaragod, Kerala. Along with hybrids, WCT, a drought tolerant tall variety extensively cultivated in West Coast of India was used as a control. The experiment was laid out in Randomized Block Design with (RBD) with two replication and six palms per replication. The planting density for this

Table 1. Details and Meteorological data of the experimental site.

Parameter	Details
Location	ICAR - CPCRI, Kasaragod, Kerala
Coordinates	12.31°N, 74.51°E
Altitude	15-17 m above MSL
Mean Temperature (Summer)	31.5°C
Mean Temperature (Winter)	21.3°C
Annual Rainfall	3400 mm
Rainfall Days	132 days
Dominant Monsoon	South-West Monsoon
Relative Humidity (Annual)	88%
Soil Type	Sandy loam
Soil pH	4.4
Soil Organic Carbon	0.58%
Soil Nitrogen	0.03%

study was set at 177 palms per hectare and standard management practices were consistently followed throughout the research period.

Anatomical parameters

For anatomical analysis, four palms per hybrid combinations were sampled. The leaflets were collected from the middle portion of the completely formed leaves (6th leaf from the top). 1cm^2 cross-sections from the mid lamina of leaflets from selected accessions were used for histological analysis. Likewise for each accession, two samples per palm were analyzed following the modified protocol by Puchtler et al. (18). Plant samples were fixed in Carnoy's solution (absolute alcohol, chloroform and glacial acetic acid) for 48 hours to localize RNA, DNA, proteins and polysaccharides. The samples were then dehydrated in a graded alcohol series (70% to 100%) followed by a reverse series with increasing concentrations of butanol (25% to 100%). After complete dehydration, the samples were infiltrated and embedded in paraffin wax by adding wax chips and incubating at 60°C. The wax was replaced with fresh molten wax over 3-4 days to remove residual butanol. The samples were then embedded in paraffin blocks using L-rods and rapidly cooled. Thin sections of 10 µm thickness were cut using a Leica RM 2145 Rotary microtome and placed on glass slides coated with 3% gelatin. After air-drying for 1-2 days, the slides were deparaffinized with xylene and butanol, stained with 1% toluidine blue, washed with water and dehydrated again with butanol. The final clearing was done using xylene. The sections were DPX-mounted and examined using a Leitz Diaplan compound microscope at 25x magnification. Microscopic images were captured using the DFC295 camera with the software, Leica Application Suite Version 4.30.

Stomatal parameters

Fresh leaflets collected from the mid-region of the 6th leaf were thoroughly washed with tap water and air dried was utilized for stomata analysis. Stomata analysis was conducted using scanning electron microscope (FEI-Quanta 250 SEM from FEI, Japan). Prior to SEM analysis, a gold layer with a thickness of 0.02 µm was applied to the samples using a sputter coater, specifically the EMITECH SC7620 from Quorum Technologies Ltd., UK.

The scanning electron micrographs of the stomata were then taken. To assess Stomatal Density (SD) on the abaxial leaf surface, a solution of nitrocellulose polymer dissolved in ethyl acetate was thinly applied to the middle portion of the lower epidermis of leaflets. As this layer dried, it adhered to the epidermal tissues, capturing the stomatal impression. The resulting nitrocellulose layer, imprinted with stomata, was carefully removed with forceps and mounted on glass slides. Stomatal density was determined by calculating the number of stomata per unit leaf area using a Leitz Diaplan compound microscope.

Eight leaflets were utilized for each hybrid and three independent counts were performed on each leaflet. The length of the stomatal pore was measured from tip to tip using a calibrated eyepiece micrometer. The width of the stomatal pore was measured as the widest distance between the thickest inner walls of the guard cells. Stomata length and width were measured and the stomatal index was calculated using the formula: stomatal index (%) = (S / (S + E)) x 100, where S and E represent the number of stomata and epidermal cells, respectively, in the microscopic view field.

Trichome parameters

A portion of lamina approximately one-third distant from the base of the leaflet from the fully emerged youngest leaf has been reckoned as the standardized region for the examination of trichomes. Observations on lamina on either side of the midrib were made, at least 5 microscopic fields per half, avoiding areas very close to the midrib and margins. The trichome count was acquired from microscopic images at 40X magnification, capturing data from five microscopic fields on each side of the leaflet lamina, resulting in a total of 20 data points per leaflet sample.

Trichome density was calculated by dividing the number of trichomes by the view field area (19).

Physiological parameters

Epicuticular wax content was determined as per the method of Rajagopal *et al.* (6). Stomatal resistance (rs) and chlorophyll fluorescence of the treatments were measured during the summer season (February to May) at weekly intervals on the adaxial surface of young, fully opened leaf using a porometer (Porometer AP4, Delta-device, Cambridge, UK) and expressed as seconds per centi meter (s cm^{-1}). Chlorophyll fluorescence indices of the leaves were measured after dark-adaption for 30 min, using a chlorophyll fluorescence meter (OS30p, OptiSciences, Hudson, MH, USA).

