South African Journal of Botany 180 (2025) 378–386

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Leaf anatomical traits of macadamia cultivars with potential links to drought tolerance

Khodani Maumela[a], Nicolette J Taylor[a], Kenneth C Oberlander[a,b], Angelique Kritzinger[a,*]

[a] Department of Plant and Soil Sciences, University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa
[b] Department of Plant and Soil Sciences, H.G.W.J. Schweickerdt Herbarium, University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa

ARTICLE INFO

Article History:
Received 23 December 2024
Revised 27 February 2025
Accepted 17 March 2025
Available online 21 March 2025

Edited by: Kamil Eugeniusz Frankiewicz

Keywords:
Macadamia
Stomatal density
Vein density
Petiole Huber value
Leaf anatomy
Drought tolerance

ABSTRACT

Selecting *Macadamia* cultivars with anatomical traits capable of withstanding drought may improve water management in their cultivation. *Macadamia integrifolia, Macadamia tetraphylla*, and their hybrids are commercially grown in South Africa for their high-value edible nuts, however, to improve yields, irrigation is required. This study examined leaf and petiole anatomical traits linked with plant water-use strategies in four commonly planted cultivars: ‘695′, ‘814′, ‘816′, and ‘A4’. Key traits investigated included stomatal density, mesophyll thickness (palisade and spongy parenchyma), vein density, and petiole Huber value. Fully matured macadamia leaves with their petioles were collected from Komatipoort, Nelspruit, and KwaZulu-Natal South Coast during the spring flush of 2021 and the summer flush of 2022 for anatomical analysis. Box plots illustrated significant variations in leaf and petiole anatomical traits among macadamia cultivars and regions. Cultivars ‘814′ and ‘695′ exhibited higher stomatal densities and higher vein densities, while cultivar ‘814′ also showed thicker mesophyll tissues and higher petiole Huber values. These traits suggest that cultivars ‘814′ and ‘695′ may have improved water transport and potential drought tolerance than cultivars ‘816′ and ‘A4’. Seasonal variation accounted for 45 % of the observed variability in leaf and petiole anatomy, highlighting a strong influence of environmental factors during leaf development. However, the anatomical variation was shaped not only by environmental factors but also by cultivar-specific characteristics. These findings suggest that these leaf and petiole anatomical traits could serve as indicators of drought tolerance and help improve the water management practices in macadamia cultivation, particularly in regions with inadequate rainfall.

1. Introduction

The genus *Macadamia* is a member of the family *Proteaceae*, originating from the subtropical coastal areas of eastern Australia (Hardner et al., 2009; Mast et al., 2008). Today, macadamia trees are commercially grown for their high-value edible nuts in tropical and subtropical regions worldwide, including Australia, Brazil, China, Colombia, Costa Rica, Guatemala, Kenya, Malawi, and South Africa (Ahmed et al., 2024; Navarro and Rodrigues, 2016). Among these countries, South Africa is the leading global producer of macadamia nuts and the industry is increasing rapidly. However, due to frequent periods of drought in the country, irrigation is necessary to maintain yields and quality (Carr, 2013; WOM, 2024). The rapid expansion of irrigated macadamia orchards calls for improved water management practices within the industry to ensure sustainable food production. Despite their economic importance, there is a considerable knowledge gap concerning the specific water requirements of macadamia trees, with most of the literature centered around the macadamia cultivar HAES ‘695′, also known as ‘Beaumont’ (Smit et al., 2020; Taylor et al., 2021). While these studies have yielded valuable insights, the water requirements for other commercially grown cultivars in South Africa remain unexplored.

Plants employ two strategies to cope with drought: avoidance and tolerance. Drought avoidance allows plants to maintain their water status by enhancing water uptake and reducing water loss, whereas drought tolerance allows the plants to maintain their physiological functions, despite the limited water availability (Ennajeh et al., 2010; Shankar et al., 2021). Both strategies rely on anatomical, morphological, and physiological adjustments. Among these, leaf anatomical traits play a crucial role in regulating transpiration and photosynthesis rates, making them key indicators of drought tolerance (Luković et al., 2009). Macadamia trees are classified as drought-tolerant species, by exhibiting hypostomatic stomata (abaxial leaf surface) and sclerophyllous leaves, which reduce transpiration and gaseous exchange. Additionally, internal self-shading minimizes water loss while maintaining photosynthesis (Shabalala et al., 2022).

Key leaf anatomical traits previously linked to drought tolerance in various plant species include higher stomatal density, thicker mesophyll tissues (palisade and spongy parenchyma), increased vein

---

[*] Corresponding author.
E-mail address: angelique.kritzinger@up.ac.za (A. Kritzinger).

https://doi.org/10.1016/j.sajb.2025.03.032
0254-6299/

density, and higher Huber value (Bosabalidis and Kofidis, 2002; Ennajeh et al., 2010; Mencuccini et al., 2019; Sack and Scoffoni, 2013). Higher stomatal density enhances carbon dioxide (CO_2) uptake, thicker palisade parenchyma provides a site for carbon fixation, and thicker spongy parenchyma promotes the diffusion of CO_2 through the intracellular spaces. Vein density defined as the total length of veins per unit leaf area (Sack et al., 2014), plays a role in the distribution of water throughout the leaf blade. Higher vein density provides a short distance for water to travel to reach the mesophyll tissues, facilitating transpiration and photosynthesis rates under water-limiting conditions. Between the major and minor veins, minor veins are the most critical in determining the leaf's hydraulic capacity and photosynthetic efficiency (Sack et al., 2012). In this study, vein density refers to minor vein density. The Huber value, as previously applied for tree stems, reflects the ratio of the stem xylem cross-sectional area to the leaf area it supplies (Tyree and Ewers, 1991). This concept has further been extended to flower pedicels and leaf petioles due to their similar functions (Blackman et al., 2024; Roddy et al., 2016). Here we defined the petiole Huber value as the ratio of the petiole xylem-cross sectional area to the leaf evaporative surface. A higher Huber value indicates a greater water supply relative to the leaf size, to support transpiration. Collectively, these traits contribute to the plant's ability to thrive under drought conditions, highlighting the importance of leaf structure in drought adaptation.

Given the importance of leaf structure in regulating transpiration and photosynthesis, which reflect the adaptation of the plant to the environment, examining the internal leaf structures can provide valuable insights into water-use strategies. This approach is cost-effective and relatively quick for assessing drought tolerance in plants. Therefore, investigating leaf anatomical traits across macadamia cultivars in South Africa could provide insights into their water-use strategies, guide future trials, and aid in selecting drought-tolerant cultivars for sustainable production. This study investigated the variability in laminar and petiolar anatomical traits across various cultivars of macadamia trees in South Africa. The study aimed to offer insights into potential variations in transpiration rates and water use efficiency, thereby enhancing water management strategies for macadamia cultivation in South Africa. Firstly, we investigated whether laminar and petiolar anatomy remains constant among diverse macadamia cultivars from different regions within South Africa. Secondly, we investigated whether differences in laminar and petiolar anatomy among macadamia cultivars could indicate potential differences in transpiration rates. We hypothesized that cultivars exhibiting higher stomatal density, thicker mesophyll tissues, increased vein density, and higher petiole Huber value would demonstrate efficient water transport capacity and potentially drought tolerance.

