Vol. 11(3): 345-359, September 2023 DOI: https://doi.org/10.23960/jsl.v11i3.757

Jurnal Sylva Lestari

P-ISSN: 2339-0913
E-ISSN: 2549-5747

Journal homepage: https://sylvalestari.fp.unila.ac.id

Full Length Research Article

Growth and Morpho-Stomatal Response of Kenaf (*Hibiscus cannabinus*) to Varying Water, Light, and Soil Conditions

Jonathan Ogayon Hernandez[1,*], Leah Grace Abalos Manese[1], Hazelyn Lacasa Lalog[1], Vrenissa Jane Valenzuela Herradura[1], Willie Payawan Abasolo[2], Lerma San Jose Maldia[1]

[1] Department of Forest Biological Sciences, College of Forestry and Natural Resources, University of the Philippines Los Baños, Los Baños, Philippines
[2] Department of Forest Products and Paper Science, College of Forestry and Natural Resources, University of the Philippines Los Baños, Los Baños, Philippines
[*] Corresponding Author. E-mail address: johernandez2@up.edu.ph

ARTICLE HISTORY:

Received: 5 July 2023
Peer review completed: 18 July 2023
Received in revised form: 20 July 2023
Accepted: 21 July 2023

KEYWORDS:

Biomass allocation
Potential conductance index
Stomatal index
Shade tolerance
Water stress

ABSTRACT

This study investigated the stomatal responses of kenaf (*Hibiscus cannabinus)* to varying water, light, and soil conditions to explain how the species acclimatize to the changes in growing conditions. Seedlings were subjected to different watering regimes (daily – CON, 2 days interval – W2, 3 days interval – W3), light intensities (high, moderate, low), and soil conditions (garden soil – GS, Mt Makiling soil – MAK, UP Land Grant soil – UPL). The biomass, stomatal density (SD), epidermal cell density (ECD), stomatal index (SI), stomatal aperture length (SAL), guard cell length (GCL), stomatal area (SA), and potential conductance index (PCI) were measured across treatments. Water and light treatments had significant effects, but soil treatment did not affect most of the parameters measured. CON and/or W2 and high light intensity resulted in a higher SD, SI, SAL, and GCL, which resulted in a higher PCI, compared with the other water and light treatments. Contrarily, W3-treated seedlings had lower SD but significantly lower SAL, GCL, PCI, and aboveground biomass, compared to CON and W2-treated ones. Biomass allocation to root was also significantly higher in W3-treated seedlings. Therefore, kenaf seedlings exhibited a degree of stomatal plasticity in response to contrasting water, light, and soil conditions.

1. Introduction

Global warming alters soil water availability through increased evapotranspiration, which dries out soils and vegetation in many regions (Lippmann et al. 2019; Vicente-Serrano et al. 2022). The light intensity reaching the ground can also be controlled indirectly by global warming *via* effects on vegetation stratification, resulting in the lower photosynthetic capacity of plants. Water, light, and soil conditions are among the most important environmental factors directly affected by global warming, which control plant survival and growth (Nurdin et al. 2022; Pressel et al. 2018). Short-term and long-term effects of changing water, light, and soil conditions are mostly associated with stomatal development and opening (Driesen et al. 2020; Hernandez et al. 2022). Stomata play a key role in plant responses to changes in environmental conditions by controlling gas exchange, water loss through transpiration, and leaf temperature. For example, stomatal

closure has been described as the major limiting factor for CO_2 assimilation in response to water shortage by diminishing the supply of CO_2 in the mesophyll cell chloroplasts (Engineer et al. 2016). Limiting water availability can lead to a reduction in leaf net photosynthesis assimilation, thus inhibiting plant growth due to stomatal limitation (Hernandez et al., 2021; Salmon et al., 2020). However, altering stomatal apertures can assist plants in enhancing photosynthesis and decreasing water loss because stomatal traits (e.g., size and density) can adjust to suit the prevailing environmental conditions (Bertolino et al. 2019). In terms of the effects of light intensity on the stomatal traits, several studies also revealed that different light intensities have significant effects on stomatal density and size, thereby influencing leaf gas exchange (Li et al. 2014; Wild and Wolf 1980). A study also reported that shade significantly affected the size and density of stomata and stomatal conductance (Kardiman and Raebild 2018). Further, soil characteristics (e.g., pH and texture) also affect plant growth through stomatal behavior regulation (Anav et al. 2018; Gentili et al. 2018). Although these environmental effects are already known in some plants, still not enough is known about the ways in which economically important annual species can acclimate to varying water, light, and soil conditions through stomatal traits regulation.

Kenaf (*Hibiscus cannabinus* L.) is one of the economically important annual species in the cotton family (i.e., Malvaceae), offering an environment-friendly alternative source of pulp to forest tree species. As the population grows, so does the demand for wood, but forests are producing less of it (Herwanti 2015); hence, it is important to consider alternatives to forest tree species as a source of pulp. Because kenaf has a shorter growth cycle compared to forest tree species, the use of annual species like kenaf can reduce pressure on natural forests, allowing a more sustainable approach to forest resource management. By lowering the demand for timber and other products (e.g., wood panels) made from forest tree wood (Hariz et al. 2023), kenaf cultivation can aid in the conservation of natural forests by deterring illegal loggers from carrying out destructives activities within the forest. It has long been recognized that the species has big potential applications in textile and building materials, pulp and paper industry, bioenergy, and animal feed (Ayadi et al. 2011). Because of its fast growth rate and economic importance, kenaf is grown commercially in various geographical regions, including China, Indonesia, USA, and Thailand, for eco-friendly fiber production (An et al. 2018). The increased demand for pulp and paper, as well as the recognition of annual and fast-growing species as good alternative fiber sources (Ayadi et al. 2011), necessitates additional research on kenaf, particularly in areas with limited forest resources. While kenaf's growth and physiological responses to environmental stresses are well-documented (An et al. 2020; Niu et al. 2016), very few experiments have been conducted about the stomatal responses of the species to different water, light, and soil conditions. Understanding the stomatal responses of kenaf to varying resource availability may provide us with information on the breeding selection methods for improving the growth and phenotypic plasticity of the plant amid climate change. Nowadays, most kenaf breeding programs worldwide are focused on developing high-yielding and drought-tolerant varieties suitable for fiber production (Kim et al. 2021).

Thus, this study investigated the growth and morpho-stomatal responses of the industrial plant kenaf to varying water, light, and soil conditions. Two hypotheses were tested; (1) the stomatal traits of kenaf seedlings are highly plastic to varying water, light, and soil conditions, and (2) there is either a positive or negative correlation between stomatal and epidermal variables that would indicate coordination of traits or trade-offs under various conditions. The findings of the present study are relevant for breeding high-yielding and resistant kenaf based on stomatal traits.