Data analysis

The mean values of all morphological traits were analyzed through Analysis of Variance (ANOVA) based on the model proposed by Panse and Sukhatme (20). The data analysis was conducted using the Web-based Agricultural Software Package (WASP). Principal component analysis was carried out using the MVSP 3.2 (Multi-Variate Statistical Package) software. The unweighted Pair Group Method using Arithmetic average (UPGMA) was used to construct the dendrogram using the MVSP software version 3.2.

Results and Discussion

Anatomical parameters

Anatomical characteristics serve as reliable indicators of drought tolerance. A positive correlation has been observed between certain xeromorphic features and the arid habitat (21). Anatomical traits indicative of adaptations to water deficit include a reduction in epidermal cells, an increased number of layers of smaller mesophyll cells and a thicker cuticle (22). In different plant species, thickness of epidermal layers (23), the width of the mesophyll cells (24) and density of xylem (25) have been reported to be associated with drought tolerance.

From previous studies, it was (26) identified that varietal distinctions in leaf thickness and tissue density among coconut cultivars, with hybrids exhibiting higher tissue density than WCT palms. Typically, coconut leaf tissue has low density due to its higher air space volume, a characteristic of C3 species. In contrast, C4 species typically display lower air space volume and higher tissue density.

In coconut, the lower portion of the leaflet was thicker and gradually tapers towards the tip. Considering the leaf anatomy of coconut, the epidermal cells are compactly arranged without any intercellular spaces. The upper epidermis in coconut exhibits a thicker structure with larger-sized cells compared to the lower epidermis. Specifically, the cuticle on the upper epidermis was twice as thick as the cuticle on the lower epidermis. Furthermore, the cuticle was even thicker at the edges of the leaflet and along the midrib. In the current study, distinctive anatomical characteristics associated with drought stress tolerance were observed (Fig. 1). Among the hybrids, COD × LCT showed the highest thickness of both upper and lower epidermis (46.02 µm and 30.86 µm, respectively), followed by WCT (30.59 µm and 27.55 µm, respectively) (Table 2). Epidermal thickness was linked to drought tolerance, with genotypes having higher epidermal thickness showing lower water loss rates and vice versa. Drought tolerance was also associated with thicker palisade mesophyll layers. COD × LCT exhibited the highest thickness in both palisade (223.30 µm) and spongy mesophyll tissues (88.04 µm) among the hybrids. A similar trend was observed for xylem diameter, with COD × LCT (44.41 µm) followed by COD × WAT (34.31 µm) having the maximum xylem diameter. A larger xylem area was also linked to the ability to maintain functional conductance during stress periods, ensuring better water potential. Variations in anatomical features of leaflets including differences in mesophyll cells among WCT, FMST, WCT x COD and PHOT was reported earlier (27).

In the current study, drought induced significant but varying changes in leaf tissue anatomy among different hybrids. The drought-resistant hybrid COD × LCT exhibited higher total epidermal thickness (46.02 µm) and upper palisade parenchyma thickness (223.3 µm). This increase in thickness should enhance the number of CO_2 assimilation sites per unit leaf area, helping to maintain high photosynthetic assimilation rates despite the low stomatal conductance caused by drought. The spongy parenchyma thickness was also higher in COD × LCT, potentially improving the diffusion of CO_2 from the sub-stomatal cavity to the mesophyll cells' outer surface through inter-cellular spaces (28). Naresh Kumar *et al.* (27) observed larger parenchyma, hypodermal and water cells in water-stress-tolerant cultivars like WCT and FMST, suggesting that upper and lower epidermal cell sizes are linked to the drought-tolerant characteristics of a cultivar. Consequently, a cumulative effect of these traits contributes to drought tolerance.

In summary, the differences in drought resistance among coconut hybrids may be attributed to leaf morpho-anatomical adaptations to water deficit conditions. The thicknesses of the epidermal layers, palisade parenchyma and spongy parenchyma are considered key structural adaptations influencing the varied response of different coconut hybrids to drought. Hence, these leaf anatomical features can be utilized as selection criteria in screening studies for drought-resistant coconut hybrids.

Stomatal parameters

Coconut leaflets are hypostomatus, meaning that stomata are completely absent on the adaxial epidermis of the coconut. Guard cells are surrounded by elongated epidermal cells along their entire length, with these epidermal cells being larger than the guard cells (27). Stomata are arranged in parallel rows, forming a Paracytic stomatal complex (29). However, the stomata are not evenly distributed; they are generally sunken below the epidermis. Stomatal traits such as density and size are considered important parameters for drought tolerance under water deficit conditions. Hence, stomatal assessment and characterization were carried out. Based on light microscope studies, the stomata of different coconut hybrids did not vary widely in appearance (Fig. 2). Among the hybrids and cultivars evaluated, stomatal density was higher in WCT (63.75/mm^2) while it was lower in MYD × WCT (39.84/ mm^2). The mean stomatal length was higher in COD × WCT (167.9 µm) and it was lower in MYD × WAT (137.1 µm) which was on par with MYD × ADOT (137.6 µm). Mean stomatal width was higher in COD × WAT (127.6 µm) and COD × WCT (127.5 µm) while it was lower in MYD × WCT (101.2 µm) followed by MYD × ADOT (106.6 µm). Stomatal aperture length was higher in COD × LCT (132.6 µm) and lower stomatal aperture length was observed in MYD × WCT (113.2 µm). Mean stomatal aperture width was higher in COD × ADOT (43.5 µm) and it was lower in MYD × LCT (27.0 µm) and MYD × WAT (28.9 µm). The stomatal index was higher in COD × WCT (13.1) while it was lower in MYD × ADOT (8.9) (Table 3). Scanning electron micrographs revealed that the stomata found sunken below abaxial epidermis and were arranged in parallel rows.

