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Image-based assessment of morphological responses and biomass allocation in cowpea seedlings: A methodological approach to drought resilience phenotyping

Avaliação de respostas morfológicas por análise de imagens e alocação de biomassa em plântulas de feijão-de-corda: Uma abordagem metodológica para a fenotipagem da resiliência à seca

Evaluación de respuestas morfológicas mediante análisis de imágenes y asignación de biomasa en plántulas de caupí: Un enfoque metodológico para la fenotipificación de la resiliencia a la sequía

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Abstract
Water deficit during the early development of cowpea (*Vigna unguiculata* (L.) Walp.) can compromise seedling establishment and reduce crop uniformity. This study aimed to evaluate morphological responses and biomass allocation in eight cowpea genotypes, including four commercial cultivars and four landraces, under two water conditions (control and deficit). A randomized block design was applied in a 2 × 8 factorial scheme. Morphological traits of roots and shoots, including length, surface area, volume, and diameter, were measured using image-based analysis. Dry biomass and root-to-shoot ratio were determined through gravimetric methods. Significant genotype-by-environment interactions were observed. Commercial cultivars tended to maintain structural attributes such as stem and root diameter, while landraces, particularly "Marronzinha" and "Verdinha", exhibited greater plasticity in root morphology and biomass accumulation under water restriction. Although the methodology allowed efficient early phenotyping, limitations such as the short stress duration and use of two-dimensional imaging may restrict broader inferences. Future studies should incorporate extended drought periods, field validation, and physiological assessments to enhance the identification of drought-resilient genotypes.
Keywords: *Vigna unguiculata* (L.) Walp.; Root length; Biomass partition; Climate change.

Resumo
O déficit hídrico durante o desenvolvimento inicial do feijão-de-corda (*Vigna unguiculata* (L.) Walp.) pode comprometer o estabelecimento das plântulas e reduzir a uniformidade da lavoura. Este estudo teve como objetivo avaliar as respostas morfológicas e a alocação de biomassa em oito genótipos de feijão-de-corda, incluindo quatro

cultivares comerciais e quatro acessos crioulos, sob duas condições hídricas (controle e déficit). Utilizou-se um delineamento em blocos casualizados, em esquema fatorial 2 × 8. As características morfológicas de raízes e parte aérea, como comprimento, área de superfície, volume e diâmetro, foram obtidas por meio de análise de imagens. A biomassa seca e a razão raiz/parte aérea foram determinadas por métodos gravimétricos. Foram observadas interações significativas entre genótipo e ambiente. As cultivares comerciais tenderam a manter atributos estruturais, como o diâmetro de raiz e caule, enquanto os acessos crioulos, especialmente "Marronzinha" e "Verdinha", apresentaram maior plasticidade morfológica e acúmulo de biomassa sob restrição hídrica. Embora a metodologia tenha permitido uma fenotipagem eficiente em estádios iniciais, limitações como o curto período de estresse e o uso de imagens bidimensionais podem restringir inferências mais amplas. Estudos futuros devem considerar períodos mais longos de déficit, validações em campo e avaliações fisiológicas para aprimorar a seleção de genótipos resilientes à seca.
Palavras-chave: *Vigna unguiculata* (L.) Walp.; Comprimento radicular; Partição de biomassa; Mudanças climáticas.

Resumen
El déficit hídrico durante el desarrollo inicial del caupí (*Vigna unguiculata* (L.) Walp.) puede comprometer el establecimiento de plántulas y reducir la uniformidad del cultivo. Este estudio tuvo como objetivo evaluar las respuestas morfológicas y la asignación de biomasa en ocho genotipos de caupí, incluidos cuatro cultivares comerciales y cuatro accesiones criollas, bajo dos condiciones hídricas (control y déficit). Se utilizó un diseño en bloques al azar con un esquema factorial 2 × 8. Los atributos morfológicos de raíces y parte aérea, como longitud, área superficial, volumen y diámetro, se evaluaron mediante análisis de imágenes. La biomasa seca y la relación raíz/parte aérea se determinaron mediante métodos gravimétricos. Se observaron interacciones significativas entre genotipos y condiciones hídricas. Los cultivares comerciales tendieron a mantener atributos estructurales, como el diámetro de raíz y tallo, mientras que las accesiones criollas, especialmente "Marronzinha" y "Verdinha", mostraron mayor plasticidad morfológica y acumulación de biomasa bajo restricción hídrica. Aunque la metodología permitió una fenotipificación eficiente en etapas tempranas, limitaciones como la corta duración del estrés y el uso de imágenes bidimensionales pueden restringir inferencias más amplias. Estudios futuros deberían incluir períodos de estrés más prolongados, validaciones en campo y evaluaciones fisiológicas para mejorar la identificación de genotipos resistentes a la sequía.
Palabras clave: *Vigna unguiculata* (L.) Walp.; Longitud de raíz; Partición de biomasa; Cambio climático.