2. Materials and Methods

2.1. Study Site and Plant Material

The experiments were carried out in the nursery of the Department of Forest Biological Sciences (DFBS), College of Forestry and Natural Resources (CFNR), University of the Philippines Los Baños (UPLB), Philippines (14°09'16.54" N, 121°14'10.98" E). The mean annual air temperature and precipitation in the study site were 27°C and 915 mm, respectively (Hernandez et al. 2022). The warmest temperature of the year is in May (i.e., 28. 1°C), while the lowest average temperature is in January (24.8°C).

The seed capsules (c.a., 1.9–2.5 cm in length and 1.4–2.0 cm in diameter) of kenaf (**Fig. 1**) were provided by the Philippine Fiber Industry Development Authority (PhilFIDA) of the Department of Agriculture in the Philippines. In January 2019, seeds were first sown in seedbeds filled with 1:1:1 (garden soil: sand: coir dust) growing media and subjected to three germination treatments, control, 24-hour soaking, and 48-hour soaking. The highest germination rate was observed in the 24-hour soaking treatment, from which seedlings were selected and potted for the experiment. The pots used were made of black polythene material and were approximately 1.5 L in volume. The soil texture of the growing medium used was clay loam, with a soil pH of 6.70–6.88.

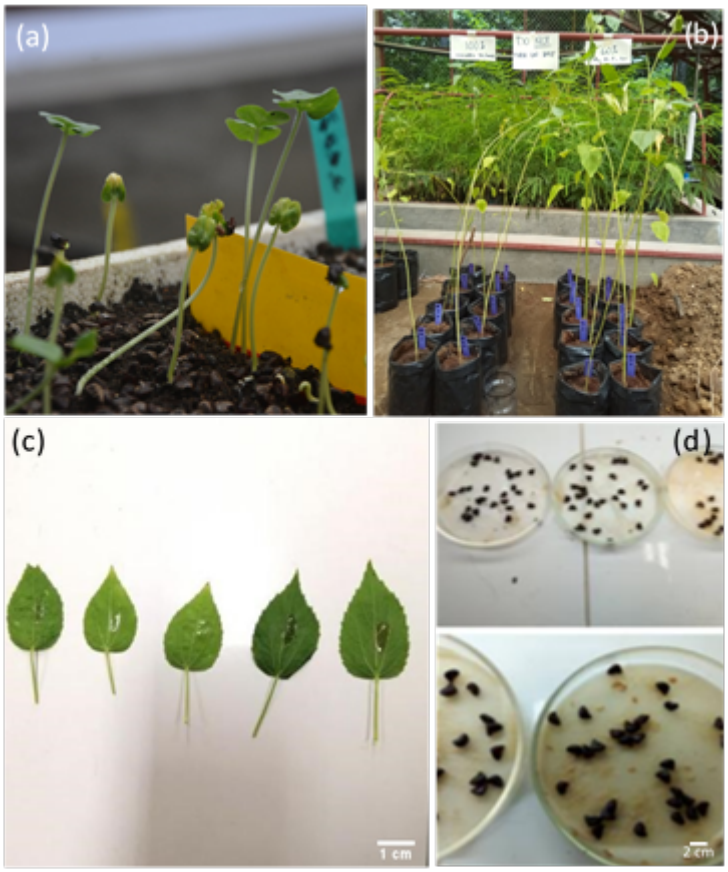

Fig. 1. (a) Two to three-day old seedlings, (b) mature seedlings, (c) leaves, and (d) seed capsules of kenaf used in the present study.

2.2. Experimental Design

After 3–4 months of acclimatization, potted seedlings were arranged in a completely randomized design in the nursery. The study consisted of three experiments (water, light, and soil),

with three treatments each. These experiments were conducted separately in the same nursery. Treatments of each experiment were replicated twice to have a total of six experimental units, and each unit was composed of fifteen pots/seedlings. The pots were arranged following a 10 cm distance between seedlings and 15 cm between rows. Pots were elevated from the ground to protect the experimental seedlings from external influences.

Three treatments were used for the watering experiment; CON (soil water is maintained at field capacity by daily watering), W2 (watering to a field capacity at 2-day intervals), and W3 (watering to a field capacity at 3-day intervals). Initially, soil moisture levels of each treatment were determined by weighing the watered pots, resulting in 75–80%, 55–60%, and 45–50% for CON, W2, and W3 treatments, respectively. The selection of these watering regimes was based on the stomata's known short-term and immediate response to low-moderate water status in most plants (Buckley 2019; Pressel et al. 2018). Because kenaf is a short-rotation woody crop, treatments were imposed when seedlings had already reached a root collar diameter (RCD) and height growth of approximately 0.5 mm and 42 cm, respectively. Roots were also fully grown at this growth stage, indicating readiness for the treatment application.

The light experiment was composed of three treatments; high (100% or 400–1600 lx), moderate (50% or 200–1200 lx), and low (25% or 100 - 1000 lx) light intensities. The light intensity outside the nursery was approximately 600 - 1800 lx without shading. Each treatment's row was covered on all sides, except the top portion, with black shading plastic (c.a., 1.5 - 2.5 m^2). The open space (hole) area at the top portion varied depending on the light intensity, i.e., small, medium, and large for low, moderate, and high light intensities, respectively. The hole was also used as a window for data collection and watering. The light measurement in lux (converted to percentage) was taken daily, 1–2 hours in the afternoon (14:00–16:00) in each treatment using a hand-held light meter (TENMARS TM-202 Lux/fc, Taipei, Taiwan). An average value of 3–5 readings was recorded and taken only on sunny days. The light sensor was facing directly upwards to accurately detect the amount of light reaching the sensor from the sky hemisphere. The time between light measurements did not exceed two minutes.

There were also three treatments used for the soil experiment, namely; GS (garden soil), MAK (Mt Makiling soil in Los Baños, Laguna), and UPLQLG (UP Laguna Quezon Land Grant in Real, Quezon). Soil samples were collected from the topsoil layer at a depth of 0–15 cm from each site using soil shovels. Prior to analysis, any debris or stones present in each sample were carefully removed. Subsequently, the soil samples were air-dried for 2–3 days and then sieved to achieve a uniform particle size. The GS treatment is a mixture of soil, coir dust, and sand, following a 1:1:1 ratio. The pH and texture of each soil treatment were analyzed using potentiometric and hydrometer methods. The soil pH of all treatments has values ranging from 5.26 to 5.70. The GS and UPL had sandy clay loam soil texture, whereas MAK had clay loam texture.