Table 2. Leaf anatomical characteristics of coconut hybrids.

Variety/ hybrid	Upper epidermis	Lower epidermis	Pallisade parenchyma	Spongy parenchyma	Width of the bundle sheath	Diameter of xylem
CODxWAT	23.29±0.69^{de}	18.17±0.75^{d}	153.25±4.72^{c}	80.09±5.99^{ab}	389.06±1.06^{e}	34.31±9.46
CODxLCT	46.02±1.51^{a}	30.86±1.87^{a}	223.30±5.28^{a}	88.04±5.99^{a}	570.71±1.77^{a}	44.41±5.97
CODxWCT	25.20±1.40^{de}	17.92±1.25^{d}	172.05±8.03^{c}	73.58±3.50^{bc}	456.52±4.07^{c}	21.01±2.92
CODxADOT	26.97±1.19^{de}	18.31±2.33^{cd}	161.47±3.93^{c}	80.12±2.95^{ab}	464.24±1.26^{c}	21.01±2.92
MYDxWCT	23.57±0.87^{de}	18.20±1.17^{cd}	169.91±3.94^{c}	62.01±6.12^{cd}	421.55±2.99^{d}	25.30±7.37
MYDxWAT	26.28±1.03^{cd}	21.60±0.91^{cd}	210.93±9.20^{ab}	58.64±1.97^{d}	503.23±5.26^{b}	30.68±9.94
MYDxLCT	25.30±1.60^{c}	23.18±0.63^{c}	172.30±9.71^{c}	74.93±3.51^{b}	517.19±13.57^{b}	27.36±6.28
MYDxADOT	23.12±0.91^{bc}	23.18±1.35^{c}	172.31±7.95^{c}	53.46±3.78^{d}	429.66±3.13^{d}	18.15±1.91
WCT	30.59±0.91	27.55±2.43^{b}	200.01±5.76^{b}	74.95±2.99^{b}	508.39±6.31^{b}	32.69±11.4
Mean	27.82	22.11	181.73	71.76	473.39	28.32
CV	4.37	3.36	19.80	12.39	24.65	NS

COD - Chowghat Orange Dwarf; WAT - West African Tall; LCT - Laccadive Ordinary Tall; WCT - West Coast Tall; ADOT - Andamon Ordinary Tall; MYD - Malayan Yellow Dwarf

