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RESEARCH ARTICLE

Screening for drought stress tolerance in traditional mango (*Mangifera indica* L.): biochemical and physiological approaches

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Abstract

This study assessed the ability of 34 mangoes (*Mangifera indica* L.) accessions and one control to tolerate drought by measuring several physiological and biochemical characteristics. There were notable variations in the relative water content among the accessions. KLM37, PTA01 and ALA23 had the highest values, suggesting that they had higher drought tolerance. Furthermore, KLM37, ALA15 and PTA01 exhibited the greatest saturated water content (SWC), indicating improved water retention during periods of drought. The analysis of specific leaf area (SLA) showed significant differences among the accessions studied. KLM37, ALA23, KLM12 and PTA01 exhibited the greatest SLA values. The accessions also showed a significant increase in epicuticular wax content (EWC). This indicates that they have improved drought adaptation by reducing water loss. The accessions exhibited an increased cell membrane stability index (CMSI), which further demonstrates their ability to withstand drought stress. The biochemical markers revealed that ALA27, ALA23 and KLM37 exhibited a noteworthy increase in proline concentration, suggesting their capacity for drought tolerance. PTA01, TVM02 and ALA27 exhibited high chlorophyll concentrations, indicating enhanced photosynthetic efficiency in drought conditions. Principal Component Analysis (PCA) revealed that RWC, CMSI and EWC are the primary characteristics that contribute to drought tolerance. PC1 accounts for 76.913% of the overall variation. The levels of proline and chlorophyll had a significant impact on the second principal component (PC2), explaining 10.556% of the variation. A drought study conducted as part of abiotic stress tolerance thus identified several mango accessions with superior drought tolerance traits, particularly KLM37, PTA01, ALA23 and KLM12. These mango accessions are promising candidates for breeding programs aimed at improving drought resilience in mangoes.

Keywords

biochemical analysis; biochemical markers; drought; mango; physiological analysis

Introduction

Mango (*Mangifera indica* L.) stands as a cornerstone of tropical and subtropical fruit production, yet its cultivation is increasingly challenged by the spectre of drought, a consequence of climate change and burgeoning deprivation. While some mango cultivars exhibit inherent resilience to water deficit, a comprehensive understanding of drought tolerance mechanisms remains elusive. This study is predicated on the notion that identifying physiological

and biochemical markers associated with drought stress tolerance can facilitate the development of water-stress-resilient mango cultivars.

Drought tolerance in plants is a complex trait, involving numerous physiological and biochemical mechanisms that enable plants to survive and maintain productivity under water-deficit conditions. Among the key physiological traits, relative water content and leaf-saturated water content are crucial indicators of a plant's water status and ability to withstand dehydration. High RWC and SWC values suggest that a plant can maintain cellular turgor and metabolic functions during periods of water scarcity (1). Specific leaf area is another important trait, reflecting the leaf's capacity to capture light and perform photosynthesis efficiently. Plants with lower SLA are often better adapted to optimize their growth and development under limited water availability (2).

Epicuticular wax content plays a vital role in reducing water loss through transpiration, acting as a protective barrier on the leaf surface (3). Enhanced EWC is a common adaptation in drought-tolerant plants, contributing to improved water-use efficiency and reduced thermal stress (4). Additionally, the cell membrane stability index is a measure of the integrity and functionality of cellular membranes under stress conditions. Higher CMSI values indicate better preservation of membrane structure and function, which is essential for maintaining cellular homeostasis during drought (5).

Biochemical markers such as proline and chlorophyll content are integral to the plant's drought response. Proline serves as an osmoprotectant, stabilizing proteins and membranes and mitigating oxidative damage during stress (6). Increased proline levels are indicative of a plant's enhanced ability to cope with drought (7). Chlorophyll content, essential for photosynthesis, often declines under drought conditions, affecting the plant's energy production and growth (8). Maintaining higher chlorophyll levels under drought conditions is crucial for sustaining photosynthetic activity and overall plant health (9).

The outcomes of this research hold significant implications for mango breeding programs focused on enhancing drought tolerance. By identifying and characterizing drought-tolerant mango accessions, we can provide valuable insights into the genetic and physiological basis of drought resistance. These findings will not only contribute to the development of resilient mango cultivars but also offer a broader understanding of plant responses to drought stress, which can be applied to other crops facing similar challenges (10). Through this study, we aim to advance our findings in mangoes which support the concept of drought stress tolerance that improves crop resilience.

