BIODIVERSITAS
Volume 27, Number 3, March 2026
Pages: 8

ISSN: 1412-033X
E-ISSN: 2085-4722
https://doi.org/10.13057/biodiv/d270334

Morpho-anatomical responses of Sertani and Trisakti upland rice genotypes to contrasting soil moisture regimes

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Manuscript received: 21 February 2026. Revision accepted: 30 March 2026.

Abstract. *Hanum C, Simatupang BD. 2026. Morpho-anatomical responses of Sertani and Trisakti upland rice genotypes to contrasting soil moisture regimes. Biodiversitas 27 (3): d270334. https://doi.org/10.13057/biodiv/d270334*. Water availability critically determines upland rice agronomic performance, necessitating characterization of genotypic responses across moisture gradients. This study evaluated morpho-anatomical responses of two upland rice genotypes (Sertani and Trisakti) under contrasting moisture regimes of 40%, 60%, and 80% field capacity (FC) during the vegetative phase, using a factorial Randomized Complete Block Design (RCBD) with three replications. As FC increased from 40% to 80%, both genotypes showed significant ($p<0.05$) progressive increases in leaf area, leaf thickness, Relative Chlorophyll Index (RCI), bulliform cell dimensions, stomatal length, stomatal width, stomatal density, root volume, and total biomass. Root volume increased nearly five-fold from 0.25 cm^3 at 40% FC to 1.23 cm^3 at 80% FC, while total plant dry weight increased 3.9-fold (0.20 to 0.78 g) over the same gradient. Anatomical cross-sections revealed moisture-dependent structural differentiation: at 40% FC, plants displayed reduced cortical aerenchyma with endodermal lignification, whereas at 80% FC, extensive aerenchyma development was observed; these observations are qualitative and descriptive. Observed root:shoot ratios were numerically highest at 40% FC (Sertani: 0.50; Trisakti: 0.82); however, as these represent treatment means only and were not subjected to replicate-level statistical testing, no inferential conclusion regarding biomass allocation shifts is drawn. Trisakti showed numerically higher mean values than Sertani for root volume (variety mean: 0.82 vs. 0.70 cm^3) and root dry weight (0.18 vs. 0.13 g), though these differences were not statistically significant (variety main effect: $F(1,15) \leq 2.36$, $p>0.05$). The sole trait showing a significant variety effect was RCI ($F(1,15) = 5.41$, $p<0.05$), with Trisakti recording a higher mean. These moisture-responsive morpho-anatomical plasticity patterns provide a phenotypic reference for future comparative studies of climate-adaptive upland rice germplasm.

Keywords: Biomass allocation, morpho-anatomical traits, plant adaptation, soil moisture regime, upland rice

Abbreviations: ABA: Abscisic Acid, FC: Field Capacity, RCA: Root Cortical Aerenchyma, RCBD: Randomized Complete Block, RCI: Relative Chlorophyll Index Design

INTRODUCTION

Rainfed dryland agricultural systems, where upland rice (*Oryza sativa* L.) cultivation predominates, are frequently characterized by unpredictable and deficient water availability, establishing moisture stress as the foremost productivity constraint and driving evolutionary selection for diverse structural adaptations within leaf and root tissues that serve as critical defenses against desiccation stress (Bhandari et al. 2023; Hassan et al. 2023). Upland rice accounts for approximately 13% of global rice harvested area and remains a critical food security crop for smallholder farming systems across sub-Saharan Africa and Southeast Asia, where irrigation infrastructure is limited or absent (Hassan et al. 2023). In Indonesia specifically, upland rice cultivation supports millions of smallholder farmers in rain-dependent dryland systems of Sumatra, Kalimantan, and Eastern Indonesia, where recurring seasonal dry spells frequently cause yield losses exceeding 40% (Mustikarini et al. 2022).

Root and leaf anatomical traits are genetically controlled and exhibit considerable genotypic variation, with root anatomical plasticity—encompassing cortical diameter, aerenchyma formation, and endodermal lignification—shaped by complex gene-environment interactions (Kadam et al. 2017; Luo et al. 2020). Indonesian local cultivars also show genotype-specific physiological responses linked to differences in osmotic adjustment capacity (Salsinha et al. 2022).

Indonesian agricultural research has successfully leveraged such genetic diversity to develop elite upland rice cultivars for water-limited environments. Sertani (released 2009) and Trisakti (released 2012) are two widely adopted cultivars among Indonesian smallholder farmers in drought-prone dryland systems, sharing drought tolerance classifications in their official release descriptors (Ministry of Agriculture 2009, 2012). However, their underlying morpho-anatomical drought response mechanisms have not been directly compared under controlled conditions. Indirect evidence suggests they may differ: Sertani's release profile emphasizes stable performance in water-limited upland conditions consistent with conservative drought avoidance, while Trisakti's broader multi-stress tolerance spectrum—encompassing drought, brown planthopper, and bacterial leaf blight—suggests a more complex stress-response profile potentially involving enhanced root plasticity and

osmotic adjustment (Miftahudin et al. 2020; Salsinha et al. 2022). Whether these contrasting release profiles reflect distinct morpho-anatomical mechanisms or shared structural plasticity under water limitation has not been directly investigated, representing a knowledge gap that the present study addresses as a preliminary exploratory assessment.