2.3. Stomatal and Epidermal Traits Measurement

For each experiment and treatment, the samples were taken in July 2019 from the 3rd - 4th youngest fully expanded leaves to minimize the effects of leaf age and nodal position on the data. Randomly, three clean leaves were sampled from five individuals per treatment and made 2 - 3 leaf imprints per leaf. The imprints were made through the leaf epidermal impression technique, also known as transparent nail polish, following the modified procedure enumerated in Millstead et al. (2020). After applying nail polish to the abaxial surface of the leaves, the dried layer was

mounted on a microscope slide with transparent tape. Thin imprints (c.a., 4 mm × 10 mm) were peeled off from the mid-area of the leaves (between 3rd and 4th secondary veins from the leaf base) to minimize possible variations in stomatal density and size. Two to three digital images were taken from each imprint using a compound light microscope at 40X magnification. The measurements were done using the ImageJ processing software (version 1.51k, MD, USA), following the procedure in some studies (Schneider et al. 2012).

The traits measured include stomatal density (SD), epidermal cell density (ECD), stomatal index (SI), stomatal aperture length (SAL), guard cell length (GCL), and stomatal area (SA). The SD expressed as the number of stomata per unit leaf area was calculated within 450 × 450 µm FOV (field of view) per image by dividing the count of stomata per FOV by the area of FOV. The number of stomata per square FOV was converted to the number per square millimeter. ECD was determined as the total area of the epidermal cells within the square FOV. The stomatal index (SI) was calculated as the ratio between stomatal density (SD) and epidermal cell density (ECD) (Zheng et al. 2013).

$$SI = \frac{SD}{SD + ECD} \quad (1)$$

The lengths of the aperture, guard cells, and area of stomata were measured from 10 stomata per image, and the values were then averaged per individual and treatment. In this study, the stomatal area was defined as the pore surrounded by two guard cells.

Lastly, the potential conductance index, which can be used as a proxy for the theoretical maximal stomatal conductance of CO_2 and water vapor, was calculated using Equation 2 (Petrik et al. 2020).

$$PCI = GCL \times SD \times 10^{-4} \quad (2)$$

where PCI is potential conductance index, SD is stomatal density, and GCL is guard cell length.

2.4. Biomass Measurement

Biomass growth and allocation were measured at the end of the experiment. Seedlings were divided into different plant parts (root, stem, and leaf) and then oven-dried at 65°C to a constant weight. Roots were washed carefully using tap water to avoid damage to fine roots while removing the soil.

2.5. Data and Statistical Analyses

Measurements of the digital images of leaf imprints were first averaged by treatment before statistical analysis, performed using R statistical software (version 3.5.1, R Core Team, Vienna, Austria) at a significance level of $\alpha = 0.05$. The normal distribution of the data was initially assessed using the "Shapiro.test". A one-way analysis of variance (ANOVA) was used to test for significant differences in stomatal and epidermal traits between treatments. When treatment effects were significant, means were compared using Tukey's HSD post hoc test. The most important traits and the relationships between traits were determined using principal component analysis (PCA) using the *prcomp* function. Only principal components (PCs) with eigenvalues of greater than 1 were used in the study. The factorextra and devtools R packages were used for PCA and correlation matrix visualization. The graphs were made in SigmaPlot version 10.0.

3. Results and Discussion

3.1. Results

3.1.1. Effects of varying water, light, and soil conditions on stomatal and epidermal traits

Watering and shading treatments had significant effects ($p < 0.05$) on the stomatal density (SD), epidermal cell density (ECD), and stomatal index (SI) of kenaf seedlings. However, soil treatment did not affect the three parameters measured (**Fig. 2**, **Fig. 6**, **Table 1**). The SD was significantly higher in CON (daily watering) and/or W2 (watering at 2-day intervals) and high light conditions than in W3 (watering at 3-day intervals) and low to moderate light treatments. A similar pattern was detected in the SI. A reverse pattern was detected in the ECD, i.e., W3 watering and low light conditions resulted in the highest epidermal cell density.

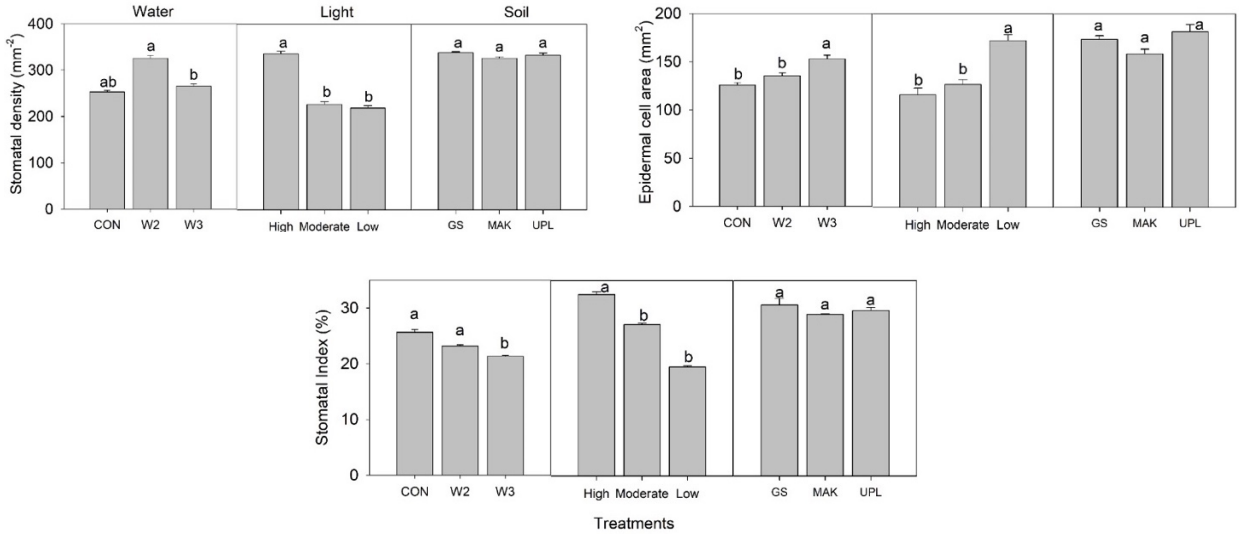

Fig. 2. Changes in the (a) stomatal density, (b) epidermal cell area, and (c) stomatal index of kenaf seedlings across different water, light, and soil conditions. Different lower-case letters indicate significant differences between treatments (n = 10).

Table 1. The *p*-values of one-way ANOVA for biomass growth and stomatal traits of kenaf planted in different water, light, and soil conditions

Parameters	Water	Treatments Light	Soil
Aboveground biomass	**0.034**		
Belowground biomass	**<0.001**		
Stomatal density	**0.058**	**0.042**	0.253
Stomatal index	**0.019**	**0.026**	0.066
Stomatal area	0.071	**0.059**	0.078
Stomatal aperture length	**<0.001**	**0.032**	0.359
Guard cell length	**0.011**	**0.028**	**0.052**
Potential conductance index	**<0.001**	**0.002**	
Epidermal cell density	**0.013**	**0.051**	0.765

Notes: Significant p-values are in bold.