Materials and Methods

Plant Material

To evaluate the drought stress tolerance of 34 traditional mangoes accessions, a screening procedure for drought tolerance was used. These accessions came from different parts of four districts in the southern region of Kerala, India: Thiruvananthapuram, Kollam, Pathanamthitta and Alappuzha (Table 1). From March 2022 to April 2024, the experiment was carried out at the Farming Systems Research Station, Kerala Agricultural University, Sadanandapuram, Kerala (8°58'54.6"N 76°48'38.5" E).

Throughout the study, the fruits were collected and the seeds were removed from the fruits. The stones from each of these accessions were immediately sown in a polyhouse environment after being washed with flowing tap water. The seedlings were raised and they were then transplanted into 30 cm diameter poly-bags (50 microns, 12 kg capacity) and maintained in the poly-bags up to 8 weeks after transplanting. Each poly-bag had 10.0 kg of planting mixture in the ratio of 1:1:1 (w/w/w) mix of sand, soil and well -rotted farmyard manure. Each pot had a uniform soil volume to ensure consistent soil moisture content. The plants were irrigated daily to field capacity for an initial period of 8 weeks to ensure uniform growth before the onset of drought stress treatment.

Drought stress induction

For each accession, three replications and one common control were used. Soil moisture content was monitored using a digital moisture meter (Delmhorst F-2000 Digital Moisture Meter, USA). Drought stress was induced by withholding water for a period of 21 days. Soil moisture content was measured daily to ensure the progressive development of drought conditions. The control plant continued to receive regular irrigation to field capacity throughout the experimental period. The youngest fully expanded leaf of all accessions was labelled and collected for the measurements and analysis in 5-day intervals.

Experimental design and data sampling

The accessions were organized into a completely randomized design (CRD) with three replicates. Data for morphological features were recorded for a maximum of 30 days when sensitive cultivars exhibited severe stress symptoms and perished.

Water status and leaf morphology

Three leaf samples were collected from each accession to assess the relative water content (11). The fresh weight (FW), Turgid weight (TW) and dried weight (DW) of leaves were measured for each accession. After measuring the fresh weight (FW) of the leaves, they were placed into containers somewhat larger than the sample, filled with distilled water and left for 24 hr until a consistent weight (TW) was achieved. Any water sticking to the leaves was absorbed using tissue paper. Turgid weight was measured for each sample. The dry weight (DW) was acquired by subjecting these leaves to a drying process at 60°C in an oven for 48 hr until a constant weight was achieved.

The calculation of RWC (%) as :

$$RWC\ (\%) = (FW-DW) / (TW-DW) \times 100$$

Specific leaf area (SLA) and leaf-saturated water content (SWC) was calculated from the samples collected for relative water content

Table 1. Site descriptors of the collected 34 traditional mango varieties used in the study