Drought triggers comprehensive structural remodeling beyond simple growth reduction. At the leaf level, adaptive modifications include strengthened cuticular barriers, altered stomatal architecture, and enhanced bulliform cell development collectively minimizing transpirational expenditure while maintaining photosynthetic function (Matkowski and Daszkowska-Golec 2023). Genetic variation in stomatal density and size significantly influences water use efficiency and drought tolerance (Pitaloka et al. 2022). Belowground, stress-tolerant genotypes characteristically exhibit increased rooting depth, expanded root volume, and elevated below-ground biomass investment supporting drought avoidance through enhanced soil water access (Uga et al. 2013).

Substantial genotypic differences in morphophysiological responses during the vegetative stage are well documented. Miftahudin et al. (2020) demonstrated that modifications in shoot growth, relative water content, chlorophyll content, and membrane lipid peroxidation varied markedly across rice cultivars under water deficit, affirming that such variation constitutes a reliable criterion for early-stage tolerance selection. Water deficit consistently induces reduced lamina expansion, enhanced tissue thickness, modified stomatal patterning, and intensified cuticle development—collectively improving stress tolerance through water conservation and functional stability (Caine et al. 2019; Pitaloka et al. 2022).

Despite these advances, integrative studies simultaneously characterizing foliar anatomy (leaf thickness, bulliform cell dimensions, stomatal density and size), root volume, and biomass allocation across precisely controlled soil moisture levels for Indonesian-released upland rice cultivars at the vegetative stage remain scarce (Mustikarini et al. 2022; Salsinha et al. 2023). This study addresses that gap by simultaneously evaluating foliar and root morpho-anatomical traits alongside biomass partitioning under three controlled FC levels (40%, 60%, 80%) for Sertani and Trisakti at the vegetative phase, providing an integrated phenotypic baseline that is currently lacking for these genotypes. Findings are intended to provide a reference for future morpho-physiological screening of climate-adaptive upland rice germplasm.

MATERIALS AND METHODS

Experimental site and duration

The experiment was conducted in a greenhouse at the Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia (±32 meter above sea level) from December 2023 to January 2024. The greenhouse maintained a mean daily temperature of 28-32°C with natural photoperiod (approximately 12 h light/12 h dark) and relative humidity of 65-85%. No supplemental lighting was used.

Soil characteristics and pot preparation

The growth medium consisted of surface soil (0-20 cm depth) classified as Typic Dystrudept (inceptisol) according to Soil Survey Staff (2022). The soil was air-dried, sieved through a 2-mm mesh, and homogenized prior to filling into polybags measuring 40 cm × 40 cm. Soil physicochemical properties were: pH 5.8, organic C 1.43%, total N 0.14%, available P 12.6 mg kg^{-1}, CEC 18.2 $cmol_n$ kg^{-1}, and loam texture (sand 32%, silt 41%, clay 27%). Each polybag received 2.33 kg of oven-dry soil and accommodated one plant per experimental unit.

Field capacity determination

Soil field capacity (FC) was determined using a saturation drainage method prior to treatment application. Soil in each pot was saturated to maximum water retention, then allowed to drain freely for 48 hours, after which gravimetric moisture content was measured. At 100% FC, the total water retained was 3.67 kg, yielding a total pot weight (soil + water + pot) of 6.0 kg. Target pot weights were calculated as: $W_{tar}{}^{g}{}_{et}$ = Wdry_pot + ($FC_{tar}{}^{g}{}_{et}$/100) × Wwater@100%FC, where Wdry_pot = 2.33 kg and Wwater@100%FC = 3.67 kg, yielding: K3 (80% FC) = 5.27 kg, K2 (60% FC) = 4.53 kg, K1 (40% FC) = 3.80 kg per pot. Accumulated plant dry weight at harvest was <0.8 g per pot (<0.5% of total pot weight), representing a negligible error source.

Plant materials and experimental design

Two upland rice (*O. sativa*) genotypes, Sertani (V1) and Trisakti (V2), were evaluated under three soil moisture levels: K1 (40% FC), K2 (60% FC), and K3 (80% FC). The K3 (80% FC) treatment served as the well-watered reference condition throughout this study. At 80% FC, soil macropores remain air-filled, supporting adequate O_2 diffusion to the root zone; at 100% FC, complete pore saturation reduces O_2 diffusion rates up to 320,000-fold relative to air, inducing rhizosphere hypoxia incompatible with upland rice root physiology (Xiong et al. 2024; Zheng et al. 2025). Therefore, K3 (80% FC) functions as the experimental control against which K1 and K2 are compared. The experiment followed a two-factor factorial RCBD with three replications, yielding 18 experimental units (2 varieties × 3 moisture levels × 3 blocks).