2. Materials and methods

2.1. Study sites and sampling

Fully matured macadamia leaves were collected from macadamia-producing regions in South Africa: Komatipoort and Nelspruit (Mpumalanga Province), and the KwaZulu-Natal South Coast (KZN). The investigated cultivars included the two hybrids ('695′ and 'A4'), and two M. *integrifolia* cultivars ('814′ and '816′). Trees were at a bearing stage, except for '814′ and '816′ in Komatipoort (2 years old). From each farm, 14 random trees per cultivar were sampled. One leaf per tree was harvested during the spring flush of 2021 and summer flush of 2022, with seven sun-exposed leaves and seven shade leaves. Sun and shade leaves were sampled to account for their well-documented anatomical differences (Dörken and Lepetit, 2018). All the leaves were collected within two weeks within each harvesting window.

Climatic data were sourced from the Agricultural Research Council (ARC), to describe the environmental conditions for each region. Saturation vapour pressure deficit (VPD, kPa) was calculated from saturation vapour pressure (daily) [es (kPa)] and average hourly actual vapour pressure [eª (kPa)]. Reference evapotranspiration (ET_O) was calculated using the FAO Penman-monteith equation for a short grass reference surface, according to Allen et al. (1998).

2.2. Leaf and petiole anatomical traits

The epidermal peel method was used to measure stomatal density (Gago et al., 2019). Fully expanded leaves from each cultivar were collected and processed on the same day. Clear nail polish was applied to the abaxial leaf surface, accompanied by a strip of transparent tape to aid in peeling. Microscopic examination was conducted at 100 × total magnification using a Nikon Eclipse E200 (TOCHIGI Nikon Corporation), and images were captured with an Olympus EP50 camera (Olympus). The variation in stomatal density across the leaf was accounted for by selecting three random zones across the leaf's center. Stomatal density (mm^{-2}) was calculated by counting all stomata within the field of view and dividing by the area of the field of view, including partially visible stomata. Additionally, stomatal size was calculated using the formula: guard cell length × guard cell width.

To determine palisade and spongy parenchyma thickness, petiole Huber value, and vein density, fully expanded leaves including their petioles were collected and immediately fixed in 70 % ethanol. Before leaf and petiole sectioning, the leaf area was determined by photographing the leaves against a white sheet using a Nikon D3100 digital camera (Nikon Corporation) and analyzing the images in ImageJ (version 1.53k) (Rasband, 2012), following the methods by Ahmad et al. (2015). Due to the sclerification of macadamia leaf and petiole tissues, classic microtomy approaches proved challenging. Therefore, leaf blades and petioles were manually sectioned using a technique described by Yeung (1998). Sections were viewed as previously described. Three petiole sections and five leaf blade sections per leaf were examined at 40 × and 100 × total magnification, respectively. Measurements for these sections were done in ImageJ and values were averaged to produce a representative measurement for each leaf as shown in (Figs. S1 and S2). The Petiole Huber value was calculated: Petiole Huber value = Petiole xylem cross-sectional area (cm^2) / Leaf area (cm^2).

To measure vein length, one cm^2 sample was taken from midway between the leaf midrib and margin, midway between the base and tip of the same leaves used for the measurements of mesophyll tissue thickness and petiole xylem cross-sectional area. Leaves were cleared in 4 % NaOH at room temperature, and the solution was changed weekly until the veins were visible (Roddy et al., 2013). To finish the clearing process, samples were soaked in distilled water and 3 % bleach, then rinsed with water and 95 % ethanol. Slides were prepared and imaged at 40x total magnification using a Nikon Eclipse E200 microscope with an Olympus EP50 camera. The length of minor veins was measured in Image J, following the steps by Pagano et al. (2016). Vein density ($mm\ mm^{-2}$) was calculated as the total vein length per unit leaf area.

2.3. Statistical analysis

Statistical analyses were conducted using R version 4.2.0 (R Core Team, 2024). To test for significant differences in stomatal density, palisade parenchyma thickness, spongy parenchyma thickness, vein density, and petiole Huber value across macadamia cultivars ('695′, '814′, '816′, 'A4'), and regions (Komatipoort, Nelspruit, and KwaZulu-Natal South Coast), linear models were fitted using the 'lm' function. Each model included all fixed effects including region, farm, cultivar season, leaf type (sun vs shade), and their interactions. Non-significant interactions were removed through a stepwise model simplification to obtain a Minimal Adequate Model. Due to model assumption violations, stomatal density, palisade parenchyma thickness, and spongy parenchyma thickness data were log-transformed. In contrast, petiole Huber value and vein density data were transformed

Table 1
Mean seasonal temperatures (°C), vapour pressure deficit (VPD, kPa), and total reference evapotranspiration (ETo, mm) during the spring flush of 2021 and summer flush of 2022 across regions.

Region	Season	Max Temp (°C)	Min Temp (°C)	VPD (kPa)	ETo (mm)
Nelspruit	Spring	28.2	14.3	1.47	338
Nelspruit	Summer	30.4	18.8	1.41	392
Komatipoort	Spring	29.7	16.0	1.49	389
Komatipoort	Summer	32.8	20.7	1.57	406
KwaZulu-Natal South Coast	Spring	23.5	15.7	0.71	296
KwaZulu-Natal South Coast	Summer	27.4	20.0	0.78	321

using Box-cox transformation. A one-way ANOVA was performed separately for each region to evaluate significant differences among cultivars. Tukey's Honest Significant Difference (HSD) ($p < 0.05$) was used to assess for significant differences between cultivars within each region. Principal Component Analysis (PCA) across all anatomical traits measured (stomatal density, palisade parenchyma, spongy parenchyma, Petiole Huber value, and vein density) was conducted to identify the factors contributing to the leaf and petiole variability across cultivars and regions. Permutational Analysis of Variance (PERMANOVA) was performed to test the major leaf and petiole anatomy patterns across seasons, regions, leaf types, farms, and cultivars.

3. Results

3.1. Seasonal weather

The climatic conditions varied across the regions (Table 1). During the spring flush, Komatipoort recorded the highest mean maximum temperatures (29.7 °C), VPD (1.49 kPa), and total ET_O (389 mm). In comparison, KwaZulu-Natal South Coast recorded the lowest mean maximum temperatures (23.5 °C), VPD (0,71 kPa), and total ET_O (296 mm). Similar trends were also recorded during the summer flush. Komatipoort had the highest mean maximum temperatures (32.8 °C), VPD (1.57 kPa), and total ET_O (406 mm), while KwaZulu-Natal South Coast recorded the lowest mean maximum temperatures (27.4 °C), VPD (0,78 kPa), and total ET_O (321 mm). Nelspruit showed intermediate values for all climatic variables.