Sl. No.	Accession No.	Agro-climatic Zone	Village/Block	District	State	Latitude	Longitude
1	ALA06	West Coast Plains and Ghat Region	Kayamkulam	Alappuzha	Kerala	9.16101	76.50613
2	ALA07	West Coast Plains and Ghat Region	Kayamkulam	Alappuzha	Kerala	9.16169	76.50541
3	ALA09	West Coast Plains and Ghat Region	Kattanam	Alappuzha	Kerala	9.18457	76.55572
4	ALA13	West Coast Plains and Ghat Region	Nilamperoor	Alappuzha	Kerala	9.48951	76.48849
5	ALA14	West Coast Plains and Ghat Region	Nilamperoor	Alappuzha	Kerala	9.48888	76.49784
6	ALA15	West Coast Plains and Ghat Region	Kainady	Alappuzha	Kerala	9.49642	76.47357
7	ALA20	West Coast Plains and Ghat Region	Kayamkulam	Alappuzha	Kerala	9.4986	76.4384
8	ALA21	West Coast Plains and Ghat Region	Kainady	Alappuzha	Kerala	9.49622	76.47384
9	ALA22	West Coast Plains and Ghat Region	Kainady	Alappuzha	Kerala	9.49426	76.47058
10	ALA23	West Coast Plains and Ghat Region	Kainady	Alappuzha	Kerala	9.49625	76.46954
11	ALA25	West Coast Plains and Ghat Region	Kainady	Alappuzha	Kerala	9.49582	76.47452
12	ALA27	West Coast Plains and Ghat Region	Harippadu	Alappuzha	Kerala	9.28216	76.44491
13	KLM03	West Coast Plains and Ghat Region	Chavara	Kollam	Kerala	8.99355	76.559
14	KLM04	West Coast Plains and Ghat Region	Kottarakkara	Kollam	Kerala	8.97242	76.73567
15	KLM10	West Coast Plains and Ghat Region	Thirumullavaram	Kollam	Kerala	8.89467	76.55787
16	KLM11	West Coast Plains and Ghat Region	Kollam	Kollam	Kerala	8.89614	76.55565
17	KLM12	West Coast Plains and Ghat Region	Kulakkada	Kollam	Kerala	8.32438	76.53449
18	KLM13	West Coast Plains and Ghat Region	Kollam	Kollam	Kerala	8.88358	76.5817
19	KLM15	West Coast Plains and Ghat Region	Thirumullavaram	Kollam	Kerala	8.89733	76.55704
20	KLM17	West Coast Plains and Ghat Region	Kollam	Kollam	Kerala	8.86784	76.84321
21	KLM20	West Coast Plains and Ghat Region	Kollam	Kollam	Kerala	8.88982	76.5753
22	KLM26	West Coast Plains and Ghat Region	Chavara	Kollam	Kerala	8.97498	76.54538
23	KLM27	West Coast Plains and Ghat Region	Chadaya-mangalam	Kollam	Kerala	8.86743	76.84198
24	KLM28	West Coast Plains and Ghat Region	Chadaya-mangalam	Kollam	Kerala	8.86743	76.84221
25	KLM29	West Coast Plains and Ghat Region	Chadaya-mangalam	Kollam	Kerala	8.86322	76.83712
26	KLM31	West Coast Plains and Ghat Region	Chadaya-mangalam	Kollam	Kerala	8.89743	76.86194
27	KLM33	West Coast Plains and Ghat Region	Thamarakulam	Kollam	Kerala	8.88338	76.58667
28	KLM35	West Coast Plains and Ghat Region	Eravipuram	Kollam	Kerala	816101	76.50613
29	KLM37	West Coast Plains and Ghat Region	Kulakkada	Kollam	Kerala	8.32518	76.42449
30	KLM38	West Coast Plains and Ghat Region	Kilikolloor	Kollam	Kerala	8.18457	76.55572
31	KLM40	West Coast Plains and Ghat Region	Vakkanadu	Kollam	Kerala	8.31143	76.43003
32	PTA01	West Coast Plains and Ghat Region	Karaykkadu	Pathanamthitta	Kerala	9.2695	76.75605
33	TVM01	West Coast Plains and Ghat Region	Navayikulam	Thiruvanantha-puram	Kerala	8.35609	76.14395
34	TVM02	West Coast Plains and Ghat Region	Kallara	Thiruvanantha-puram	Kerala	8.46083	76.88945

SLA ($cm^2\ g^{-1}$) = A/M_L

Where, A is the area of a given leaf or all leaves of a plant and M_L is the dry mass of those leaves.

Leaf-saturated water content was calculated using the formula

SWC (g $H_2O\ g^{-1}$ DW) = (TW-DW) / DW

Cell membrane stability Index

The cell membrane stability index was determined following the procedure outlined by (12). The samples were thoroughly washed three times in deionized water to eliminate any electrolytes that were sticking to the surface as a result of all the treatments. A 10 mL sample of deionized water was placed in a sealed 20 mL vial and left at room temperature for 24 hr in the absence of light. The conductivity was determined using a conductivity meter. Subsequently, the vials were subjected to autoclaving, resulting in the termination of leaf tissue viability for 15 min, thereby liberating the electrolytes. The second measurement of conductivity was obtained after the sample had been cooled. Both measures have been conducted separately for all treatments. The cell membrane stability index was determined and represented as a percentage using the following formula.

CMS (%) = [1 - (T1 / T2) / 1 - (C1 / C2)] x 100,

T1 and T2 are treatment conductivities before and after autoclaving and C1 and C2 are the respective control conductivities.

Epicuticular wax content

The modified and standardized method (13) was employed to estimate the epicuticular wax content of the leaf in mango. Chloroform (10 mL) was used to immerse five leaf segments (3 cm^2 area) from fully opened matured mango leaves. The leaves were vigorously shaken for 30 seconds and the chloroform was promptly transferred to a glass vial. The chloroform was evaporated until the vial was entirely dry. The potassium dichromate reagent (5 mL) was then added to the vial and the vial was subsequently placed in a boiling water immersion for 30 minutes. The optical density was determined at 590 nm using a UV-VIS spectrophotometer (AU2701 - Double Beam UV-VIS Spectrophotometer, Systronics, India) after the final volume was increased to 12 mL using distilled water. ECW expressed as mg cm^{-2} leaf area.