Moisture treatment and regulation

Moisture treatments were imposed one week after transplanting (14 DAS) by maintaining soil water content at target FC percentages using the gravimetric method. Pots were weighed daily using a digital balance, and distilled water was added as needed to restore each pot to its predetermined target weight. A tolerance of ±5% of the target weight was maintained throughout. Moisture treatments were sustained continuously until harvest at 40 days after transplanting (DAT).

Sampling scheme

For each experimental unit (one plant per pot), anatomical measurements were conducted on a single fully expanded leaf per plant (the second leaf below the flag leaf). A single

measurement session was performed per plant at harvest; replication (n = 3 per treatment combination) reflects the three independent pots per treatment, each representing one block in the RCBD design—not repeated measurement sessions on the same plant. Three transverse hand-sections were prepared per leaf, and three fields of view per section were analyzed for lamina thickness, cuticle thickness, bulliform cell length and width, and epidermis thickness, giving nine measurements per experimental unit per trait. Stomatal impressions were taken at three positions per leaf (base, middle, apex) using nail polish replicas applied to an approximately 1 cm² area of the abaxial leaf surface at each position. Three microscope fields per impression were counted for stomatal density, and three individual stomata per field were measured for length and width, yielding nine measurements per experimental unit. All anatomical measurements were made by a single observer; intra-observer reliability was assessed by re-measuring 10% of images on separate occasions, yielding a coefficient of variation <5% for all traits.

Physiological and anatomical measurements

Leaf area and Relative Chlorophyll Index (RCI, scale 0-1) were quantified using the Petiole Pro mobile application (version 2.7) on a Samsung Galaxy A52 smartphone at a fixed distance of 30 cm under consistent diffuse indoor lighting; three readings per leaf were averaged. RCI values are not equivalent to conventional SPAD meter readings and are used solely for between-treatment comparisons. Leaf cross-sections were prepared manually from the second leaf below the flag leaf, mounted on glass slides with distilled water without staining. Tissue boundaries (cuticle, epidermis, bulliform cells) were identified based on cell morphology and relative position in the cross-section under bright-field illumination; measurements were accepted only when boundaries were unambiguous. Leaf lamina thickness (µm), cuticle thickness (µm), bulliform cell length and width (µm), and stem epidermis thickness (µm) were measured from photomicrographs using ZEN 2012 imaging software (Carl Zeiss, Germany). Stomatal impressions were obtained using the nail polish replica method applied to the abaxial leaf surface. Stomatal density (no./mm²) and dimensions (length and width, µm) were measured using an Olympus BX41 light microscope (Olympus, Japan) at 100× (density) and 400× (dimensions) magnification, analyzed with ZEN 2012 software.

For qualitative root anatomical documentation, fresh root segments (approximately 5 mm in length) were excised from the middle third of seminal roots at harvest, hand-sectioned transversely, and mounted on glass slides with distilled water without staining. Cross-sections were photographed under bright-field illumination at 100× magnification. Observations were restricted to qualitative description of cortical aerenchyma extent and endodermal cell morphology; no quantitative measurements were extracted from root cross-section images. Root number was not recorded in this study; this constitutes a recognized limitation, as root number data would have strengthened the quantitative characterization of root system development across moisture regimes. Future studies should incorporate root number counts alongside image-based morphometry of percentage root cortical aerenchyma (%RCA), stele diameter, and metaxylem dimensions.

Growth and biomass measurements

At harvest (40 DAT), plants were separated into root and shoot components. The vegetative stage was selected as the sampling point because root system architecture and biomass allocation during early growth are critical determinants of subsequent drought avoidance capacity (Gupta et al. 2020; Li et al. 2022). It is acknowledged that drought responses can differ substantially between vegetative and reproductive stages, with reproductive-stage responses being more directly linked to yield outcomes; characterization of reproductive-stage responses is recommended for future studies. Root volume was measured using the water displacement method. All tissues were oven-dried at 70°C for 72 hours to constant weight. Root:shoot (R:S) ratios were computed as root dry weight divided by shoot dry weight and are reported as descriptive treatment means only; no replicate-level statistical testing was performed for this derived variable.