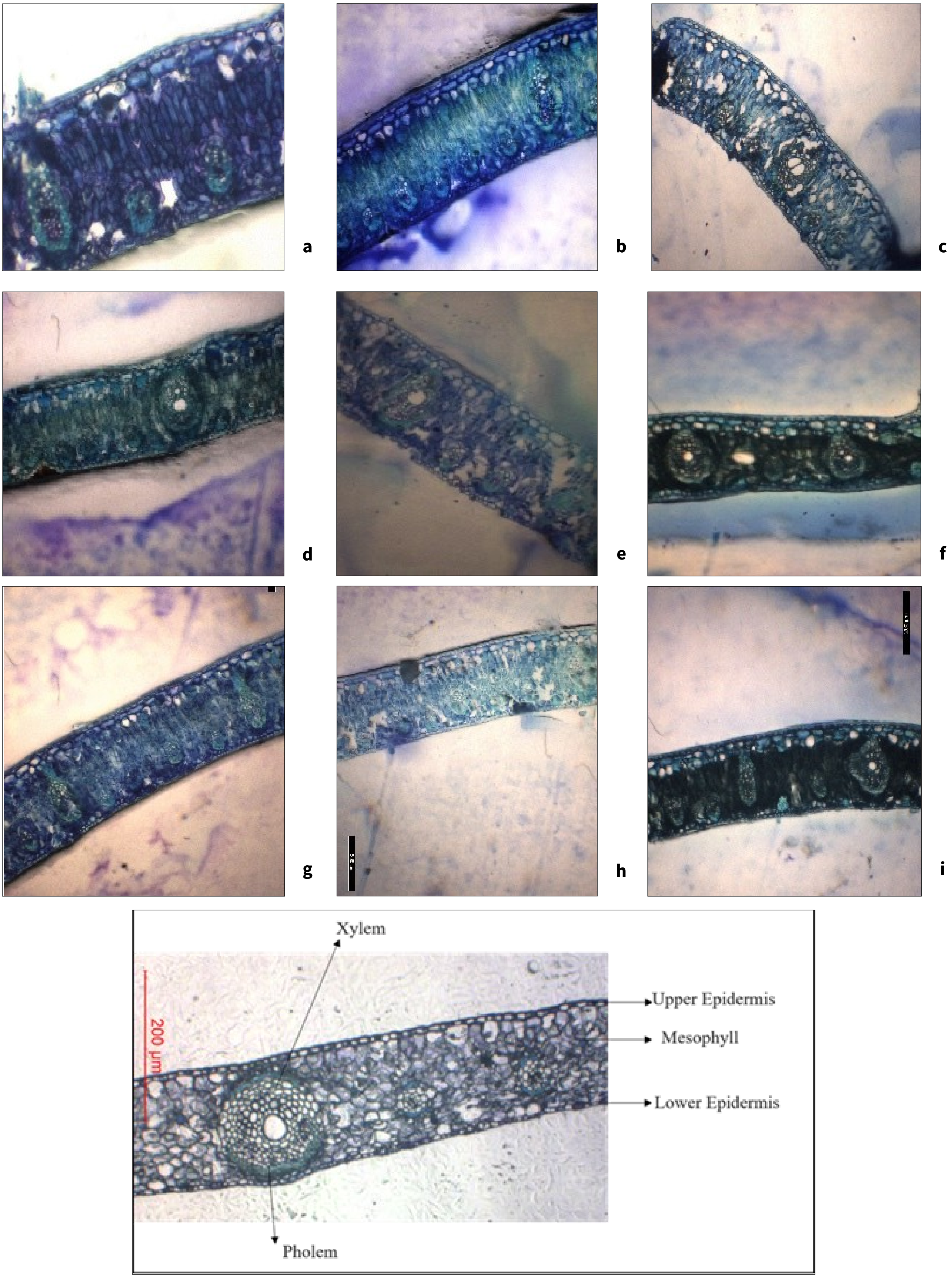


Fig 1. Leaf anatomy of coconut hybrids and WCT. a) CODxWAT, b) CODxLCT, c) CODxWCT, d) CODxADOT, e) MYDxWCT, f) MYDxWAT, g) MYDxLCT, h) MYDxADOT and, i) WCT, j) Leaf cross sections showing the Upper epidermis, Lower epidermis, Mesophyll, Xylem and Phloem under a light microscope.