3.2. Leaf and petiole anatomy across cultivars and regions

Microscopy images of macadamia leaves and petioles were used to examine the investigated anatomical traits (Fig. 1). The abaxial leaf surface had paracytic stomata, each surrounded by a pair of guard cells (Fig. 1A). The leaf cross-section revealed the mesophyll consisting of palisade parenchyma, with two layers of closely packed columnar cells, and spongy parenchyma, distinguished by irregular cells,

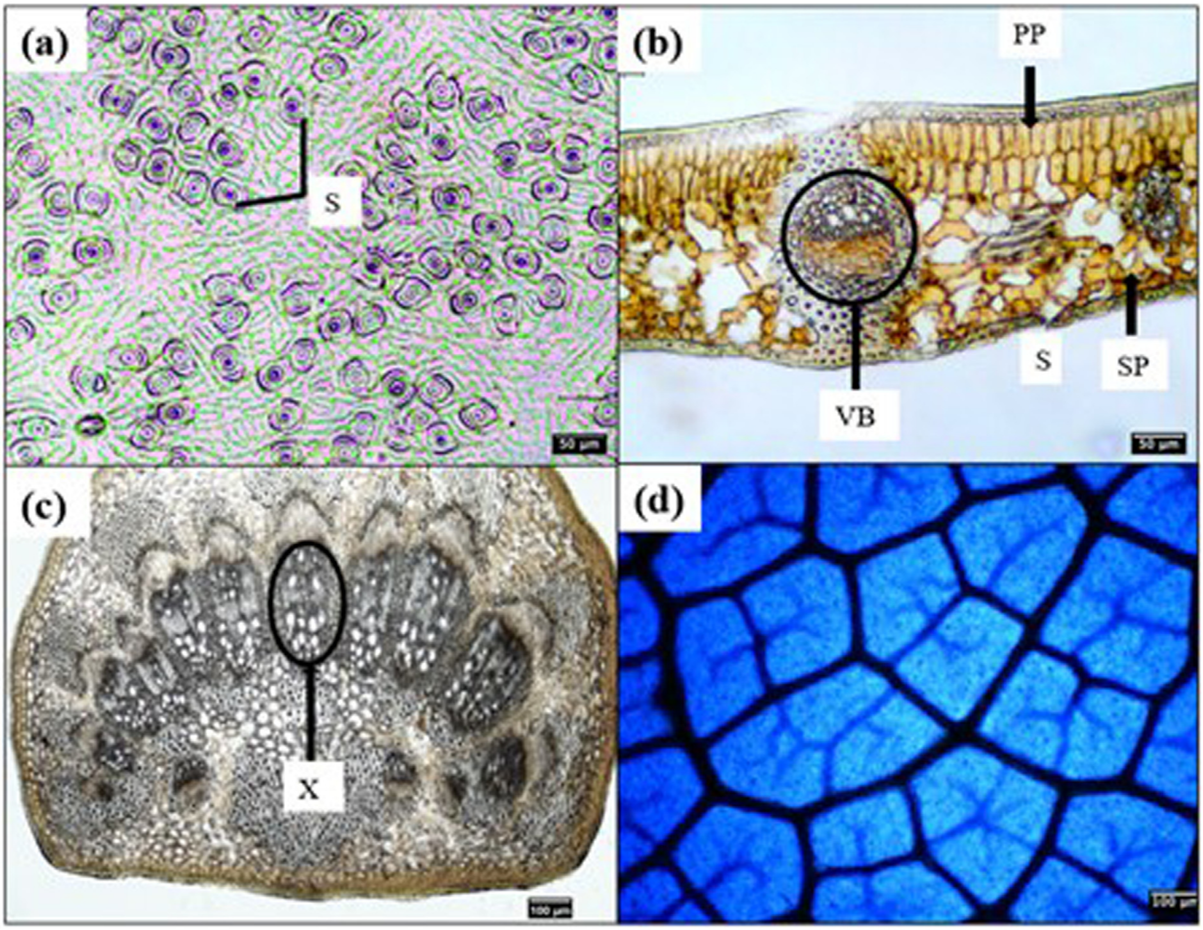

Fig. 1. Leaf and petiole anatomical traits of macadamia. A, stomata; B, cross-section of macadamia leaf; C, cross-section of macadamia petiole; D, minor veins on macadamia leaf. Arrows: s = stomata; pp = palisade parenchyma; sp = spongy parenchyma; vb = vascular bundle; x = xylem. Scale bars: A and B = 50 μm; C and D = 100μm.