Generally, the stomatal aperture length (SAL), guard cell length (GCL), and stomatal area (SA) varied significantly across the water and light treatments (**Fig. 3**, **Fig. 6**, **Table 1**). The highest SAL was observed in W2 (10.15 µm), intermediate in CON (8.95 µm), and the lowest in W3 (4.25 µm). The moderate light condition had the highest SAL, followed by high and low light treatments. The GCL showed a decreasing pattern in both watering (i.e., CON > W2 > W3) and shading (i.e.,

high > moderate > low) treatments. While watering had no significant effect on SA, the low light condition resulted in the highest SA among the treatments. Here, the effects of soil treatments on the stomatal aperture length (SAL) and stomatal area (SA) of the seedling were also not significant (**Fig. 3**, **Table 1**). However, GCL was significantly higher in both garden soil (GS) and UP Laguna Quezon Land Grant (UPLQLG) soil types than that in Mt Makiling soil (MAK).

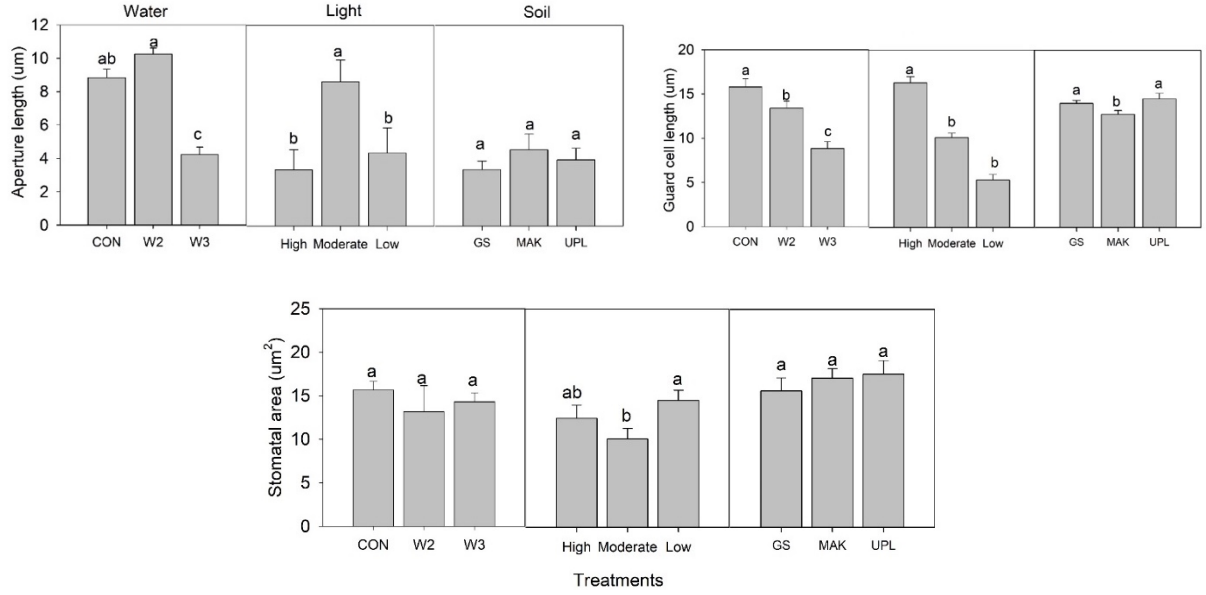

Fig. 3. Changes in the (a) stomatal aperture length, (b) guard cell length, and (c) stomatal area of kenaf seedlings across different water, light, and soil conditions. Different lower-case letters indicate significant differences between treatments (n = 10).

3.1.2. Potential conductance index of kenaf seedlings in different water and light conditions

In this study, seedlings planted at W3 and low light treatments had a significantly lower PCI than other water and light treatments (**Fig. 4**, **Table 1**). Seedlings watered daily and with abundant light availability had the highest PCI among the treatments.

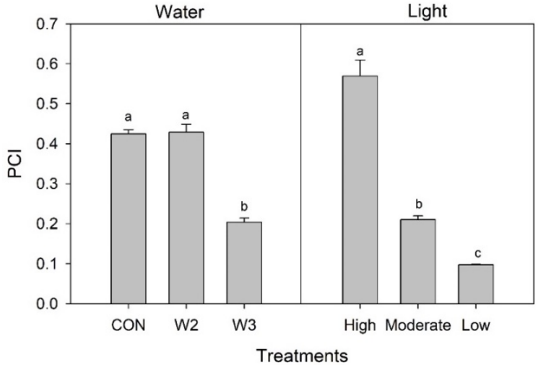

Fig 4. Potential conductance index (PCI) of kenaf seedlings in different water and light conditions. Different lower-case letters indicate significant differences between treatments (n = 10).

3.1.3. Biomass of kenaf in different watering regimes

The effects of watering treatments had a significant effect ($p < 0.05$) on the biomass growth of kenaf (**Fig. 5**, **Table 1**). The variation in biomass in all the plant components was significantly higher in CON and W2 than in W3 treatments. Overall, the aboveground biomass decreased

significantly in W3-treated seedlings. However, these seedlings yielded significantly higher root biomass than seedlings in the other treatments.

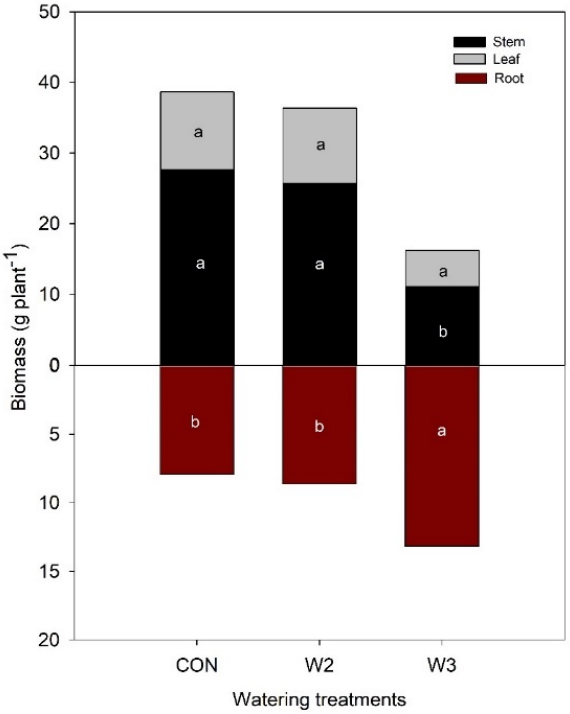

Fig. 5. Biomass of kenaf in daily (CON), 2-day interval (W2), and 3-day interval (W3) treatments. Different lower-case letters indicate significant differences in biomass by plant component (stem, leaf, and root) between treatments (n = 10).