Statistical analysis

All data were analyzed using the linear model: Response ~ Variety (V) + Moisture Regime (K) + V×K + Block. ANOVA was performed for each response variable with the pot as the experimental unit (n = 3 per treatment combination). Three replications are consistent with the standard level applied in preliminary controlled-environment factorial studies (Gomez and Gomez 1984); however, this replication level may have been insufficient to detect subtle inter-genotypic differences, particularly for anatomical traits with inherently high biological variability—a recognized limitation of the present study that future investigations should address by employing five or more replications. The V×K interaction was tested prior to interpreting main effects; as it was non-significant for all variables, main effects were interpreted independently. Where the K main effect was significant, mean separation was performed using Duncan's Multiple Range Test (DMRT) at the 5% level. Normality and homogeneity of variance assumptions were verified prior to analysis. A compact ANOVA summary table is provided as Table 4.

RESULTS AND DISCUSSION

Foliar anatomical responses to contrasting moisture regimes

Leaf anatomical parameters were assessed across three soil moisture levels: K1 (40% FC), K2 (60% FC), and K3 (80% FC, well-watered reference). Table 1 presents leaf area, tissue thickness, Relative Chlorophyll Index (RCI), and bulliform cell dimensions for Sertani and Trisakti. Moisture regime (K) was the primary driver of variation across all leaf anatomical parameters ($p<0.05$), while the variety main effect (V) was non-significant for most traits ($F(1,15)\leq 2.36$, $p>0.05$; 2.36 represents the maximum observed F-value for variety across all non-significant traits),

and V×K interactions were non-significant throughout (Table 4).

Both Sertani and Trisakti showed consistent positive associations between soil moisture availability (K1→K3) and all measured leaf anatomical parameters. Leaf area increased from 3.93 cm² at K1 to 13.17 cm² at K3, reflecting moisture-dependent lamina expansion. Leaf thickness increased progressively from 179.66 µm at K1 to 246.54 µm at K3, consistent with turgor-driven mesophyll expansion under favorable hydration. RCI increased from a mean of 0.22 at K1 to 0.31 at K3. Variety (V) had a significant effect on RCI (V: $F(1,15) = 5.41$, $p<0.05$), with Trisakti recording a higher mean RCI (0.27) than Sertani (0.25); the non-significant V×K interaction ($F(2,15) = 0.64$, $p>0.05$) indicates this difference was consistent across moisture levels. Bulliform cell length and width both increased significantly with FC (K: $F(2,15) = 43.95$, $p<0.05$ for width), reflecting turgor-driven cell expansion under well-hydrated conditions. Under water-limited conditions (K1), reduced bulliform cell size reflects turgor loss, triggering inward leaf rolling as a drought avoidance mechanism that reduces exposed leaf surface area and attenuates transpirational water loss (Gunnula et al. 2023; Latif et al. 2023).

Bulliform cell responses

Microscopic observations of bulliform cells in both Sertani and Trisakti confirmed a clear positive association between soil moisture availability and cell dimensions (Figure 1). At K1 (40% FC), cells appeared reduced in size due to turgor loss, triggering leaf rolling as a mechanism to reduce transpiration. At K2 (60% FC), partial turgor recovery was evident, while at K3 (80% FC), cells were the largest and most turgid, consistent with hydration-driven expansion. This turgor-dependent response is consistent with the known physiological function of bulliform cells: their maximum size occurs under adequate hydration, while size reduction under deficit conditions contributes to leaf rolling as a drought avoidance strategy (Gunnula et al. 2023). The absence of a significant V×K interaction for bulliform cell dimensions indicates that both varieties responded similarly to each moisture level.

Stomatal morphological responses to soil moisture regimes

Stomatal morphology responded significantly to soil moisture availability in both upland rice varieties (Table 2). Moisture regime (K) was the primary determinant of stomatal length (K: $F(2,15) = 44.38$, $p<0.05$), width (K: $F(2,15) = 82.72$, $p<0.05$), and density (K: $F(2,15) = 67.53$, $p<0.05$). Variety main effects and V×K interactions were non-significant for all stomatal traits (Table 4).