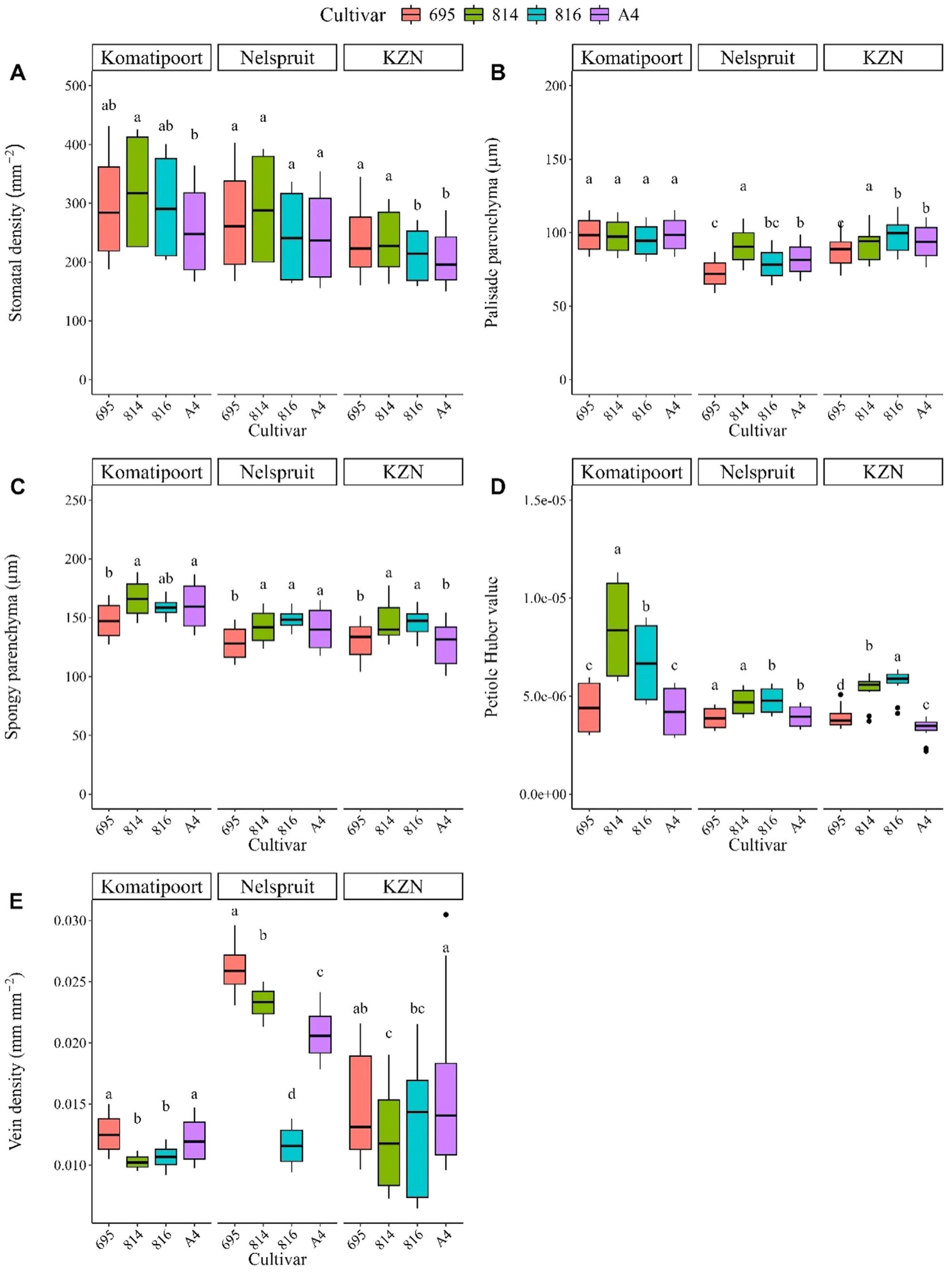

Fig. 2. Box plots illustrating the variation in stomatal density (A), palisade parenchyma thickness (B), spongy parenchyma (C), petiole Huber value (D), and vein density (E) among macadamia cultivars ('695', '814', '816', and 'A4' across different regions of South Africa: Komatipoort, Nelspruit, and KZN. Lowercase letters above boxplots indicate significant differences among cultivars ($p < 0.05$).

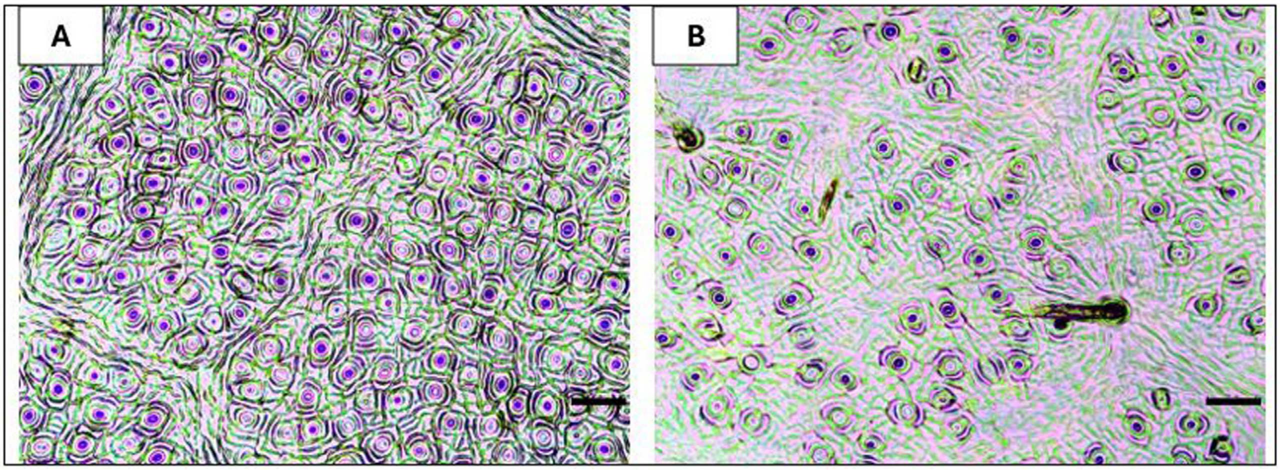

Fig. 3. Stomata on the abaxial leaf surface of macadamia cultivars '814′ (A) and 'A4' (B). Scale bars = 50 μm.

with intracellular air spaces (Fig. 1B). The petiole cross-section displayed collateral vascular bundles, with variations in xylem number among cultivars (Fig. 1C) (Abubaker et al., 2018). Additionally, leaf venation patterns exhibited a network of minor veins (Fig. 1D).

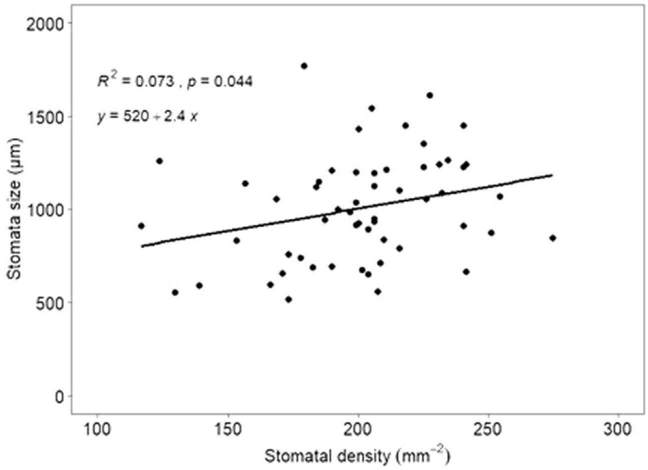

Fig. 4. Relationship between stomatal density (mm^{-2}) and stomata size (μm).

Leaf and petiole anatomy varied significantly among macadamia cultivars and across regions (Fig. 2). Stomatal density varied significantly among cultivars in KZN ($p < 0.001$), whereas no significant differences were detected in Nelspruit (p = 0.142) and Komatipoort (p = 0.074) (Fig. 2A). Across regions, cultivars '814′ and '695′ exhibited higher stomatal densities, while cultivars A4' and '816′ showed the lowest (Fig. 3). There was a weak positive correlation between stomatal density and stomatal size (R^2 = 0.073 and p = 0.044) (Fig. 4). Palisade parenchyma thickness was significantly different among cultivars in Nelspruit and KZN ($p < 0.001$), but no significant differences were observed in Komatipoort (p = 0.605) (Fig. 2B). Cultivar '814′ had the thickest palisade parenchyma, whereas cultivars '695′, '814′, and 'A4' had the thinnest (Fig. 5). Spongy parenchyma thickness varied significantly among cultivars in all three regions ($p < 0.001$), (Fig. 2C), with cultivar '814′ exhibiting thicker spongy parenchyma. Petiole Huber values varied significantly across cultivars in all regions ($p < 0.001$), with cultivars '814′ consistently exhibiting higher petiole Huber values than cultivars '816′, '695′ and 'A4' (Fig. 2D). Vein density also varied significantly across cultivars in all regions ($p < 0.001$), with cultivar '695′ having the highest vein density across regions compared to cultivars '814′, '816′, and 'A4'.