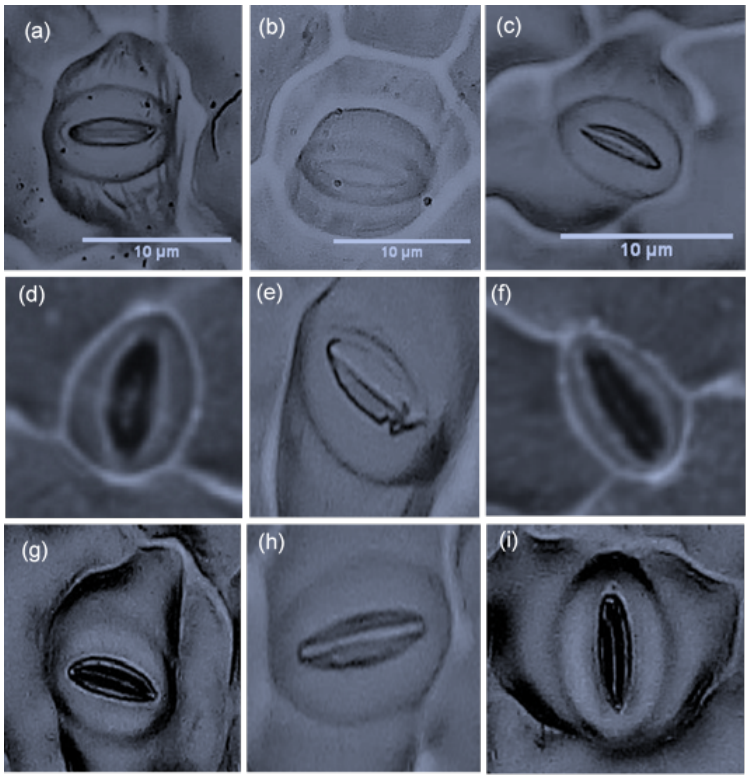

Fig. 6. Representative stomata of kenaf seedlings grown in (a) daily watering (b) every 2 days interval watering, W2 (c) every 3 days interval watering, W3 (d) high light (e) moderate light (f) low light intensity (g) garden soil, GS (h) Mt Makiling soil, MAK, and (i) UP LQLG soil, UPL.

3.1.4. Multivariate analysis

All measured stomatal and epidermal parameters were used in the principal components analysis (**Fig. 7**, **Table 2**). Through the biplot, the magnitude of the influence of water, light, and soil treatments on the stomatal characteristics of kenaf could be observed. The PCA revealed that the first two components accounted for 66.3% of the variation in the dataset. Specifically, PC1 accounted for 41.7% of the variation and was highly and positively related to SD (0.88) and SA (0.84). PC 2 accounted for 34.6 % of the variation and was highly and positively correlated with GCL (0.77) and negatively correlated with ECD (-69). In the upper right quadrat shows that GCL, SA, and SD had been strongly influenced by high light intensity. Garden soil (GS) showed a marginal effect on GCL. GCL was also influenced by watering treatments (i.e., CON and W2), whereas effects on SAL were mainly driven by W3 treatment and moderate light intensity. The ECD was driven mainly by low-intensity treatment. The effects of MAK and UPL soil treatments on the stomatal characteristics were generally weak.

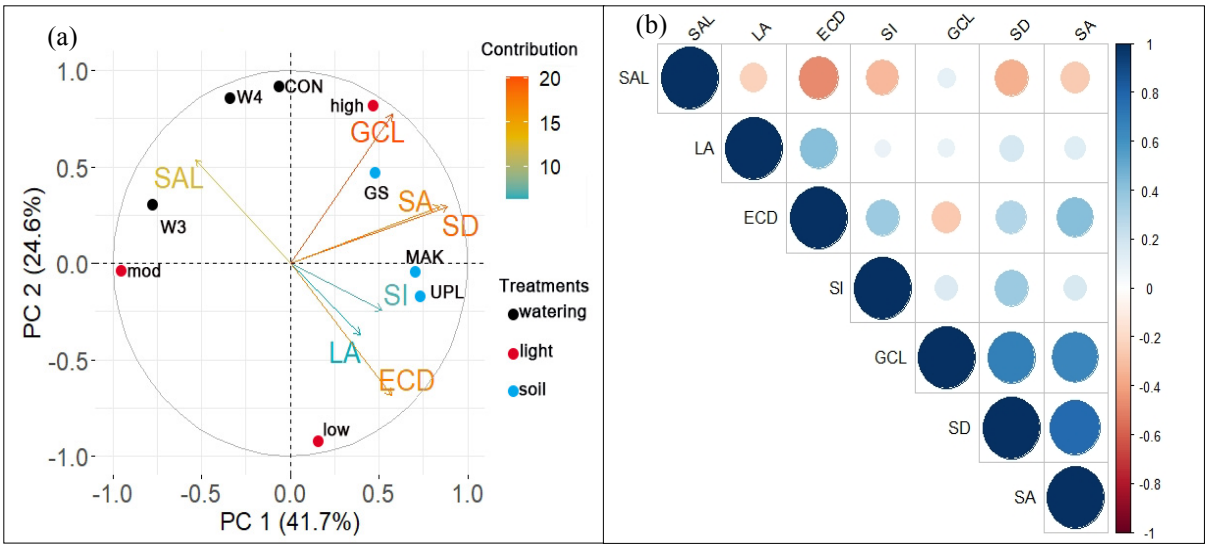

Fig. 7. (a) Loading plot of the stomatal characteristics of kenaf across different water, light, and soil conditions and (b) correlation matrix between variables. Abbreviations: CON- control or daily, W2- two-day interval, and W3- three-day interval watering; mod- moderate light intensity; GS- garden soil, MAK- Mt Makiling soil, UPL- UPLQLG soil; GCL- guard cell length, SA- stomatal area, SD- stomatal density, SI- stomatal index, LA- leaf area, ECD- epidermal cell density, SAL- stomatal aperture length. The length of the vector of variables corresponds to their contribution to the dimensions.