Proline Content

Proline content was determined per the procedure described by (11). 0.5g of mid-leaf portion was homogenized with 3% aqueous sulphosalicylic acid having a volume of 10 mL and centrifugation was done at 3000 rpm for 15 min (14). The supernatant (2 mL) was taken and blended with an equal amount of acid ninhydrin and glacial acetic acid. The mixture was kept at 100°C for one hr in the water bath. By keeping it in an ice bath for 10 min the reaction was terminated. The reaction mixture was mixed with 4 mL toluene using a vortex mixture for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the optical density was read at 520 nm. with toluene as blank. A standard curve was drawn using concentration versus absorbance. The concentration of proline was determined from a graph and expressed as µg /g tissue = {[(µg proline / mL) x ml toluene] / 115.5} x (5/ g sample), where, 115.5 is the molecular weight of proline.

Total Chlorophyll content

A portable chlorophyll meter (at LEAF® CHL BLUE chlorophyll meter, USA) was used to measure the SPAD (Chlorophyll meter reading) SCMR units, which is a measure of the chlorophyll status of the leaf. The SPAD reading was measured after the instrument was fastened to the leaf at various positions and on various leaves of the plant. The average SPAD values were calculated by subtracting the mean of the SPAD value.

Statistical Analysis

A complete randomized block design with three replications was implemented in the experiment. F values were computed using SPSS 22.0 and all data were subjected to an analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) using GRAPES (13). The threshold for statistical significance was set at $P < 0.05$.

Results

Water status indicators

The analysis of relative water content (RWC) under induced drought stress in 34 mango accessions and one control revealed significant differences among treatments ($P < 0.05$). Among the accessions, KLM37 (89.39%), PTA01 (88.62%) and ALA23 (88.52%) demonstrated the highest RWC, indicating superior drought tolerance (Fig. 1a).

The analysis of saturated water content (SWC). Notably, KLM37 (1.567 mg cm^{-2}), ALA15 (1.530 mg cm^{-2}) and PTA01 (1.493 mg cm^{-2}) showed the top highest SWC values among the accessions, indicating better water retention under drought conditions (Fig. 1b).

Leaf morphology traits and cellular integrity

An analysis of variance (ANOVA) on the specific leaf area revealed extremely significant differences across the treatments ($F(34, 70) = 768.77$, $P < 0.05$). Out of the accessions, KLM33, KLM-38, TVM01, KLM10 and KLM-27 exhibited lower specific leaf area (SLA) values of 112.51, 113.17, 113.5, 113.60 and 113.87 cm^2/g, respectively (Fig. 2a). These accessions formed different groups according to the DMRT test (Duncan Multiple Range Test), indicating their superior ability to adapt to drought conditions. Genotypes with low SLA perform better with low leaf area whereby the transpiration is reduced.

In the same manner, the examination of Epicuticular Wax Content showed significant variance among the different treatments. Notably, KLM37, PTA01, ALA23 and KLM12 displayed increased wax levels, measuring 65.28, 64.96, 64.51 and 61.78 µg/cm^2, respectively. The accessions were clustered, showing their exceptional ability to tolerate drought due to increased wax deposition (Fig. 2b). Accessions such as KLM13, KLM31 and KLM35 displayed elevated levels of wax content, which further sets them apart as being more resistant to drought compared to other accessions.