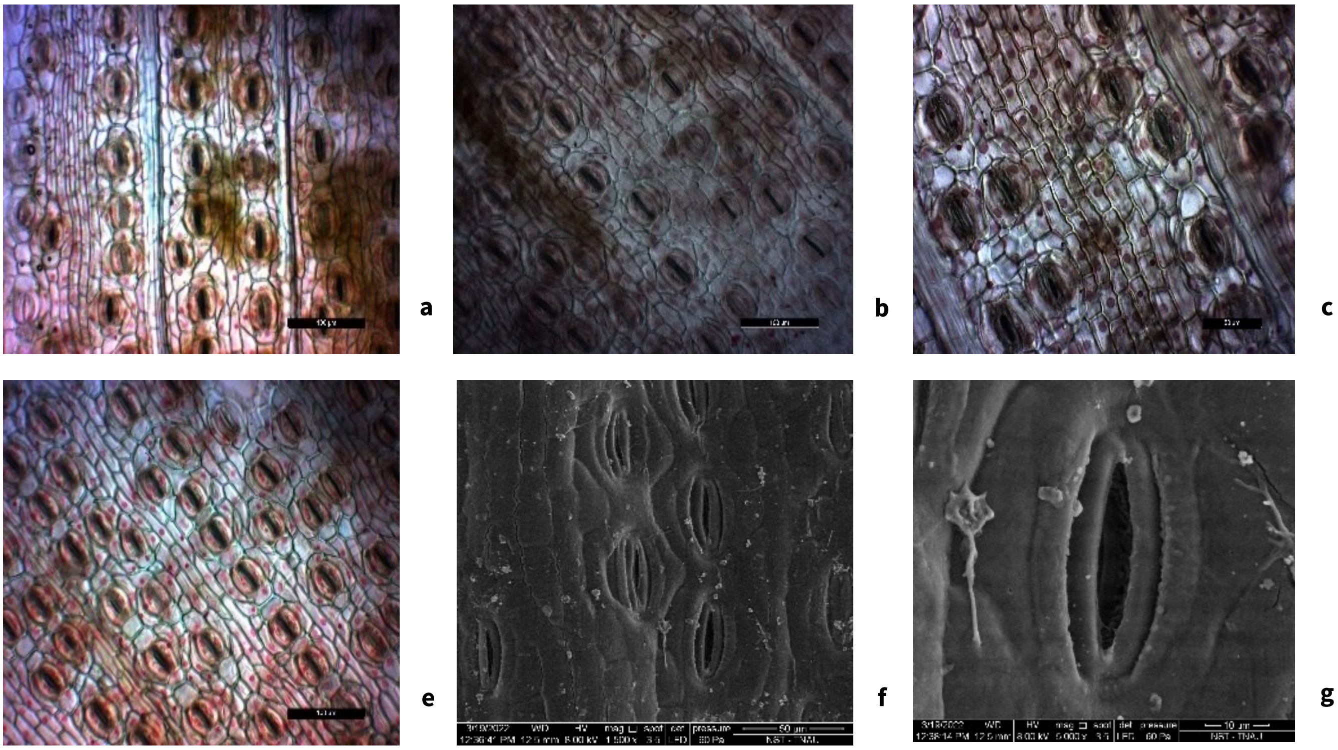


Fig 2. Stomata on leaf abaxial surface a. MYDxWAT; b. MYDxWCT; c. MYDxLCT; d. WCT; e & f. SEM of stomata at abaxial leaf surface of coconut (1500 & 5000x)

Table 3. Leaf stomatal and trichome characteristics of coconut hybrids

Variety/ Hybrid	Stomatal density (No./mm^2)	Stomatal index	Stomatal Aperture length (µm)	Stomatal Aperture width (µm)	Stomatal length (µm)	Stomatal width (µm)	Trichome density (No./mm^2)	Cuticular wax content (µg/cm^2)
CODxWAT	47.81	9.03	128.29	32.58	156.25	127.61	39.24	156.11
CODxLCT	55.78	10.99	137.56	32.79	164.72	122.23	30.88	161.48
CODxWCT	51.79	13.11	124.92	34.28	167.90	127.55	47.61	329.26
CODxADOT	45.82	11.34	126.47	43.49	156.25	112.82	36.85	141.67
MYDxWCT	39.84	9.34	113.17	33.54	150.69	101.18	45.42	200.74
MYDxWAT	47.81	10.76	128.42	28.88	137.07	126.84	55.78	132.78
MYDxLCT	43.82	10.14	121.98	26.99	139.32	111.62	72.71	212.22
MYDxADOT	55.78	8.94	122.67	31.56	137.59	106.61	32.87	364.63
WCT	63.75	12.24	121.09	36.05	147.34	132.78	43.23	459.81
Mean	50.24	10.65	124.95	33.35	150.79	118.80	44.95	239.86
CV	18.4	7.01	8.46	17.82	7.82	9.66	11.09	1.06

COD - Chowghat Orange Dwarf; WAT - West African Tall; LCT - Laccadive Ordinary Tall; WCT - West Coast Tall; ADOT - Andamon Ordinary Tall; MYD - Malayan Yellow Dwarf

In mild to moderate drought conditions, limitations at the stomatal level reduce coconut yield more significantly than limitations at the non-stomatal (biochemical) level of the assimilation process, as noted by Nainanayake and Morrison (30). The number and distribution of the stomata per unit leaf area plays a crucial role in assimilation processes by regulating the exchange of O_2, CO_2 and moisture between the leaves and the atmosphere. In the current study, it was observed that MYD × WCT had a lower stomatal density. Jones (31) proposed a hypothesis suggesting that breeding for stomatal characters and our understanding of the physiological and ecological role of stomata in relation to drought tolerance was currently incomplete. Drought resistance involves a complex system with various possibilities regarding adaptive mechanisms. While increasing assimilation rates could potentially maximize productivity and yield, it ultimately depends on stomatal conductance. On the other hand, a low stomatal conductance can limit plant water losses, serving as a crucial survival strategy during drought but potentially restricting yield.

In a general context, tolerant cultivars tend to efficiently decrease water loss by reducing stomatal density and size, thereby avoiding dehydration effects. According to Mehri *et al.* (32), drought-tolerant wheat genotypes exhibit fewer stomata, while sensitive genotypes have a higher stomatal count. Similarly, a study by Kusvuran *et al.* (33) on melons in Turkey demonstrated that cultivars with greater drought tolerance have lower stomatal density in leaf tissues under control conditions.

Physiological and anatomical changes in stomatal density and size, stomatal conductance and relative leaf water content have been considered as significant measures for yield improvement and desirable expression of these characters has to be selected to maintain both adaptation and optimal yield of crops under drought stress environments (34). Baloch *et al.* (35) screened the wheat genotypes for drought tolerance and observed that cultivars were quite variable for stomatal density in drought stress environment and drought tolerant cultivars recorded minimum stomatal density suggesting less transpiration rate under drought conditions. According to

Hasanuzzaman's report in 2018, barley genotypes that exhibit tolerance transpire a lesser amount of water due to their lower stomatal density under normal growth conditions. This characteristic suggests that these genotypes have a better ability to conserve water, particularly under drought stress conditions.