1. Introduction

Among cultivated grain legumes, cowpea (*Vigna unguiculata* (L.) Walp.) stands out as a vital crop for semi-arid regions due to its inherent drought tolerance, high nutritional value, and capacity to form symbiotic associations with nitrogen-fixing bacteria (Omomowo & Babalola, 2021). As such, this species plays a crucial role in promoting food security, particularly within smallholder farming systems across various tropical and subtropical countries (Santos et al., 2023). However, despite its adaptive potential, cowpea productivity is often constrained by irregular rainfall patterns and extended periods of water scarcity, challenges that are especially pronounced in key production areas such as Brazil (Almeida et al., 2017).

Expanding on this, the seedling stage represents a particularly vulnerable phase in the cowpea life cycle, exhibiting heightened sensitivity to drought stress. Thus, water scarcity during this critical period can severely restrict root development, hinder seedling establishment, and ultimately lead to stand failure or necessitate reseeding (Agbicodo et al., 2009; Goufo et al., 2017). These establishment failures are especially detrimental in smallholder farming systems, as they reduce plant population , diminish yield potential, and escalate production costs. Furthermore, the increasing frequency and intensity of drought events, driven by climate change, have amplified these constraints, highlighting need for the development of cowpea cultivars with enhanced resilience to water deficit during the early stages of growth (Farooq et al., 2022; Martínez-Nieto et al., 2022).

Alongside these considerations, root system architecture (RSA) has emerged as a critical morphological attribute to predict crops drought adaptation (Afonso et al., 2025; Barros et al., 2021). RSA encompasses traits such as root length, diameter, and volume, which directly influence a plant's capacity to explore deeper soil layers and access water and nutrients. Moreover, roots are the first organs to detect changes in water availability, triggering adaptive responses that include morphological, physiological, and biochemical adjustments (Kalra et al., 2024). Studies on grain legumes have confirmed the central role of RSA in drought resilience, enabling plants to survive water-limited conditions by developing deeper and more efficient root systems (Umburanas et al., 2019). These findings highlight the importance of root phenotyping as a strategic tool for identifying genotypes with improved drought tolerance, particularly in legumes, where studies on root traits remain scarce (Jarvonik et al., 2025; Umburanas et al., 2019; Wang et al., 2024).