Table 2. Eigenvalues of each principal component (PC). Only PCs with greater than 1 Eigenvalues were considered important components in the present study

Components	Eigenvalue	Variance percent	Cumulative variance percent
PC1	2.87	41.04	41.04
PC2	1.72	24.64	65.69
PC3	0.94	13.45	79.14
PC4	0.71	10.19	89.33
PC5	0.55	7.98	97.32
PC6	0.17	2.53	99.85
PC7	0.00	0.14	100.00

3.2. Discussion

Results of the present study generally support our hypothesis that kenaf seedlings would adjust their stomatal traits in response to varying water, light, and soil conditions. The CON and/or W2 watering and high light intensity resulted in a higher SD compared with W3 watering and low to moderate light treatments. Our result agrees with the findings of Idris et al. (2019), who reported that high light intensity influences the stomatal density of the studied plants. It was also reported that stomatal density increased with increasing water stress (Xu et al. 2008). A significant positive relationship between aridity ecodistance and stomatal density, which reflects an adaptive trait, was also found in tree species (Petrik et al. 2022). Our results can be attributed to the strong positive correlation between SD and SA based on the PCA biplot and correlation matrix. The observed higher SI of seedlings under CON and/or W2 also supports the positive correlation between SD and SA. Our previous study also reported that the density of stomata per unit leaf area is restricted by size depending on the prevalent environmental conditions (Hernandez et al. 2022). A review reported that stomatal movement (opening/closure) is also controlled by light conditions (Yang et al. 2020). Plants with dense and small stomata tend to have higher rates of photosynthesis but have lower water-use efficiency (Drake et al. 2013). In the present study, the aboveground biomass was also higher in CON and/or W2 than in W3 treatments, possibly indicating higher rates of photosynthesis through enhanced SD. Although the photosynthesis and water-use efficiency were not included in this study, the result of Drake et al. (2013) can be explained by the observed larger GCL in CON and/or W2 and high light treatments compared with those of the other treatments because such a pattern indicates larger stomatal opening. The GCL at CON and/or W2 and high light intensity was also negatively correlated with the LA of the seedlings under moderate to low light intensity treatments. The guard cells at CON and/or W2 may have regulated leaf gas exchange and, thus, the leaf area growth in response to prevalent water and light conditions. This explains why seedlings under CON and/or W2 had lower belowground growth and instead allocated more carbon to the aboveground system to capture more light because water availability was not limited, as it was in the W3 treatment. In addition, CON and/or W2 and high light intensity treatments had the highest PCI among the other treatments, indicating maximal stomatal opening. An increase in PCI may increase stomatal and leaf hydraulic conductance, which might improve photosynthetic capacity. However, under prolonged exposure to high light conditions, higher PCI may increase water loss through transpiration, leading to low water-use efficiency. Stomata generally open in response to light to allow the passage of carbon dioxide for photosynthesis, but too much pore opening may result in higher water consumption relative to carbon gain. This explains the strong positive loadings of CON and/or W2 and high light intensity treatments, which, based on the PCA plot, cluster with GCL. This means that as light intensity increases with sufficient water supply, so does stomatal pore opening via GCL expansion (**Fig. 2 a, b**). Rapid changes in stomatal pore characteristics under different light conditions have been reported as one of the immediate responses of plants to light (Martins et al. 2014). The result suggests that the observed modulation of the stomatal activity of CON- and W2-treated seedlings may help to enhance their productivity but may require high water consumption. Thus, low to moderate light conditions may be better for well-watered kenaf seedlings to efficiently achieve a suitable balance between carbon gain and water consumption. The GCL and SA of kenaf tended to decrease at a moderate light intensity, and this indicates lower water consumption via stomatal closure and more efficient transpiration.

This study revealed some stomatal mechanisms which are possibly associated with drought plasticity in kenaf seedlings. Although the W3-treated seedlings have lower SD, they had significantly lower SAL and GCL than CON and W2-treated ones. This observation was already illustrated in kenaf leaves, i.e., the stomata closed almost completely under restricted soil water availability (Patanè and Cosentino 2013). Such results indicate that kenaf is able to control stomatal opening under a limited water supply that can prevent water loss and increase intercellular CO_2. This can be explained by the observed lower PCI in W3-treated seedlings compared with the other treatments because lower PCI indicates effective stomatal closure. Plants under diminishing water supply might acclimate by exhibiting a water conservation strategy with decreasing SD and PCI (Petrik et al. 2020), which are shown in the stomata of W3-treated kenaf seedlings. Studies have shown that drought plasticity development in plants is predominantly induced by closing stomata (Iqbal et al. 2022). Crops with the ability to acclimate to the changes in soil water availability are sought for production in many countries. However, such an adaptive response of the stomata of kenaf may imply a limited carbon accumulation, which can be seen in the observed significantly lower total biomass in W3 in the present study. Stomatal closure was the main limiting factor for CO_2 assimilation in response to mild water stress via a reduction in the availability of CO_2 in the mesophyll cells (Engineer et al. 2016), resulting in reduced plant growth. Further, although W3-treated seedlings had the lowest total aboveground growth, they had the highest root biomass allocation among the treatments. Several studies concluded that improved root-to-shoot biomass allocation was an important adaptive strategy of plants for experiencing abiotic stress (Aaltonen et al. 2016; Qi et al. 2019). When plant roots detect a water deficiency, they generally send stress signals to the rest of the plant, causing developmental changes in response to drought stress (Kang et al. 2022). Such stress signaling can be evident in the reduction in leaf and stem biomass allocations of kenaf to redirect carbon investment to belowground systems because root organs are more needed to enhance water absorption than leaf or stem. This further explains the lower LA and ECD of seedlings under W3-treated kenaf seedlings.

PCA and ANOVA results showed that the SAL of kenaf was highly responsive to water limitation and shade conditions. The W3-treated and moderate light-treated seedlings had a significantly lower SAL than the other treatments, ascribed to a significant increase in the ECD of seedlings under W3 and moderate-low light treatments. This is also illustrated in the correlation matrix (i.e., a strong negative relationship between SAL and ECD). The osmotic potential of the leaf epidermal cells of W3-treated and moderate light-treated seedlings may have increased, resulting in water consumption from the guard cells. When this happens, the uptake of water of the guard cells *via* suction pressure may decrease, promoting stomatal closure *via* decreases in stomatal aperture size (McAdam et al. 2015; Papanatsiou et al. 2016). Distinct stomatal movements are associated with adjacent epidermal cells (Gray et al. 2020). Also, the reduced size and availability of adjacent epidermal cells could explain the significant alterations in stomatal behavior in plants (Dow et al. 2013).

5. Conclusions

In conclusion, the stomatal traits (aperture, guard cell, and subsidiary cells) of kenaf are highly responsive to varying water and light availability but not to varying soil conditions, indicating stomatal trait plasticity to environmental changes. Hence, seedlings of kenaf may be planted in different areas, including those with low to high water and light availability.

Specifically, kenaf seedlings may survive in water-deficient areas because of their ability to increase root biomass growth for more efficient water absorption and uptake. The present findings are of great ecophysiological significance for the propagation and growth of kenaf. The result also provides information on how kenaf may acclimate to the ever-changing climate.

Acknowledgments

Authors of the present study would like to thank the Philippine Fiber Industry Development Authority (PhilFIDA), Department of Agriculture for providing the seeds used in the experiments.