3.3. Principal components analysis (PCA)

The first two principal components explained 56.5 % of the variability. PC1 (36.5 %) was driven by palisade parenchyma (PP), spongy

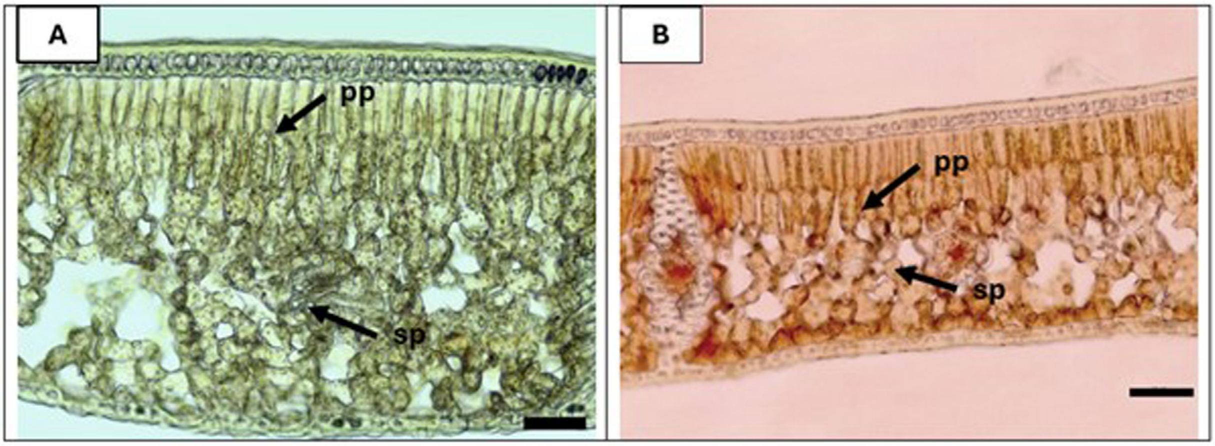

Fig. 5. Cross-sections of the leaves of macadamia cultivars '814′ (A) and '695′ (B). pp= palisade parenchyma; sp= spongy parenchyma. Scale bars = 50 μm.

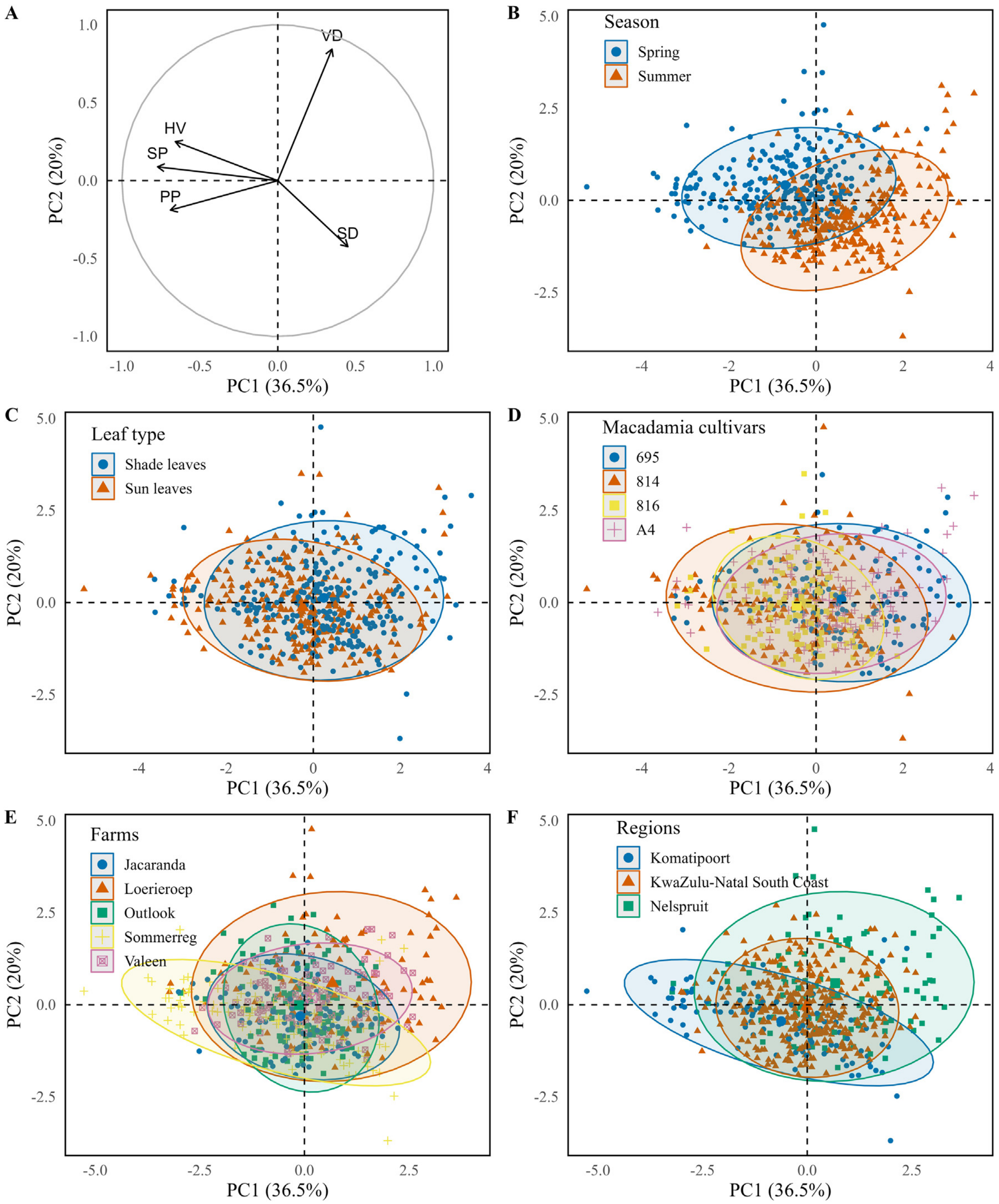

Fig. 6. Principal Components analysis of macadamia leaf and petiole anatomical traits. A: PCA plot showing the relationship between stomatal density (SD), palisade parenchyma (PP), spongy parenchyma (SP), vein density (VD), and petiole Huber value (HV). B: PCA plots clustered by season (B), leaf type (C), cultivars (D), farms (E), and regions (E). Ellipses represent a 95 % confidence interval.

parenchyma (SP), and petiole Huber value (HV), while PC2 (20 %) was driven by stomatal density (SD) and vein density (VD) (Fig. 6A). Clustering patterns were strongest for the season, with spring and summer showing distinct grouping (Fig. 6B). Additional clustering was observed for leaf type, farm, region, cultivar, and region (Fig. 6C–F). PERMANOVA analysis confirmed that season was the strongest driver of variability (R^2 = 0.455, P = 0.001), followed by region and cultivar effects (Table 2). Additionally, there were significant interactions (Table 2).

4. Discussion

This study examined whether leaf and petiole anatomical traits vary across macadamia cultivars and regions in South Africa, and explored how these traits could potentially influence transpiration rates and water-use strategies. Significant differences in stomatal density, palisade parenchyma thickness, spongy parenchyma thickness, petiole Huber value, and vein density across macadamia cultivars and regions were observed. Seasonal variation emerged as the

Table 2
Permutation analysis of variance (PERMANOVA) table showing the significant effect of season, region, farm, cultivar, leaf type, and interactions on leaf and petiole anatomy. Number of permutations = 999.