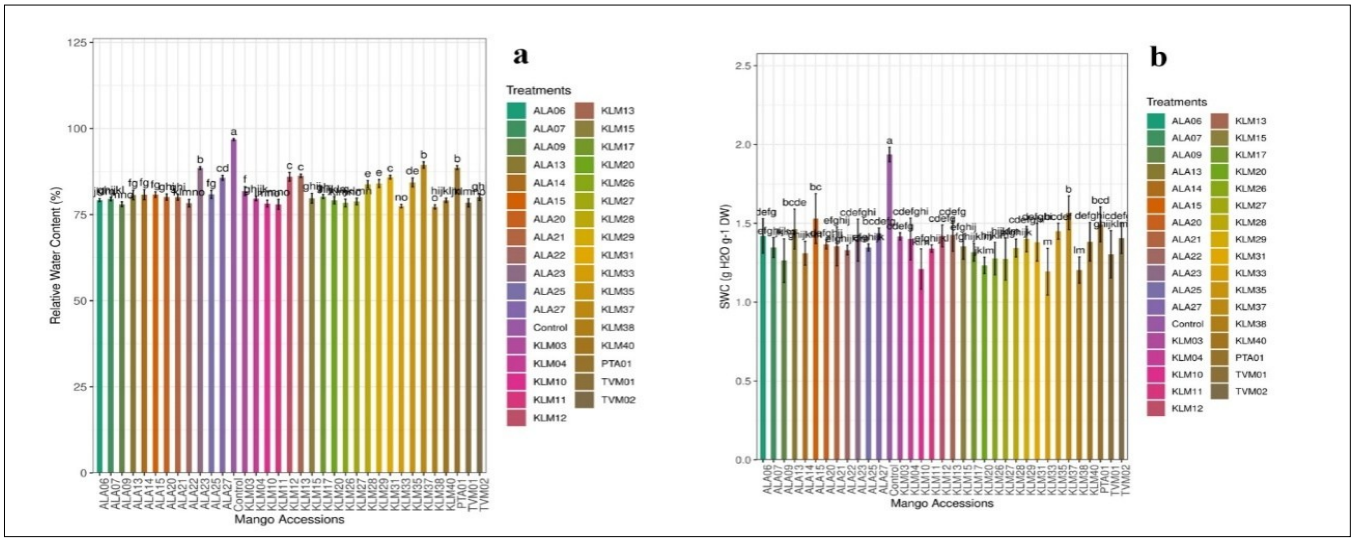

Fig. 1. Accumulation statistics **(a)** Relative water content and **(b)** Leaf saturated water content of two month old plants, replicated thrice.

The CMS analysis confirmed the substantial variations between treatments ($F(34, 70) = 34296.3$, $P < 0.05$). Notably, KLM37, PTA01, ALA23 and KLM12 showed considerable CMS values at 65.28%, 64.96%, 64.51% and 61.78% respectively (Fig. 2c). These accessions, along with KLM13, KLM31 and KLM35, demonstrated enhanced cell membrane integrity in response to drought stress, indicating their resilience. The notable differences in SLA, Epicuticular Wax Content and CMS among the accessions under drought stress highlight the potential of specific mango accessions, particularly KLM37, ALA23, KLM12 and PTA01.