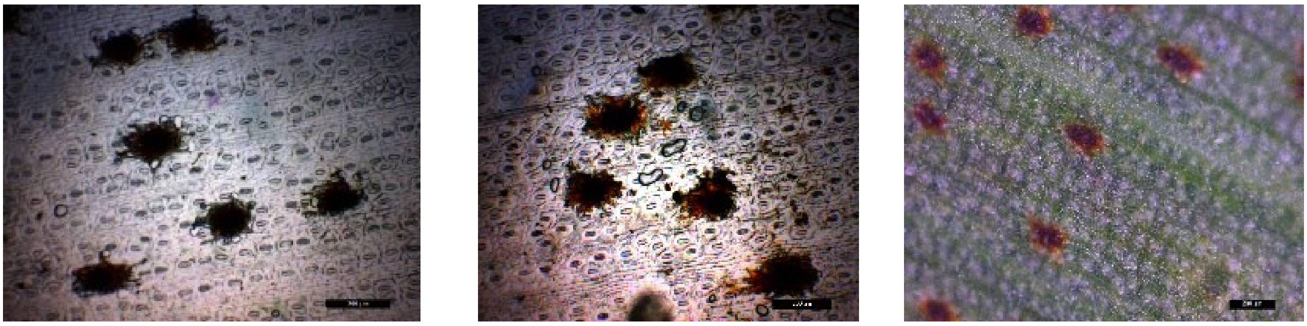
Trichome parameter

To adapt to water deficit, plants exposed to drought conditions require specific physiological mechanisms to store sufficient water for normal metabolic processes. The majority of water loss in plants occurs through transpiration and this rate is significantly influenced by diffusion resistance. Trichomes, being specialized structures covering the plant's surface, play a role in enhancing the diffusion resistance to transpiration, as supported by Fu *et al.* (36). The barrier effect of trichomes plays a crucial role in reducing excessive transpiration and photoinhibition by influencing CO_2 and H_2O exchange, as noted by Fu *et al.* (36). Additionally, trichomes are known to diminish the absorption of solar radiation by the plant, lower its temperature by expanding the leaf surface boundary layer and thereby provide protection against drought stress. In this study, trichome density among the D × T hybrids was evaluated. In coconut, the petiole, rachis, leaf sheath, abaxial lamina, spathe, peduncle, spikes and even the exposed portion of young ovary are covered with trichomes/ hairs of different sizes and shapes which vary in intensity. Coconut trichomes on the leaf lamina are multicellular base of cutinized cells which produce a shield like expanse of thin-walled cells (37). Observations on distribution of trichomes on leaves of eight D × T hybrids, along with WCT, showed that coconut trichomes are distributed only on the abaxial surface of leaves, ranging of 32.87 to 72.71/mm^2. The highest trichome density was observed in MYD × LCT followed by MYD ×WAT (55.78/mm^2) (Table 3) (Fig. 3).

The role of trichomes in drought tolerance and adaptation to water stress has been documented across various plant species. For instance, in tomatoes, plants with a high trichome density demonstrated greater tolerance to water stress compared to plants with lower trichome density, as reported by Ewas *et al.* (38). Similarly, in watermelon, wild genotypes that are drought-tolerant exhibit increased trichome density compared to domesticated, drought-sensitive varieties, as observed in the study by Mo *et al.* (39). In barley, brinjal and olive, there was increased trichome density in plants grown under water stress (36, 40). The observed phenomenon can be attributed to the fact that trichomes may limit to water loss through transpiration by increasing the leaf-air boundary layer resistance, as suggested by studies such as Guerfel *et al.* (41) and Mo *et al.* (36). Trichomes also serve to protect leaves from UV-related photoinhibition, either by reflecting or absorbing UV radiation through pigmented molecules, as indicated by Galmés *et al.* (42). This dual function helps prevent the overheating of leaves, as highlighted by Holmes and Keiller (43). These findings collectively emphasize the significant role of trichomes play in plant-water relations.

physiological parameters

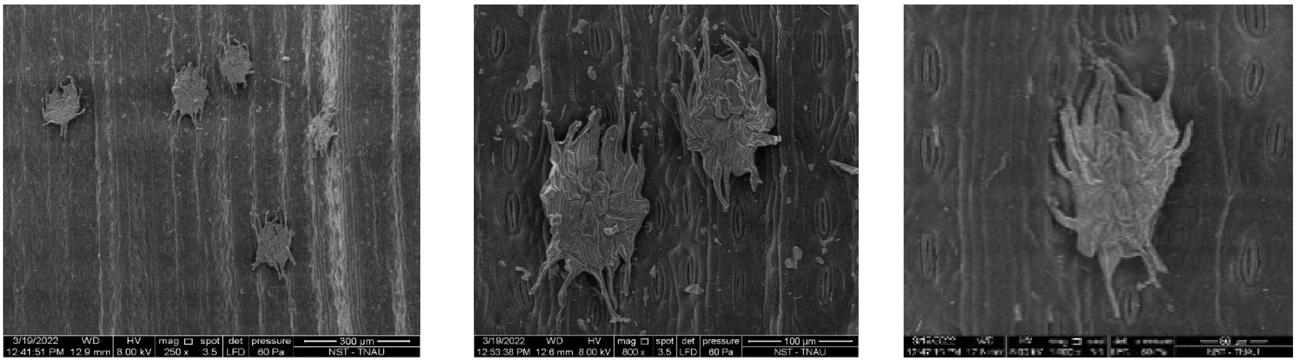
Leaf cuticular waxes cover all the plant surfaces and form a protective barrier between a plant and its environment. These cuticular waxes play an important role in plant resistance mechanism against various biotic and abiotic stresses. Earlier studies show that drought stress can cause an increase in the amount of wax deposited per unit leaf surface area in various plants. Further, depositions of increased amounts of cuticular waxes were associated with improved drought tolerance.