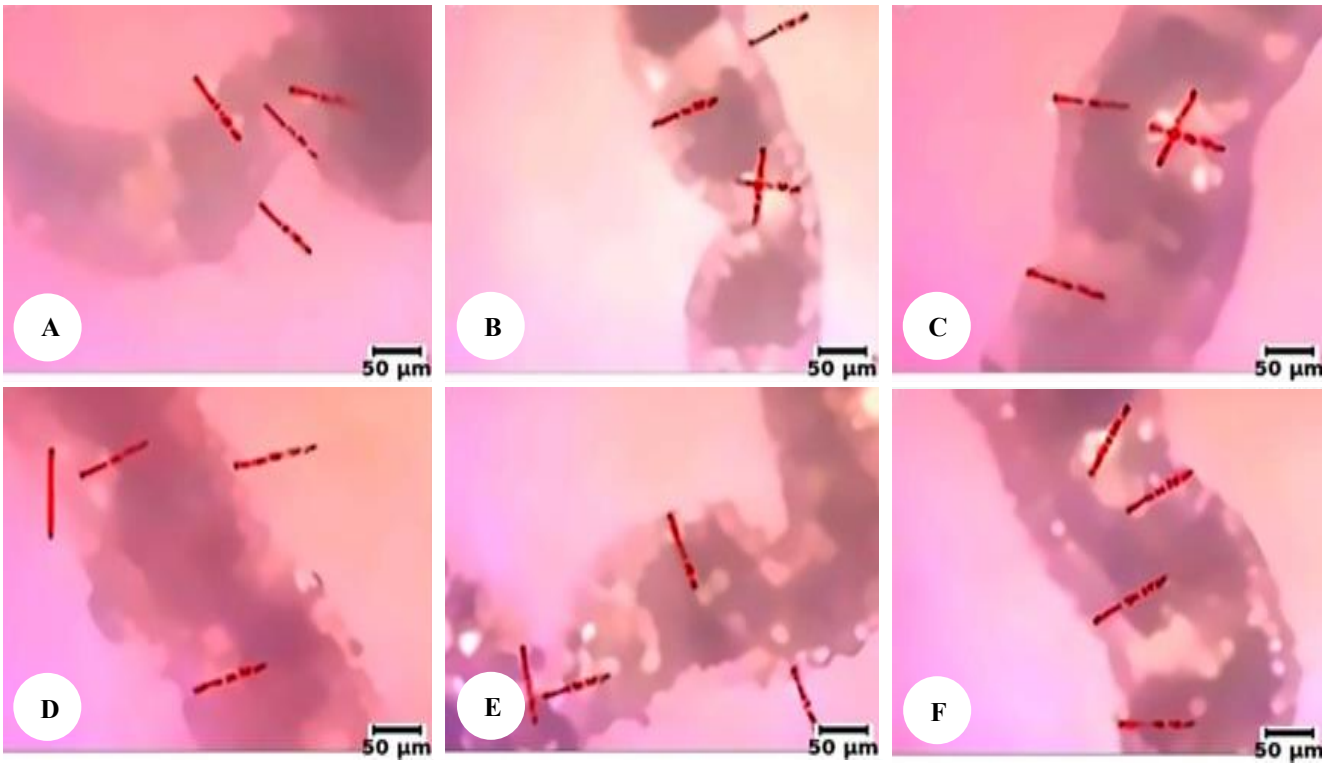

Figure 1. Bulliform cells. A. Sertani K1 (40% FC), B. Sertani K2 (60% FC), C. Sertani K3 (80% FC), D. Trisakti K1 (40% FC), E. Trisakti K2 (60% FC), F. Trisakti K3 (80% FC). Scale bar: 50 µm; Magnification: 400×

Table 1. Leaf anatomical characteristics of two upland rice varieties under contrasting soil moisture regimes

Parameter	Variety	K1 (40% FC)	K2 (60% FC)	K3 (80% FC)	Variety mean	Unit
Leaf area	Sertani	4.04±1.31	6.87±2.00	13.88±3.26	8.26	cm²
	Trisakti	3.83±1.66	8.00±1.62	12.46±2.76	8.10	
	K Mean	3.93±0.49c	7.44±0.63b	13.17±1.02a	-	cm²
Leaf thickness	Sertani	176.66±23.96	210.99±24.45	253.90±16.96	213.85	µm
	Trisakti	182.66±31.58	223.62±19.91	239.17±35.36	215.15	
	K Mean	179.66±9.24c	217.30±7.68b	246.54±9.49a	-	µm
Leaf greenness (RCI)	Sertani	0.20±0.08	0.26±0.02	0.30±0.08	0.25	0-1
	Trisakti	0.24±0.02	0.26±0.02	0.33±0.04	0.27*	
	K Mean	0.22±0.01c	0.26±0.01b	0.31±0.01a	-	0-1
Bulliform cell length	Sertani	32.88±7.62	49.58±8.77	61.71±9.88	48.05	µm
	Trisakti	34.45±5.05	43.18±2.77	57.74±6.69	45.12	
	K Mean	33.66±2.14c	46.38±2.45b	59.72±2.86a	-	µm
Bulliform cell width	Sertani	23.71±5.70	35.59±9.79	48.09±6.44	35.80	µm
	Trisakti	21.70±6.51	32.79±2.40	49.11±7.11	34.53	
	K Mean	22.71±2.04c	34.19±2.39b	48.60±2.23a	-	µm

Note: DMRT letters (a, b, c) in the 'K Mean' rows compare moisture-level means within each trait; different letters indicate significant differences at the 5% level. Variety means (rightmost column) are provided for descriptive comparison; variety main effect was non-significant for most traits ($p>0.05$). *: Trisakti variety mean for RCI was significantly higher than Sertani (V: $F(1,15) = 5.41$, $p<0.05$). RCI: Relative Chlorophyll Index (Petiole Pro, scale 0-1). Values are means ±SD of three replicates