Factor	Df	SumOfSqs	R^2	F	Pr(>*F*)
Season	1	2,263,796,18	0,45	1136,90	0,001*
Region	2	557,604,28	0,11	140,02	0,001*
Farm	2	22,342,88	0,00	5,61	0,001*
Cultivar	3	177,776,70	0,04	29,76	0,001*
Leaf type	1	152,671,05	0,03	76,67	0,001*
Season: Region	2	256,667,24	0,05	64,45	0,001*
Season: Farm	2	28,935,68	0,01	7,27	0,001*
Season: Cultivar	3	24,083,89	0,00	4,03	0,005*
Region: Cultivar	6	43,234,35	0,01	3,62	0,002*
Farm: Cultivar	6	41,822,42	0,01	3,50	0,001*
Season: Leaf type	1	19,533,05	0,00	9,81	0,001*
Region: Leaf type	2	5971,44	0,00	1,50	0,202
Farm: Leaf type	2	1360,15	0,00	0,34	0,81
Cultivar: Leaf type	3	65,182,23	0,01	10,91	0,001*
Season: Region: Cultivar	6	53,222,67	0,01	4,45	0,001*
Season: Farm: Cultivar	6	67,890,67	0,01	5,68	0,001*
Season: Region: leaf type	2	1770,24	0,00	0,44	0,702
Season: Farm: Leaf type	2	14,177,84	0,00	3,56	0,019*
Season: Cultivar: Leaf type	3	6069,87	0,00	1,02	0,392
Region: Cultivar: Leaf type	6	122,558,20	0,02	10,26	0,001*
Farm: Cultivar: Leaf type	6	17,303,89	0,00	1,45	0,185
Season: Region: Cultivar: Leaf type	6	65,121,30	0,01	5,45	0,001*
Season: Farm: Cultivar: Leaf type	6	11,882,81	0,00	0,99	0,442
Residuals	480	955,776,95	0,19		
Total	559	4,976,755,98	1		

* Significant p values.

strongest driver of the anatomical differences, accounting for 45 % of the observed variability, as shown in Table 2. While previous studies have investigated macadamia leaf anatomical traits (Abubaker et al., 2018; Wakefield et al., 2021), their relationship to its water-use strategies remains unexplored. Our findings provide new insights into their potential drought tolerance adaption, marking this study the first to assess leaf and petiole anatomical traits across macadamia cultivars in South Africa.

Stomata play a key role in regulating gaseous exchange for photosynthesis and transpiration, making them good indicators of plant water-use efficiency (Bertolino et al., 2019). In macadamia, stomata are located only on the abaxial leaf surface, covered by a protective layer of trichomes and cuticle (Abubaker et al., 2018). These features are associated with drought-tolerant species (Boughalleb and Hajlaoui, 2011). Our findings revealed that cultivars ‘814′ and ‘695′ had higher stomatal densities than cultivars ‘816′ and ‘A4’. Higher stomatal density typically enhances CO_2 uptake and photosynthetic efficiency while regulating transpiration rates (Bosabalidis and Kofidis, 2002). Higher stomatal densities in drought-tolerant cultivars have also been reported in other evergreen species such as olives (Bosabalidis and Kofidis, 2002; Ennajeh et al., 2010; Guerfel et al., 2009). Based on stomatal density alone, cultivars ‘814′ and ‘695′ may be well suited for drier climates than cultivars ‘816′ and ‘A4’.

Typically, higher stomatal density is associated with smaller stomata, allowing rapid closure and minimizing excessive water loss (Liu et al., 2018; Zhao et al., 2015). However, our results deviated from this pattern, as there was a weak positive correlation between stomatal density and stomatal size ($R^2 = 0.073$ and $p = 0.044$). This deviation could suggest that macadamia trees exhibit unique water-use strategies, as stomatal traits vary across different species (Bertolino et al., 2019). Further investigation is needed to determine the underlying mechanism driving these deviations.

The mesophyll tissues play a crucial role in photosynthesis and water-use regulation, with the palisade parenchyma facilitating carbon fixation while the spongy parenchyma enables the gaseous exchange (Bacelar et al., 2004; Boughalleb and Hajlaoui, 2011). In several plant species, thicker palisade and spongy parenchyma have been associated with enhanced drought tolerance (Bosabalidis and Kofidis, 2002; Ennajeh et al., 2010; Rivera et al., 2017). Our findings revealed that cultivar ‘814′ exhibited thicker palisade parenchyma and spongy parenchyma than cultivars ‘814′, ‘816′, and ‘A4’. This combination of thicker mesophyll tissues in cultivar ‘814′ increases surface area for carbon fixation and improves the internal diffusion of CO_2 due to increased intracellular spaces (Ennajeh et al., 2010). These traits suggest that cultivar ‘814′ may possess adaptions associated with improved drought tolerance.

Vein density is closely related to leaf hydraulic conductance and photosynthetic efficiency (Wang et al., 2024). A higher vein density reduces the distance water travels to reach the mesophyll tissues, improving photosynthesis and transpiration (Scoffoni et al., 2011). Our findings revealed that cultivar ‘695′ exhibited higher vein density than ‘814′, ‘816’, and ‘A4’, a typical adaption for plants growing in drier environments, allowing efficient water transport throughout the leaf (Dunbar Co et al., 2009; Sack and Scoffoni, 2013; Zhang et al., 2022). This suggests that cultivar ‘695′ may likely have improved hydraulic efficiency, making it a potential drought-tolerant cultivar.

The Petiole xylem transports water from the stem into the leaf lamina. The ability of the stem xylem to transport water to a specific leaf area is termed the Huber value, which is the ratio of the xylem sapwood -cross-sectional area relative to the leaf area in tree stems (Tyree and Ewers, 1991). Since the petiole performs the same function as the stem xylem, we applied the Huber value concept to petioles here. Under water-limited conditions, plants tend to increase their Huber values, increasing the xylem area relative to the leaf area to maintain the water supply and demand during transpiration (Sanchez–Martinez et al., 2020; Schultz and Matthews, 1993). In our study cultivar ‘814′ showed the highest petiole Huber value across regions, potentially enhancing its drought tolerance compared to other cultivars. The observed leaf and petiole anatomical differences suggest different adaptation strategies among macadamia cultivars. Cultivars ‘814′ and ‘695′ exhibit higher stomatal density and higher vein density, while cultivar’814’ also shows thicker mesophyll tissues and higher petiole Huber value, ensuring efficient water transport and gas exchange throughout the leaf blade, making them potential drought tolerant cultivars compared to cultivars ‘816′ and ‘A4’.