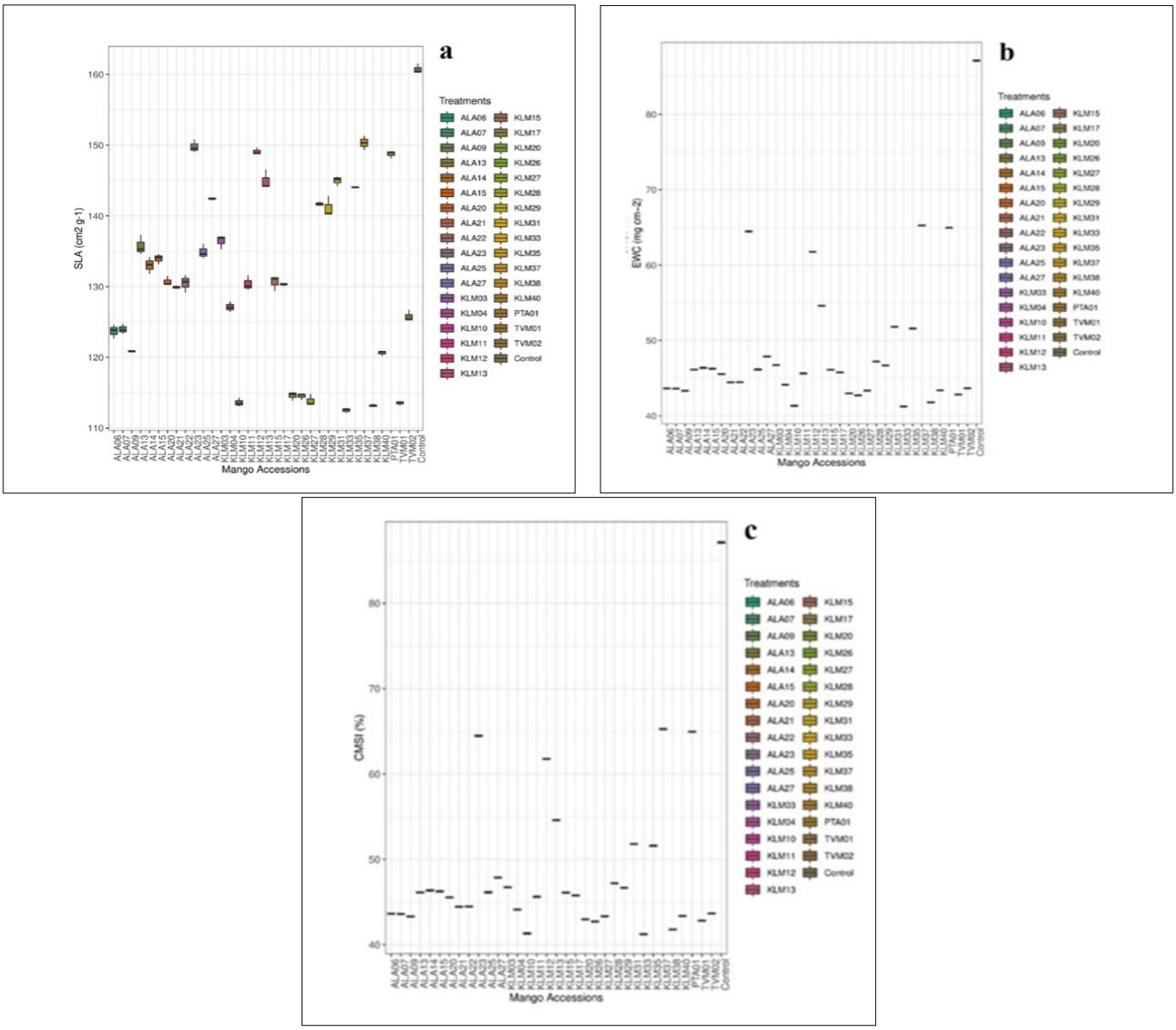

Fig. 2. DMRT Box plot showing variations in **(a)** Specific leaf area, **(b)** Epicuticular wax content and **(c)** Cell membrane stability index.

Biochemical Markers

Out of the accessions, ALA27 (2.487), ALA23 (2.48) and KLM37 (2.283) exhibited significantly elevated Proline content, establishing separate groupings (Fig. 3a). These accessions, including KLM13 (2.217) and KLM20 (2.167), exhibited increased Proline accumulation, indicating their potential for drought tolerance. Significantly, accessions ALA15 (1.227) and TVM02 (1.423) had the lowest Proline content, indicating a reduced ability to respond to stress.

Furthermore, accessions such as PTA01 (55.103), TVM02 (55.093) and ALA27 (54.17) exhibited increased chlorophyll levels and efficiency has to be quantified by photosynthetic systems instruments (Fig.3b). Accessions KLM29 (54.027) and KLM37 (53.75) exhibited elevated levels of chlorophyll, clearly distinguishing them and emphasizing their ability to withstand drought conditions. KLM11 (43.04) and TVM01 (43.083) had the lowest chlorophyll concentration.

Principal Component analysis

Principal Component Analysis (PCA) was conducted to assess the relative contributions of multiple physiological and biochemical parameters (Fig. 4). The first principal component (PC1) explained 76.913% of the total variation. This means that PC1 contains most of the important information from the original variables. The traits RWC, CMSI and EWC exhibited the highest loadings on PC1, with values of 0.423, 0.416 and 0.416, respectively. This indicates that these traits make a major contribution to the overall variation and are crucial indicators of drought tolerance.

PC2, which accounts for an additional 10.556% of the variance, shows that proline and chlorophyll contents have significant loadings (0.681 and 0.682, respectively). This suggests that proline and chlorophyll are important factors in distinguishing the accessions based on their response to drought. The following principal components (PC3 to PC7) accounted for decreasing proportions of the variation, with PC3 capturing 5.754% and the remaining components contributing only minimally. The significant eigenvalues and percentage contributions of factors on PC1 highlight the importance of RWC, CMSI and EWC in evaluating the ability of mango accessions to tolerate drought stress. The significant positive correlations between these factors and PC1 (0.981, 0.965 and 0.965, respectively) emphasize their interrelated function in preserving cellular integrity and hydration retention during stressful circumstances (supplementary file 1).