References

Aaltonen, H., Lindén, A., Heinonsalo, J., Biasi, C., and Pumpanen, J. 2016. Effects of Prolonged Drought Stress on Scots Pine Seedling Carbon Allocation. *Tree Physiology* 37(4): 418–427. DOI: 10.1093/treephys/tpw119

An, X., Jin, G., Zhang, J., Luo, X., Chen, C., Li, W., Ma, G. Y., Jin, L., Dai, L., Shi, X., Wei, W., Zhu, G. 2018. Protein Responses in Kenaf Plants Exposed to Drought Conditions Determined using Itraq Technology. *FEBS Open Bio* 8(10): 1572–1583. DOI: 10.1002/2211-5463.12507

An. X., Jin, G., Luo, X., Chen, C., Li, W., and Zhu, G. 2020. Transcriptome Analysis and Transcription Factors Responsive to Drought Stress in *Hibiscus cannabinus*. *PeerJ* 8: e8470. DOI: 10.7717/peerj.8470

Anav, A., Proietti, C., Menut, L., Carnicelli, S., De Marco, A., and Paoletti, E. 2018. Sensitivity of Stomatal Conductance to Soil Moisture: Implications for Tropospheric Ozone. *Atmospheric Chemistry and Physics* 18(8): 5747–5763. DOI: 10.5194/acp-18-5747-2018

Ayadi, R., Hamrouni, L., Hanana, M., Bouzid, S., Trifi, M., and Khouja, M. L. 2011. In Vitro Propagation and Regeneration of an Industrial Plant Kenaf (*Hibiscus cannabinus* L.). *Industrial Crops and Products* 33(2): 474–480. DOI: 10.1016/j.indcrop.2010.10.025

Bertolino, L. T., Caine, R. S., and Gray, J. E. 2019. Impact of Stomatal Density and Morphology on Water-Use Efficiency in a Changing World. *Frontiers in Plant Science* 10: 225. DOI: 10.3389/fpls.2019.00225

Buckley, T. N. 2019. How do Stomata Respond to Water Status? *New Phytologist* 224(1): 21–36. DOI: 10.1111/nph.15899

Dow, G. J., Berry, J. A., and Bergmann, D. C. 2013. The Physiological Importance of Developmental Mechanisms that Enforce Proper Stomatal Spacing in a *Abidopsis thaliana*. *New Phytologist* 201(4): 1205–1217. DOI: 10.1111/nph.12586

Drake, P. L, Froend, R. H., and Franks, P. J. 2013. Smaller, Faster Stomata: Scaling of Stomatal Size, Rate of Response, and Stomatal Conductance. *Journal of Experimental Botany* 64(2): 495–505. DOI: 10.1093/jxb/ers347

Driesen, E., Van den Ende, W., De Proft, M., and Saeys, W. 2020. Influence of Environmental Factors Light, CO_2, Temperature, and Relative Humidity on Stomatal Opening and Development: A Review. *Agronomy* 10 (12): 1975. DOI: 10.3390/agronomy10121975

Engineer, C. B., Hashimoto-Sugimoto, M., Negi, J., Israelsson-Nordström, M., Azoulay-Shemer, T., Rappel, W. J., Iba, K., and Schroeder, J. I. 2016. CO_2 Sensing and CO_2 Regulation of

Stomatal Conductance: Advances and Open Questions. *Trends in Plant Science* 21(1): 16–30. DOI: 10.1016/j.tplants.2015.08.014

Gentili, R., Ambrosini, R., Montagnani, C., Caronni, S., and Citterio, S. 2018. Effect of Soil pH on the Growth, Reproductive Investment and Pollen Allergenicity of *Ambrosia artemisiifolia* L.. *Frontiers in Plant Science* 9: 1335. DOI: 10.3389/fpls.2018.01335

Gray, A., Liu, L., and Facette, M. 2020. Flanking Support: How Subsidiary Cells Contribute to Stomatal Form and Function. *Frontiers in Plant Science* 11: 881. DOI: 10.3389/fpls.2020.00881

Hariz, T. M., Hadi, Y. S., Lubis, M. A., Maulana, M. I., Sari, R. K., and Hidayat, W. 2023. Physical and Mechanical Properties of Cross-Laminated Timber Made of a Combination of Mangium-Puspa Wood and Polyurethane Adhesive. *Jurnal Sylva Lestari* 11(1): 37–65. DOI: 10.23960/jsl.v11i1.645

Hernandez, J. O., and Park, B. B. 2022. The Leaf Trichome, Venation, and Mesophyll Structural Traits Play Important Roles in the Physiological Responses of Oak Seedlings to Water-Deficit Stress. *International Journal of Molecular Sciences* 23(15): 8640. DOI: 10.3390/ijms23158640

Hernandez, J. O., Tolentino, C. A., Quiñones, L. E., Villancio, G. B., Maldia, L. S. J., and Combalicer, M. S. 2022. Growth and Morpho-Physiology of *Tectona philippinensis* under Different Water Stress and Soil Conditions. *Biodiversitas* 23(1): 507–513. DOI: 10.13057/biodiv/d230154

Hernandez, J. O., An, J. Y., Combalicer, M. S., Chun, J. P., Oh, S. K., and Park, B. B. 2021. Morpho-Anatomical Traits and Soluble Sugar Concentration Largely Explain the Responses of Three Deciduous Tree Species to Progressive Water Stress. *Frontiers in Plant Science* 12: 738301. DOI: 10.3389/fpls.2021.738301

Herwanti, S. 2015. Potensi Kayu Rakyat pada Kebun Campuran di Desa Pesawaran Indah Kabupaten Pesawaran. *Jurnal Sylva Lestari* 3(1): 113–120. DOI: 10.23960/jsl13113-120

Idris, A., Linatoc, A., and Abu Bakar, M. F. 2019. Effect of Light Intensity on the Photosynthesis and Stomatal Density of Selected Plant Species of Gunung Ledang, Johor. *Malaysian Applied Biology* 48(3):133–140.