The variability in leaf and petiole anatomy observed across macadamia cultivars was influenced by environmental conditions and cultivar genetic differences. Traits such as stomatal density exhibited regional plasticity, while palisade parenchyma thickness, spongy parenchyma thickness, vein density, and petiole Huber value appeared to be more cultivar-specific. The seasonal effect (45 %) highlights the role of environmental factors such as temperatures, VPD, and total reference evapotranspiration in shaping leaf and petiole anatomy (Table 2). The highest temperatures and VPD were recorded in Komatipoort and Nelspruit, where there was a significant increase in stomatal density, especially on cultivars ‘814′ and ‘695′ (Table 1; Fig. 2A). This suggests that stomatal density in macadamia may be plastic, allowing macadamia cultivars to adjust the number of stomata on their leaves in response to environmental conditions (Bertolino et al., 2019; Zhao et al., 2015). Such adjustments may influence gaseous exchange and enhance transpiration cooling (Carlson et al., 2016). Cultivars grown in KZN exhibited lower stomatal densities, indicating reduced transpiration demand in humid conditions (Table 1; Fig. 2A). Furthermore, the petiole Huber value was higher in the drier region (Komatipoort), particularly in cultivar ‘814′ suggesting increased water transport under high evaporative demand. The lower plasticity of mesophyll tissues and vein density may suggest that these traits may be influenced by genetic factors, rather than short-term environmental variations.

This study revealed that cultivars '814' and '695' demonstrate traits linked to improved water use efficiency and drought tolerance. Seasonal variation emerged as the primary driver of leaf and petiole anatomical differences, highlighting the influence of environmental factors, particularly temperatures, and VPD in shaping macadamia leaf anatomy. These findings offer valuable insights into the selection of drought-tolerant cultivars and improving water management strategies, particularly in regions facing water scarcity due to climate change. However, while these anatomical traits may serve as indicators of drought tolerance, the absence of physiological measurements limits our ability to make conclusions about their drought tolerance. Future studies should integrate physiological measurements, such as stomatal conductance, photosynthetic rates, and transpiration rates under controlled conditions, to assess the performance of these cultivars and draw conclusions on macadamia water-use strategies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Khodani Maumela: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nicolette J Taylor:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition. **Kenneth C Oberlander:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Angelique Kritzinger:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Acknowledgments

This research was funded by the Water Research Commission (WRC) (Project C2021/2022-00787) with co-funding from Macadamias South Africa (SAMAC). The authors would like to thank the farmers who generously allowed us access to their farms for leaf sampling and the Agricultural Research Council (ARC) for providing us with the meteorological data.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2025.03.032.

References

Abubaker, M., El Hawary, S.S., Mahrous, E.A., El-Kader, E., 2018. Botanical study and DNA fingerprinting of Macadamia integrifolia maiden and Betche cultivated in Egypt. J. Pharm. Res. 12 (3), 298–304.

Ahmad, S., Ali, H., Ur Rehman, A., Khan, R.J.Z., Ahmad, W., Fatima, Z., Abbas, G., Irfan, M., Ali, H., Khan, M.A., 2015. Measuring leaf area of winter cereals by different techniques: a comparison. Pak. J. Life Soc. Sci. 13 (2), 117–125.

Ahmed, M.F., Popovich, D.G., Whitby, C.P., Rashidinejad, A., 2024. Phenolic compounds from macadamia husk: an updated focused review of extraction methodologies and antioxidant activities. Food Bioprod. Process.. https://doi.org/10.1016/j.fbp.2024.09.014.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration-Guidelines for Computing Crop Water Requirements-FAO Irrigation and Drainage Paper 56, 300. FAO, Rome, p. D05109. http://www.fao.org/docrep/X0490E/X0490E00.htm.

Bacelar, E.A., Correia, C.M., Moutinho-Pereira, J.M., Gonçalves, B.C., Lopes, J.I., Torres-Pereira, J.M., 2004. Sclerophylly and leaf anatomical traits of five field-grown olive cultivars growing under drought conditions. Tree Physiol. 24 (2), 233–239. https://doi.org/10.1093/treephys/24.2.233.

Bertolino, L.T., Caine, R.S., Gray, J.E., 2019. Impact of stomatal density and morphology on water-use efficiency in a changing world. Front. Plant Sci. 10, 225. https://doi.org/10.3389/fpls.2019.00225.

Blackman, C.J., Halliwell, B., Hartill, G.E., Brodribb, T.J., 2024. Petiole XLA (xylem to leaf area ratio) integrates hydraulic safety and efficiency across a diverse group of eucalypt leaves. Plant Cell Environ. 47 (1), 49–58. https://doi.org/10.1111/pce.14713.

Bosabalidis, A.M., Kofidis, G., 2002. Comparative effects of drought stress on leaf anatomy of two olive cultivars. Plant Sci. 163 (2), 375–379. https://doi.org/10.1016/S0168-9452(02)00135-8.

Boughalleb, F., Hajlaoui, H., 2011. Physiological and anatomical changes induced by drought in two olive cultivars (cv Zalmati and Chemlali). Acta Physiol. Plant. 33, 53–65. https://doi.org/10.3390/plants13192812.

Carlson, J.E., Adams, C.A., Holsinger, K.E., 2016. Intraspecific variation in stomatal traits, leaf traits, and physiology reflects adaptation along aridity gradients in a South African shrub. Ann. Bot. 117 (1), 195–207. https://doi.org/10.1093/aob/mcv146.

Carr, M., 2013. The water relations and irrigation requirements of macadamia (Macadamia spp.): a review. Exp. Agric. 49 (1), 74–90. https://doi.org/10.1017/S0014479712000804.

Dörken, V.M., Lepetit, B., 2018. Morpho-anatomical and physiological differences between sun and shade leaves in Abies alba Mill. (Pinaceae, Coniferales): a combined approach. Plant Cell Environ. 41 (7), 1683–1697. https://doi.org/10.1111/pce.13213.

Dunbar Co, S., Sporck, M.J., Sack, L., 2009. Leaf trait diversification and design in seven rare taxa of the Hawaiian Plantago radiation. Int. J. Plant Sci. 170 (1), 61–75. https://doi.org/10.1086/593111.

Ennajeh Vadel, A., Cochard, H., Khemira, H., 2010. Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. J. Hortic. Sci. Biotechnol. 85 (4), 289–294. https://doi.org/10.1080/14620316.2010.11512670.

Gago, P., Conejero, G., Martínez, M.C., This, P., Verdeil, J.L., 2019. Comparative anatomy and morphology of the leaves of grenache Noir and Syrah grapevine cultivars. S. Afr. J. Enol. Vitic. 40 (2), 1–9. https://doi.org/10.21548/40-2-3031.

Guerfel, M., Baccouri, O., Boujnah, D., Chaïbi, W., Zarrouk, M., 2009. Impacts of water stress on gas exchange, water relations, chlorophyll content, and leaf structure in the two main Tunisian olive (Olea europaea L.) cultivars. Sci. Hortic. 119 (3), 257–263. https://doi.org/10.1016/j.scienta.2008.08.006.

Hardner, C.M., Peace, C., Lowe, A.J., Neal, J., Pisanu, P., Powell, M., Schmidt, A., Spain, C., Williams, K., 2009. 1 Genetic resources and domestication of Macadamia. Hortic. Rev. 35 (4), 1–125. https://doi.org/10.1002/9780470593776.ch1.

Liu, C., He, N., Zhang, J., Li, Y., Wang, Q., Sack, L., Yu, G., 2018. Variation of stomatal traits from cold temperate to tropical forests and association with water use efficiency. Funct. Ecol. 32 (1), 20–28. https://doi.org/10.1111/1365-2435.12973.