Discussion

Differences in RWC as part of the drought stress study among the accessions under induced drought stress ($P < 0.05$) highlight the variability in drought tolerance. Notably, accessions KLM37 (89.39%), PTA01 (88.62%) and ALA23 (88.52%) demonstrated the highest RWC, indicating their superior ability to maintain high water status under water-stressed conditions. High RWC is critical for maintaining cell turgor and metabolic functions during drought stress, corroborating findings from studies on other crops such as wheat and rice (14). The relative water content of wheat

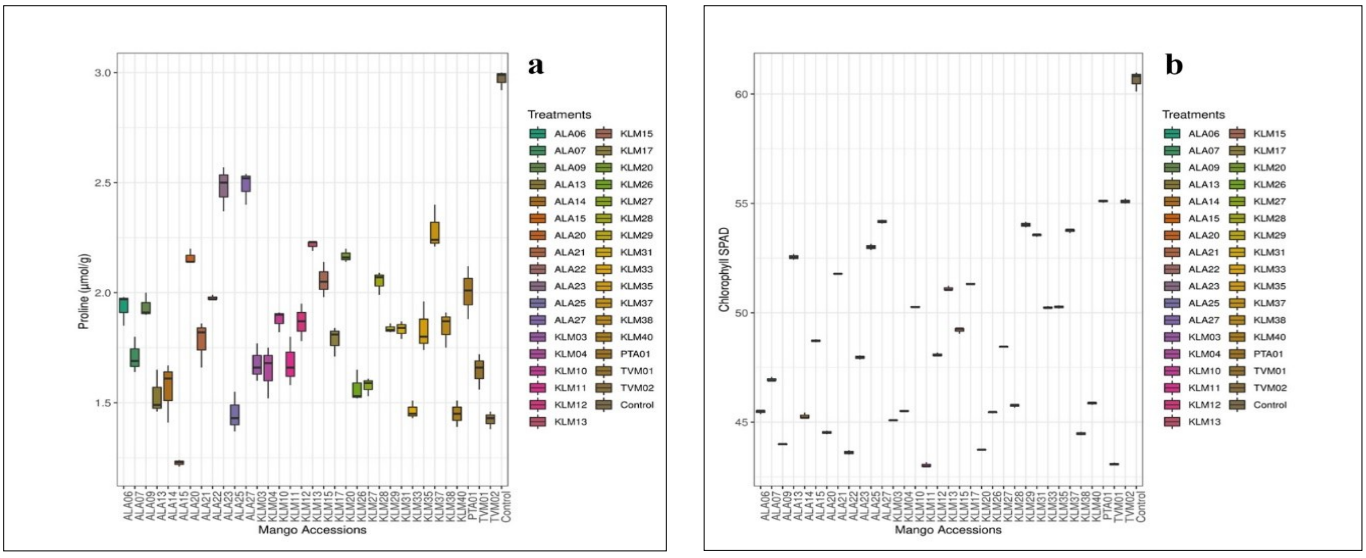

Fig. 3(a) Proline and **(b)** Chlorophyll content in mango accessions.

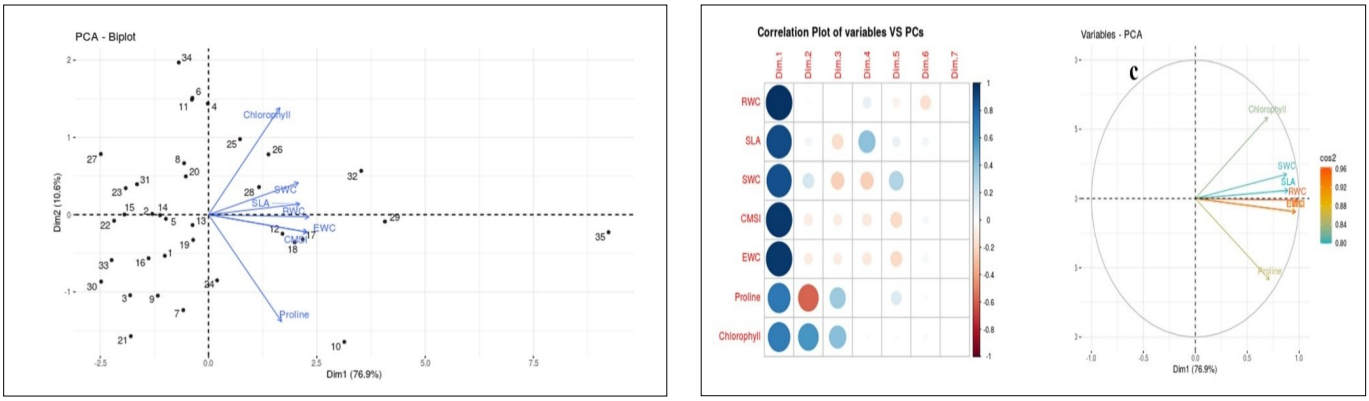

Fig. 4. Correlation analysis of physio-chemical parameters of mango accessions.

leaves was higher initially during leaf development and decreased as the dry matter accumulated and the leaf matured.