Table 2. Stomatal size (length and width) and stomatal density of upland rice under different varieties and soil moisture regimes

Parameter	Variety	K1 (40% FC)	K2 (60% FC)	K3 (80% FC)	n†
Stomatal length (µm)	Sertani	13.71±1.09	17.83±0.74	21.21±2.88	9
	Trisakti	13.54±0.86	17.64±0.44	21.93±3.37	9
	K mean ± SE	13.62±0.09c	17.74±0.10b	21.57±0.36a	
Stomatal width (µm)	Sertani	7.20±0.79	9.41±0.64	11.70±0.31	9
	Trisakti	6.41±0.43	9.15±0.56	11.62±1.30	9
	K mean ± SE	6.81±0.40c	9.28±0.13b	11.66±0.04a	
Stomatal density (no./mm²)	Sertani	104.62±7.43	143.85±5.96	177.31±19.11	9
	Trisakti	118.08±9.26	137.69±8.10	179.62±10.39	9
	K mean ± SE	111.35±6.73c	140.76±3.08b	178.46±1.16a	

Note: DMRT letters (a, b, c) compare K means within each trait; different letters indicate significant differences at the 5% level (K: $F(2,15) \geq 44.38$, $p<0.05$ for all stomatal traits). Variety main effects and V×K interactions were non-significant ($p>0.05$). SE = standard error of K mean ($n = 6$ per K level). Values for individual varieties are means ±SD of three replicates. K Mean ±SE values are derived from pooled variety means within each FC level. † n = number of measurements per experimental unit (3 fields × 3 stomata per field for dimensions; 3 fields × 1 count per field for density)

Stomatal length increased significantly from 13.62 µm at K1 to 21.57 µm at K3, and stomatal width from 6.81 µm to 11.66 µm, reflecting stomatal development and expansion under favorable hydration. Stomatal density increased from 111.35 no./mm² at K1 to 178.46 no./mm² at K3. An important interpretive distinction is warranted: the observed moisture-dependent increases in stomatal dimensions and density reflect developmental plasticity during organogenesis under progressively favorable soil water conditions, not dynamic stomatal regulation as a drought avoidance response per se. Plants at K1 developed constitutively smaller and fewer stomata as a consequence of impaired turgor-driven cell expansion during leaf formation (Kim et al. 2020; Matkowski and Daszkowska-Golec 2023), consistent with published reports that chronic water deficit suppresses stomatal development via pathways consistent with ABA signaling—though ABA was not measured here and no direct mechanistic inference is made. Future studies imposing water deficit on fully formed leaves would better isolate the dynamic regulatory component of stomatal drought response. Trisakti showed marginally higher stomatal density than Sertani under K1, though this difference was not statistically significant.

Qualitative structural observations of root cortical aerenchyma

Root anatomical cross-sections of Sertani and Trisakti revealed qualitative differences in cortical aerenchyma formation across moisture regimes (Figure 2). At K3 (80% FC), extensive cortical aerenchyma with large, interconnected air spaces was observed in both varieties, consistent with reports that adequate cellular turgor and energy availability support aerenchyma formation under well-hydrated conditions (Fonta et al. 2022). At K1 (40% FC), cortical aerenchyma appeared less extensively developed compared to K3, with air spaces that were visibly smaller and less interconnected; cortical cells showed a relatively denser arrangement. Apparent differences in cell wall thickness and endodermal

features were also noted, consistent with published descriptions of water-stress-induced hydraulic modifications (Kadam et al. 2017); however, histochemical confirmation (e.g., lignin staining) was not performed and these structural observations cannot be confirmed from bright-field microscopy alone. It should be noted that the gradient of aerenchyma development from K1 to K3 reflects moisture-dependent tissue development rather than drought-specific anatomical adaptation: aerenchyma develops most extensively when turgor and metabolic energy support active cell differentiation. These observations are strictly qualitative and descriptive; quantitative measurements of %RCA, stele diameter, and metaxylem dimensions were not performed. Root number was not recorded, which is acknowledged as a limitation—quantitative root system characterization including root number would have substantially strengthened the interpretation of biomass data. Future studies should incorporate image-based morphometry and root count data.

Root volume, plant dry weight, and root dry weight

Root volume, plant dry weight, and root dry weight all increased significantly ($p<0.05$) with increasing soil moisture availability from K1 to K3 (Table 3). Moisture regime (K) was the primary determinant of plant dry weight (K: $F(2,15) = 25.30$, $p<0.05$) and root dry weight (K: $F(2,15) = 14.90$, $p<0.05$). Variety main effects and V×K interactions were non-significant for all biomass parameters (Table 4).