a. MYDxLCT

b. MYDxWAT

c. Trichomes in abaxial side of leaf (8x)



d, e & f. SEM of coconut Trichomes at abaxial leaf surface (250x, 800x & 1000x)

Fig 3. Trichomes on leaf abaxial surface a. MYDxLCT; b. MYDxWAT; c. Trichomes in abaxial side of leaf (8x); d, e & f. SEM of coconut Trichomes at abaxial leaf surface (250x, 800x &1000x).

In the present study, cuticular wax content was determined in all the hybrids and WCT and its value ranged from 132.78 to 459.8µg/cm^2. Highest wax content was recorded in WCT (459.8µg/cm^2) followed by MYD x ADOT (364.6 µg/cm^2). Similar results were observed by Rajagopal *et al.* (11), where three-to-four-fold increases in cuticular wax content during the dry season in some of the coconut hybrids *viz.*, WCT x COD, WCT x GBGD, LCT x COD and LCT x GBGD (Table 3). Earlier studies demonstrated that greater cuticular wax deposition was often associated with enhanced drought tolerance and/or reduced transpiration rate in many and diverse crops. The ability to alter cuticular waxes in response to drought provides one mechanism whereby plants might limit transpiration, improve water conservation and maintain more vigorous growth in water-limiting environments.

Chlorophyll fluorescence is one of the most common methods used to measure and, in some cases, categorise a range of stressors impacting the photosynthetic processes. It has been used extensively to identify stress and stress responses in plants. Also, plants uptake CO_2 from air and transpire water through stomata. Under water scarcity, plants regulate transpiration by adjusting the stomatal conductance. Therefore, plants with lowered stomatal conductance are thought to have better adaptability under drought stress conditions. In the present study, the chlorophyll fluorescence indices and stomatal conductance significantly decreased during the drought period. There were significant variations in these parameters among different hybrids. The ratio of variable to maximal fluorescence (Fv/Fm) was an important indicator of drought tolerance. Among the hybrids, COD x WCT and MYD x WCT showed higher Fv/Fm ratio. Higher chlorophyll index was recorded in COD x WCT and COD x ADOT. Hybrids COD x ADOT and MYD x ADOT are characterised by high stomatal conductance (Table 4).

Principal component analysis (PCA)

PCA (Principal Component Analysis) was acknowledged as a robust method for data analysis and interpretation. It has been effectively employed as a multivariate analysis tool to cluster genotypes based on their stress tolerance levels, as assessed by various phenotypes under field conditions, as demonstrated by Liu *et al.* (40). In this study, the responses of coconut hybrids to drought were assessed using PCA. The first two principal components (PCI and PCII), based on the scree plot, explained 90.7% of total variations between the coconut hybrids for anatomical, stomatal parameters, trichome density and yield. The eigenvectors for PCI had high positive scores for thickness of the palisade mesophyll cells, width of the bundle sheath and number of nuts per palm whereas, the eigenvector for PC2 had high positive scores for stomata and stomatal pore length and width, thickness of the upper epidermal layers as well as spongy mesophyll cells and xylem width (Table 5). Based on the results, the hybrids were grouped into four categories. Group I, characterized by higher values of PC I and II, included COD × LCT, COD x ADOT and MYD x WCT. Group II, with a higher value of PC I, included MYD x ADOT and WCT. Group III, with a higher value of component II, included COD × WAT and COD × WCT. Group IV, with the least amounts in both principal components, included MYD × LCT and MYD × WAT. Since COD × LCT, COD × ADOT and MYD × WCT had the maximum amounts of PC II, principal component I, characterized by a high value of thickness of palisade mesophyll cells, width of bundle sheath cells, number of nuts, stomatal length and width, thickness of spongy mesophyll cells and width of the xylem, could effectively distinguish between tolerant and susceptible hybrids and also determines most discriminant variables that can identify drought tolerance levels of cultivars. The combined analysis of the different parameters evaluated through a PCA allowed a clear identification of the most tolerant hybrid combination. This approach, based on the parameters observed, may assist the field-level identification of drought-stress tolerant cultivars.