Luković, J., Maksimović, I., Zorić, L., Nagl, N., Perčić, M., Polić, D., Putnik-Delić, M., 2009. Histological characteristics of sugar beet leaves potentially linked to drought tolerance. Ind. Crops Prod. 30 (2), 281–286. https://doi.org/10.1016/j.indcrop.2009.05.004.

Mast, A.R., Willis, C.L., Jones, E.H., Downs, K.M., Weston, P.H., 2008. A smaller Macadamia from a more vagile tribe: inference of phylogenetic relationships, divergence times, and diaspore evolution in Macadamia and relatives (tribe Macadamieae; Proteaceae). Am. J. Bot. 95 (7), 843–870. https://doi.org/10.3732/ajb.0700006.

Mencuccini, M., Rosas, T., Rowland, L., Choat, B., Cornelissen, H., Jansen, S., Kramer, K., Lapenis, A., Manzoni, S., Niinemets, Ü., 2019. Leaf economics and plant hydraulics drive leaf: wood area ratios. New Phytol. 224 (4), 1544–1556. https://doi.org/10.1111/nph.15998.

Navarro, S.L., Rodrigues, C.E., 2016. Macadamia oil extraction methods and uses for the defatted meal byproduct. Trends Food Sci. Technol. 54, 148–154. https://doi.org/10.1016/j.tifs.2016.04.001.

Pagano, M., Corona, P., Storchi, P., 2016. Image analysis of the leaf vascular network: physiological considerations. Photosynthetica 54 (4), 567–571. https://doi.org/10.1007/s11099-016-0238-2.

R Core Team, 2024. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rasband, W., 2012. ImageJ - Image Processing and Analysis in Java. 1. Astrophysics Source Code Library, 06013.

Rivera, P., Villaseñor, J.L., Terrazas, T., 2017. Meso-or xeromorphic? Foliar characters of Asteraceae in a xeric scrub of Mexico. Bot. Stud. 58, 1–16. https://doi.org/10.1186/s40529-017-0166-x.

Roddy, A.B., Brodersen, C.R., Dawson, T.E., 2016. Hydraulic conductance and the maintenance of water balance in flowers. Plant Cell Environ. (39), 2123–2132. https://doi.org/10.1111/pce.12761.

Roddy, A.B., Guilliams, C.M., Lilittham, T., Farmer, J., Wormser, V., Pham, T., Fine, P.V.A., Feild, T.S., Dawson, T.E., 2013. Uncorrelated evolution of leaf and petal venation patterns across the angiosperm phylogeny. J. Exp. Bot. 64 (13), 4081–4088. https://doi.org/10.1093/jxb/ert247.

Sack, L., Caringella, M., Scoffoni, C., Mason, C., Rawls, M., Markesteijn, L., Poorter, L., 2014. Leaf vein length per unit area is not intrinsically dependent on image magnification: avoiding measurement artifacts for accuracy and precision. Plant Physiol. 166 (2), 829–838. https://doi.org/10.1104/pp.114.237503.

Sack, L., Scoffoni, C., 2013. Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. New Phytologist 198, 983–1000. https://doi.org/10.1111/nph.12253.

Sack, L., Scoffoni, C., McKown, A.D., Frole, K., Rawls, M., Havran, J.C., Tran, H., Tran, T., 2012. Developmentally based scaling of leaf venation architecture explains global ecological patterns. Nat. Commun. 3 (1), 1–10. https://doi.org/10.1038/ncomms1835.

Sanchez-Martinez, P., Martínez-Vilalta, J., Dexter, K.G., Segovia, R.A., Mencuccini, M., 2020. Adaptation and coordinated evolution of plant hydraulic traits. Ecol. Lett. 23 (11), 1599–1610. https://doi.org/10.1111/ele.13584.

Schultz, H.R., Matthews, M.A., 1993. Xylem development and hydraulic conductance in sun and shade shoots of grapevine (Vitis vinifera L.): evidence that low light uncouples water transport capacity from leaf area. Planta 190 (3), 393–406. https://doi.org/10.1007/BF00196969.

Scoffoni, C., Rawls, M., McKown, A., Cochard, H., Sack, L., 2011. Decline of leaf hydraulic conductance with dehydration: relationship to leaf size and venation architecture. Plant Physiol. 156 (2), 832–843. https://doi.org/10.1104/pp.111.173856.

Shabalala, M., Toucher, M., Clulow, A., 2022. The Macadamia bloom–What are the hydrological implications? Sci. Hortic. 292, 110628. https://doi.org/10.1016/j.scienta.2021.110628.

Shankar, A., Singh, A., Chaudhary, S., Prasad, V., 2021. Desiccation-tolerant rhizobacteria: a possible approach for managing agricultural water stress. Agricultural Water Management. Elsevier, pp. 81–100. https://doi.org/10.1016/B978-0-12-812362-1.00006-0.

Smit, T.G., Taylor, N.J., Midgley, S.J., 2020. The seasonal regulation of gas exchange and water relations of field grown macadamia. Sci. Hortic. 267, 109346. https://doi.org/10.1016/j.scienta.2020.109346.

Taylor, N., Smit, T., Smit, A., Midgley, S., Clulow, A., Annandale, J., Dlamini, K., & Roets, N. (2021). Water Use of Macadamia Orchards. WRC Report No. 2552/2/21. Water Research Commission, Pretoria, South Africa. Available from: https://www.wrc.org.za/wp-content/uploads/mdocs/2552%20Vol%202.pdf. [Accessed September 13, 2023].

Tyree, M.T., Ewers, F.W., 1991. The hydraulic architecture of trees and other woody plants. New Phytol. 119 (3), 345–360. https://doi.org/10.1111/j.1469-8137.1991.tb00035.x.

Wakefield, S., Topp, B., Alam, M., 2021. Aspect and rootstock genotype influence leaf stomatal density in Macadamia sp. †. Biol. Life Sci. Forum 2021, 1–15. https://doi.org/10.3390/xxxxx.

Wang, X., Chen, S., Yang, X., Zhu, R., Liu, M., Wang, R., He, N., 2024. Adaptation mechanisms of leaf vein traits to drought in grassland plants. Sci. Total Environ. 917, 170224. https://doi.org/10.1016/j.scitotenv.2024.170224.

WOM. (2024). Industry data. Available from: https://www.worldmacadamia.com/industry-data/. [Accessed December 2, 2024].

Yeung, E.C., 1998. Chapter 9 A beginner ' s guide to the study of plant structure. Structure 125–143 March.

Zhang, M., Gao, H., Chen, S., Wang, X., Mo, W., Yang, X., Wang, X., Wang, Z., Wang, R., 2022. Linkages between stomatal density and minor leaf vein density across different altitudes and growth forms. Front. Plant Sci. 13, 1064344. https://doi.org/10.3389/fpls.2022.1064344.

Zhao, W., Sun, Y., Kjelgren, R., Liu, X., 2015. Response of stomatal density and bound gas exchange in leaves of maize to soil water deficit. Acta Physiol. Plant. 37 (1), 1–9. https://doi.org/10.1007/s11738-014-1704-8.