Root volume expanded from 0.25 cm^3 at K1 to 1.23 cm^3 at K3 (well-watered reference), and root dry weight from 0.08 g to 0.29 g, reflecting improved root system development under greater water availability. Total plant dry weight increased from 0.20 g at K1 to 0.78 g at K3. Although absolute biomass values increased with FC, descriptive root:shoot (R:S) ratios were highest at K1 for both Sertani (0.50) and Trisakti (0.82) compared to K2 and K3, suggesting a relative shift in biomass partitioning toward root tissue under water-limited conditions, consistent with allometric partitioning theory (Eziz et al. 2017). This pattern should be interpreted cautiously as it is based on treatment means only and was not subjected to statistical testing. Trisakti showed numerically higher root volume (0.82 vs. 0.70 cm^3) and root dry weight (0.18 vs. 0.13 g) than Sertani, though these differences were not statistically significant (V: $F(1,15) = 2.15$, $p>0.05$). The absence of statistically significant inter-varietal differences across nearly all morpho-anatomical and biomass traits—despite numerical trends favoring Trisakti in root parameters—likely reflects in part the limited statistical power of the three-replication design, and should not be interpreted as evidence of physiological equivalence between the two cultivars.

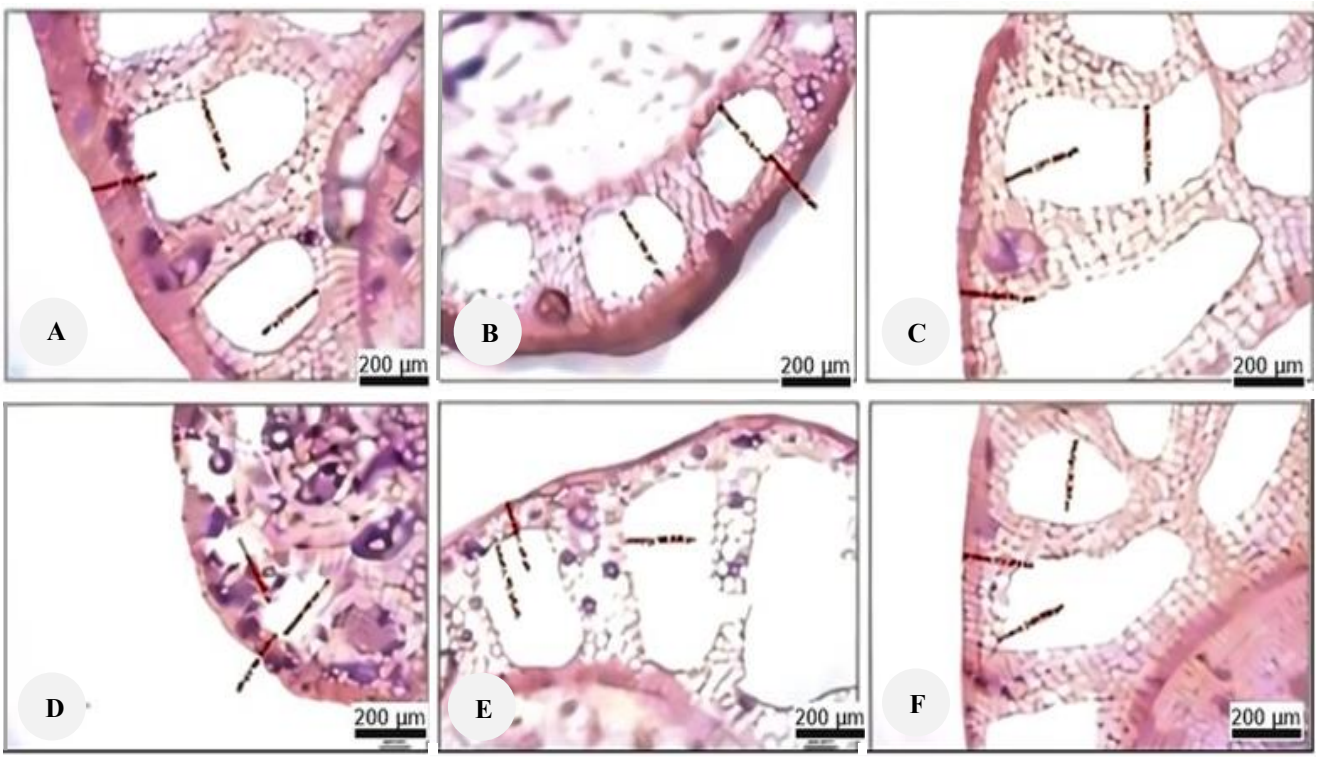

Figure 2. Root anatomical cross-sections. A. Sertani K1 (40% FC), B. Sertani K2 (60% FC), C. Sertani K3 (80% FC), D. Trisakti K1 (40% FC), E. K2 (60% FC), F. K3 (80% FC). Scale bar: 200 µm