Iqbal, S., Wang, X., Mubeen, I., Kamran, M., Kanwal, I., Díaz, Ga., Abbas, A., Parveen, A., Atiq, M. N., Alshaya, H., Zin, E. L., Abedin, T. K., and Fahad, S. 2022. Phytohormones Trigger Drought Tolerance in Crop Plants: Outlook and Future Perspectives. *Frontiers in Plant Science* 12: 799318. DOI: 10.3389/fpls.2021.799318

Kang, J., Peng, Y., and Xu, W. 2022. Crop Root Responses to Drought Stress: Molecular Mechanisms, Nutrient Regulations, and Interactions with Microorganisms in the Rhizosphere. *International Journal of Molecular Sciences* 23(16): 9310. DOI: 10.3390/ijms23169310

Kardiman, R., and Ræbild, A. 2018. Relationship between Stomatal Density, Size and Speed of Opening in Sumatran Rainforest Species. *Tree Physiology* 38(5): 696–705. DOI: 10.1093/treephys/tpx149

Kim, J., Han, G. D., Muthukathan, G., Rodrogues, R., Hyun, D. Y, Kim, S. H., Yu, J. K., Park, J., Yoo, S. C., and Chung, Y. S. 2021. What Traits Should be Measured for Biomass in Kenaf. *Plants* 10(7): 1394. DOI: 10.3390/plants10071394

Li, T., Liu, L. N., Jiang, C. D., Liu, Y. J., and Shi, L. 2014. Effects of Mutual Shading on the Regulation of Photosynthesis in Field-Grown Sorghum. *Journal of Photochemistry and Photobiology B: Biology* 137: 31–38. DOI: 10.1016/j.jphotobiol.2014.04.022

Lippmann, R., Babben, S., Menger, A., Delker, C., and Quint, M. 2019. Development of Wild and Cultivated Plants under Global Warming Conditions. *Current Biology* 29(24): 1326–1338. DOI: 10.1016/j.cub.2019.10.016

Martins, S. C., Galmés, J., Cavatte, P. C., Pereira, L. F., Ventrella, M. C., and DaMatta, F. M. 2014. Understanding the Low Photosynthetic Rates of Sun and Shade Coffee Leaves: Bridging the Gap on the Relative Roles of Hydraulic, Diffusive and Biochemical Constraints to Photosynthesis. PLoS ONE 9(4): e95571. DOI: 10.1371/journal.pone.0095571

McAdam, S. A. M., and Brodribb, T. J. 2015. The Evolution of Mechanisms Driving the Stomatal Response to Vapor Pressure Deficit. *Plant Physiology* 167(3): 833–843. DOI: 10.1104/pp.114.252940

Millstead, L., Jayakody, H., Patel, H., Kaura, V., Petrie, P. R., Tomasetig, F., and Whitty, M. 2020. Accelerating Automated Stomata Analysis through Simplified Sample Collection and Imaging Techniques. *Frontiers in Plant Science* 11: 580389. DOI: 10.3389/fpls.2020.580389

Niu, X., Chen, M., Tao, A., and Qi, J. 2016. Salinity and Drought Treatment Assays in Kenaf (*Hibiscus cannabinus* L.). *Bio-Protocol* 6(17): e1918. DOI: 10.21769/BioProtoc.1918

Nurdin, A. S., Hadun, R., Setiawan, B. D., Irmayanti, L., and Ashari, R. 2022. Effects of Initial Water Temperature and Soaking Duration on the Germination of Canarium (*Canarium indicum*) Seeds. *Jurnal Sylva Lestari* 10(2): 244–253. DOI: 10.23960/jsl.v10i2.571

Papanatsiou, M., Amtmann, A., and Blatt, M. R. 2016. Stomatal Spacing Safeguards Stomatal Dynamics by Facilitating Guard Cell Ion Transport Independent of the Epidermal Solute Reservoir. *Plant Physiology* 172(1): 254–263. DOI: 10.1104/pp.16.00850

Patanè, C., and Cosentino, S. L. 2013. Yield, Water Use and Radiation Use Efficiencies of Kenaf (*Hibiscus cannabinus* L.) under Reduced Water and Nitrogen Soil Availability in a Semi-Arid Mediterranean Area. *European Journal of Agronomy* 46: 53–62. DOI: 10.1016/j.eja.2012.12.002

Petrík, P., Petek, A., Konôpková, A., Bosela, M., Fleischer, P., Frýdl, J., and Kurjak, D. 2020. Stomatal and Leaf Morphology Response of European Beech (*Fagus sylvatica* L.) Provenances Transferred to Contrasting Climatic Conditions. *Forests* 11(12): 1359. DOI: 10.3390/f11121359

Petrik, P., Petek-Petrik, A., Kurjak, D., Mukarram, M., Klein, T., Gömöry, D., Střelcová, K., Frýdl, J., and Konôpková, A. 2022. Interannual Adjustments in Stomatal and Leaf Morphological Traits of European Beech (*Fagus sylvatica* L.) Demonstrate Its Climate Change Acclimation Potential. *Plant Biology* 24(7): 1287–1296. DOI: 10.1111/plb.13401

Pressel, S., Renzaglia, K. S., Clymo, R. S., and Duckett, J. G. 2018. Hornwort Stomata Do Not Respond Actively to Exogenous and Environmental Cues. *Annals of Botany* 122(1): 45–57. DOI: 10.1093/aob/mcy045

Qi, Y., Wei, W., Chen, C., and Chen, L. 2019. Plant Root-Shoot Biomass Allocation Over Diverse Biomes: A Global Synthesis. *Global Ecology and Conservation* 18: e00606. DOI: 10.1016/j.gecco.2019.e00606

Salmon, Y., Lintunen, A., Dayet, A., Chan, T., Dewar, R., Vesala, T., and Hölttä, T. 2020. Leaf Carbon and Water Status Control Stomatal and Nonstomatal Limitations of Photosynthesis in Trees. *New Phytologist* 226(3): 690–703. DOI: 10.1111/nph.16436

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 Years of Image Analysis. *Nature Methods* 9: 671–675.

Vicente-Serrano, S. M., Peña-Angulo, D., Beguería, S., Domínguez-Castro, F., Tomás-Burguera, M., Noguera, I., Gimeno-Sotelo, L., and El Kenawy, A. 2022. Global Drought Trends and Future Projections. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 380: 20210285. DOI: 10.1098/rsta.2021.0285

Wild, A., and Wolf, G. 1980. The Effect of Different Light Intensities on the Frequency and Size of Stomata, the Size of Cells, the Number, Size and Chlorophyll Content of Chloroplasts in the Mesophyll and the Guard Cells during the Ontogeny of Primary Leaves of *Sinapis alba*. *Zeitschrift Für Pflanzenphysiologie* 97(4): 325–342. DOI: 10.1016/s0044-328x(80)80006-7

Xu, Z., and Zhou, G. 2008. Responses of Leaf Stomatal Density to Water Status and Its Relationship with Photosynthesis in a Grass. *Journal of Experimental Botany* 59(12): 3317–3325. DOI: 10.1093/jxb/ern185

Yang, J., Li, C., Kong, D., Guo, F., and Wei, H. 2020. Light-Mediated Signaling and Metabolic Changes Coordinate Stomatal Opening and Closure. *Frontiers in Plant Science* 11: 601478. DOI: 10.3389/fpls.2020.601478

Zheng, Y., Xu, M., Hou, R., Shen, R., Qiu, S., and Ouyang, Z. 2013. Effects of Experimental Warming on Stomatal Traits in Leaves of Maize (*Zea may* L.). *Ecology and Evolution* 3(9): 3095–3111. DOI: 10.1002/ece3.674