The analysis of SWC further supported these results, with KLM37 (1.567 mg cm^{-2}), ALA15 (1.530 mg cm^{-2}) and PTA01 (1.493 mg cm^{-2}) exhibiting the highest SWC values. This indicates these accessions' ability to retain water more effectively under drought stress, underscoring their potential for improved drought resilience. Previous research has emphasized the importance of water retention capabilities in drought-tolerant genotypes, suggesting that high SWC contributes to maintaining physiological activities during water deficit conditions (15).

Significant differences in specific leaf area (SLA) ($F(34, 70) = 768.77$, $P < 0.05$) among accessions, particularly in KLM37, ALA23, KLM12 and PTA01, suggest that these accessions have a superior ability to adapt their leaf morphology to drought conditions. Higher SLA values are associated with a greater surface area for light capture and gas exchange, enhancing photosynthetic efficiency under limited water availability (16). This adaptation is crucial for sustaining growth and productivity in drought-prone environments, as leaves with higher SLA can maximize photosynthesis while transpiration loss is high. Hence leaves with less SLA are proffered.

Epicuticular wax content (EWC) and cell membrane stability index (CMSI) also showed substantial variance among the accessions. Accessions KLM37, PTA01, ALA23 and KLM12 exhibited significantly higher EWC and CMSI values, indicating their enhanced ability to tolerate drought through increased wax deposition and improved cell membrane integrity. Epicuticular waxes reduce transpiration by forming a barrier against water loss and increasing reflectance to reduce leaf temperature (17). Higher CMSI values indicate better cell membrane stability, which is vital for maintaining cellular functions under stress conditions, as reported by Bajji et. al. (18, 19). The above mentioned genotypes showed high water status also as these are high in wax so prevent transpiration loss.

Biochemical markers such as proline and chlorophyll content further distinguished the accessions' drought responses. Accessions ALA27 (2.487), ALA23 (2.48) and KLM37 (2.283) exhibited significantly elevated proline levels, which play a crucial role in osmotic adjustment and protection of cellular structures under stress. Proline accumulation is a common response to drought stress and has been widely documented as a marker for stress tolerance in various plant species (20). High chlorophyll content in accessions like PTA01 (55.103), TVM02 (55.093) and ALA27 (54.17) suggests better maintenance of photosynthetic activity under drought conditions. Chlorophyll stability under drought stress is essential for sustaining photosynthesis and, consequently, growth and yield (21). Genotypes mentioned for proline and chlorophyll content are either showing the highest values for these parameters or on par with the accessions showing the highest value and they were selected as drought stress tolerant ones.

Principal Component Analysis (PCA) indicates that all the traits are crucial indicators of drought tolerance and contribute significantly to the overall variation among the accessions. The substantial positive correlations between these traits and PC1 emphasize their interrelated roles in maintaining cellular integrity and hydration during drought stress. Studies on other crops have similarly identified RWC, membrane stability and epicuticular wax as key traits associated with drought tolerance (22). Characters chlorophyll content, SWC, SLA, RWC, EWC, CMS and proline content are the characters contributing to PC1 and it is contributing up to 76.9%.

The second principal component (PC2), explaining an additional 10.556% of the variance, highlighted the importance of proline and chlorophyll contents in distinguishing the accessions' drought responses. This suggests that these biochemical markers are critical for understanding the physiological mechanisms underlying drought tolerance. Proline's role in osmo protection and reactive oxygen species scavenging (23).

Conclusion

The study identified several mango accessions with superior drought tolerance traits, particularly KLM37, PTA01, ALA23 and KLM12. These accessions having water-saving ability exhibited high RWC, SWC, SLA, EWC, CMSI, proline and chlorophyll content, making them promising candidates for breeding programs aimed at improving drought resilience in mangoes but in the future, further confirmatory studies need to be conducted at the genetic level.

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Authors' contributions

BB was responsible for conception and design. BB and SS contributed to the analysis and interpretation of the data. SS played a key role in the drafting of the article. BB, RB and SS carried out the critical revision of the article for important intellectual content. RB, AM and SS performed the statistical expertise. BB obtained funding to carry out the work. BB, AM and SS took part in the collection and assembly of data.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical issues: None

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