Table 3. Root volume, plant dry weight, root dry weight, and root: Shoot ratio of upland rice under different varieties and soil moisture regimes

Parameter	Variety	K1 (40% FC)	K2 (60% FC)	K3 (80% FC)	Variety mean
Root volume (cm³)	Sertani	0.20±0.06	0.78±0.17	1.13±0.10	0.70a
	Trisakti	0.30±0.05	0.83±0.22	1.33±0.13	0.82a
	K Mean	0.25±0.03c	0.80±0.07b	1.23±0.05a	-
Plant dry weight (g)	Sertani	0.21±0.12	0.33±0.10	0.74±0.13	0.43
	Trisakti	0.20±0.08	0.31±0.09	0.81±0.30	0.44
	K Mean	0.20±0.03b	0.32±0.03b	0.78±0.08a	-
Root dry weight (g)	Sertani	0.07±0.04	0.08±0.03	0.23±0.10	0.13
	Trisakti	0.09±0.04	0.11±0.05	0.34±0.14	0.18
	K Mean	0.08±0.01b	0.10±0.01b	0.29±0.03a	0.16
Root:shoot ratio (descriptive)†	Sertani	0.50	0.32	0.45	0.42
	Trisakti	0.82	0.55	0.72	0.70
	K Mean	0.66	0.44	0.59	0.56

Note: DMRT letters (a, b, c) in the 'K Mean' rows compare moisture-level means within each trait; different letters indicate significant differences at the 5% level. Variety means followed by the same letter are not significantly different. Values for individual varieties are means ±SD of three replicates. † Root:Shoot Ratio is presented as descriptive evidence only (treatment means, n = 1 composite per treatment); no replicate-level statistical testing was performed

Table 4. ANOVA summary: p-values for variety (V), moisture regime (K), and V×K interaction effects for all response variables, with variety means

Trait	V (p)	K (p)	V×K (p)	Sertani mean	Trisakti mean
Leaf area	0.887 ns	<0.001 *	0.453 ns	8.26 cm²	8.10 cm²
Leaf thickness	0.906 ns	<0.001 *	0.531 ns	213.85 µm	215.15 µm
RCI	0.038 *	<0.001 *	0.537 ns	0.25	0.27
Bulliform cell length	0.362 ns	<0.001 *	0.424 ns	48.05 µm	45.12 µm
Bulliform cell width	0.630 ns	<0.001 *	0.960 ns	35.80 µm	34.53 µm
Stomatal length	0.726 ns	<0.001 *	0.854 ns	17.58 µm	17.71 µm
Stomatal width	0.393 ns	<0.001 *	0.303 ns	9.43 µm	9.06 µm
Stomatal density	0.576 ns	<0.001 *	0.208 ns	141.92 no./mm²	145.13 no./mm²
Root volume	0.308 ns	<0.001 *	0.801 ns	0.70 cm³	0.82 cm³
Plant dry weight	0.891 ns	<0.001 *	0.624 ns	0.43 g	0.44 g
Root dry weight	0.211 ns	<0.001 *	0.549 ns	0.13 g	0.18 g

Note: *p<0.05 (significant), ns: not significant (p>0.05). Table 4 presents ANOVA p-values for the full two-variety dataset; the Sertani Mean and Trisakti Mean columns show overall variety means across all FC levels for reference. p-values for V: F (1,15); K: F (2,15); V×K: F (2,15). Exact F-statistics reported in text per trait

In conclusion, soil moisture regime was the dominant driver of morpho-anatomical variation in Sertani and Trisakti upland rice at the vegetative stage, with increasing field capacity (40% to 80% FC, the well-watered reference) progressively enhancing leaf area, tissue thickness, stomatal dimensions and density, root volume, and total biomass ($p<0.05$); observed increases in stomatal dimensions and density reflect developmental plasticity during organogenesis rather than dynamic drought regulation. Both varieties responded similarly across nearly all measured traits, with the sole statistically significant genotypic difference observed in RCI, where Trisakti recorded higher values than Sertani ($F(1,15) = 5.41$, $p<0.05$); qualitative root cross-sections and descriptive root:shoot ratios provided supporting structural context but require quantitative morphometric and statistical confirmation in future work. These findings establish a preliminary morpho-anatomical baseline for both cultivars, pending validation through multi-location field trials, inclusion of reproductive-stage assessments, and studies with broader genotypic panels and greater replication.

ACKNOWLEDGEMENTS

The authors acknowledge the Faculty of Agriculture, Universitas Sumatera Utara, Indonesia, for providing greenhouse facilities. We also thank the Biotechnology Laboratory, Faculty of Agriculture, Universitas Sumatera Utara, for technical assistance and laboratory support.

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