Table 5. Eigen value, percent of variance, cumulative percentage and components weight of the first two components extracted from PCA analysis of drought tolerance indices in coconut hybrids

Eigenvalues	Axis 1	Axis 2
Eigenvalues	3719.10	325.99
Percentage	83.446	7.315
Cum. Percentage	83.446	90.761
PCO case scores		
Stomatal Density	0.006	0.014
Stomatal Length	0.006	0.495
Stomatal width	0.057	0.333
Aperture length	0.057	0.199
Aperture width	-0.013	0.101
Stomatal Index	0.011	0.024
Trichome density	0.012	-0.443
Wax content	0.00	0.005
Upper epidermis	0.096	0.139
Lower Epidermis	0.062	0.005
Palisade mesophyll	0.340	-0.188
Spongy mesophyll	0.069	0.548
Width of the bundle sheath	0.924	-0.033
Width of the xylem	0.080	0.203
Number of nuts/palm	0.158	0.448

Table 4. Chlorophyll index (CI), chlorophyll fluorescence (Fv/Fm) and stomatal resistance (rs) in coconut hybrids

Hybrids	Chlorophyll Index (CI)	Chlorophyll Fluorescence (Fv/Fm)	Stomatal resistance (rs) (s cm^{-1})
COD x LCT	52.27^{de}	0.405^{d}	42.33^{cd}
COD x WCT	49.17^{e}	0.494^{bc}	65.70^{a}
COD x WAT	59.86^{bc}	0.581^{a}	58.06^{ab}
COD x ADOT	58.28^{bcd}	0.421^{d}	37.57^{d}
MYD x WAT	67.15^{a}	0.461^{cd}	24.43^{e}
MYD x WCT	53.06^{cde}	0.446^{cd}	50.60^{bc}
MYD x LCT	67.37^{a}	0.456^{cd}	36.53^{d}
MYD x ADOT	57.16^{bcd}	0.549^{ab}	64.23^{a}
WCT	63.23^{ab}	0.437^{cd}	62.73^{a}
Mean	58.62	0.472	49.13
CD (0.05%)	7.08	0.064	8.61

Further, it was also important to mention that different genotypes have different physiological mechanisms to combat drought stress and the identified tolerant hybrids, as well as the discriminating variables, may provide ample opportunity for coconut breeders to develop high-yielding drought-resilient hybrids.

Cluster analysis

Unweighted pair group method (UPGMA) cluster analysis using Euclidean distance coefficient was performed to group the selected cultivars based on their response to water stress (Fig. 4). Based on the analysis, the hybrids were divided into four main clusters (Fig. 4). Eight coconut hybrids grouped in to four clusters includes cluster I (COD × ADOT, COD × LCT and MYD × WCT), cluster II (COD × WCT, COD × WAT), cluster III (MYD × ADOT and WCT) and cluster IV (MYD × LCT and MYD × WAT). The cultivars under cluster I were more drought tolerant than the other hybrids based on the stomatal, trichome, anatomical, physiological parameters and yield. However, drought tolerance is a complex quantitative trait controlled by many mechanisms and is considered one of the most difficult traits to study and characterize. Also coconuts have various mechanisms that help them to survive and grow in the presence of drought. It may combine multiple adaptation mechanisms to survive. Thus, this is the preliminary study for the identification of drought-tolerant hybrids and these identified hybrids may be screened for other important parameters.

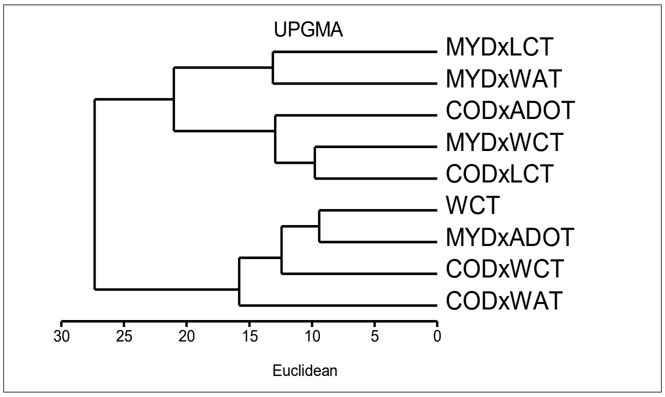


Fig. 4. Cluster analysis of eight coconut hybrids based on UPGMA

The variation uncovered through cluster analysis suggests the presence of distinct gene pools among the investigated coconut hybrids concerning drought tolerance. Further analysis at the molecular level, along with measurements of additional biochemical and physiological traits, was crucial to validate the definitive clustering of the cultivars regarding drought tolerance. Nonetheless, the clustering obtained from the measured traits holds significant importance for researchers as it delineates similarities and differences among the studied hybrids, offering valuable insights for commercial and scientific purposes under field conditions.

Conclusion

Presence of the genetic variability among all the hybrids for all tested indices/ parameters suggested that COD × LCT, COD × ADOT and MYD × WCT were more drought-tolerant compared to the other studied hybrids. These selected hybrids can be further utilized for in-depth studies on drought stress and for developing drought-tolerant coconut varieties. All the evaluated parameters/indices proved to be reliable indicators of drought stress. Despite the complexity of drought tolerance in coconut, the hybrids identified in this study serve as a reference point for future research aimed at developing new hybrids with enhanced drought resistance in the West Coast regions of India.

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Authors' contributions

R.S, V.N and K.S designed the study. R.S, M.N and V.A conducted the experiment and analysed the data with support from V.S. R.S wrote the original draft, with reviewing and editing support from M.N and AMR

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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