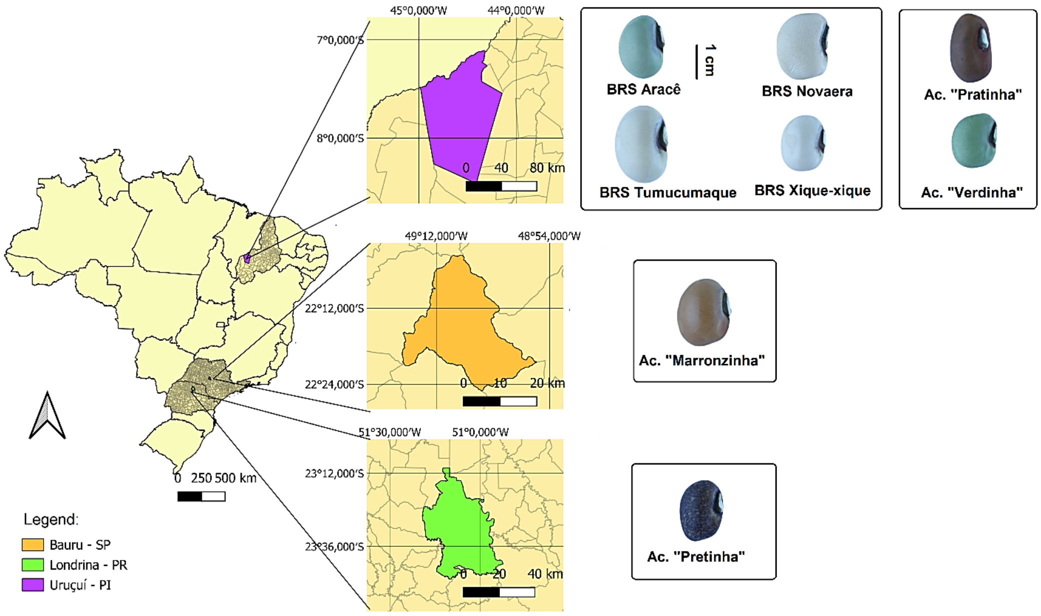
In this context, evaluating cowpea performance under controlled drought conditions during the early developmental stages could be a promising way for identifying morphological traits linked to this stress tolerance. Our hypothesis is that, when combined with standardized experimental protocols, high replication, and digital image analysis, these evaluations can support the development of robust and scalable phenotyping platforms. Tus, these methodologies are especially valuable for screening and selecting drought-resilient varieties early in breeding programs, where precision and time efficiency are essential (Hall, 2012; Muchero et al., 2013). Furthermore, this approach generates reliable phenotypic data that can be integrated with genotypic information, thereby supporting genomic selection strategies to accelerate genetic gains and enhance crop breeding. This study aimed to evaluate morphological responses and biomass allocation in eight cowpea genotypes, including four commercial cultivars and four landraces, under two water conditions (control and deficit). A randomized block design was applied in a 2 × 8 factorial scheme.

2. Material and Methods

A mixed research was carried out, partly in the field and partly in the laboratory, in a mixed investigation: qualitative and quantitative in nature (Pereira et al., 2018) with the use of statistical analysis (Vieira, 2021). The present study was conducted at the Seed Analysis Laboratory of the "Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP), located in Piracicaba, São Paulo, Brazil (22° 42′ S, 47° 38′ W; altitude 546 m). A randomized complete block design was adopted, arranged in a 2 × 8 factorial scheme with 70 replications. In this context, each experimental unit consisted of a single seedling, a strategy aimed at enhancing the precision in detecting the effects of water deficit on the morphological traits of the evaluated varieties. Moreover, the use of individual seedlings as replicates is a well-established practice in seed analysis experiments and phenotyping studies for plant breeding programs, as reported by Umburanas et al. (2019). Regarding the treatment structure, the first factor (W) comprised two levels of water availability, based on the water-holding capacity of the substrate: 30% (water deficit) and 60% (control treatment), respectively. It is important to emphasize that water restriction was applied only at the beginning of the experiment (day zero), with no further irrigation throughout the experimental period. The second factor (V) consisted of eight cowpea (*V. unguiculata*) varieties, including four commercial cultivars (BRS Aracê, BRS Novaera, BRS Xique-xique, and BRS Tumucumaque) and four landraces ("Verdinha", "Pratinha", "Marronzinha", and "Pretinha") (Figure 1).

Sowing was carried out in plastic trays (46 × 28 × 9 cm) filled with medium-textured sand (2 mm particle size), using 50 seeds per tray, totaling 100 seeds per treatment. Thus, for the control treatment, the substrate's water-holding capacity was adjusted to 60%, the level recommended for the seedling emergence test of *V. unguiculata*, according to the Brazilians Rules for Seed Testing (BRASIL, 2009). Thus, seeds were sown on July 22, 2024, in Piracicaba, São Paulo, Brazil, under partially controlled environmental conditions. After ten days, destructive sampling of the seedlings was conducted: the substrate was saturated with water to facilitate the complete removal of the shoot and root systems. For subsequent analyses, 70 normal seedlings were randomly selected per treatment (totaling 1120 experimental units). The criteria for defining a normal seedling, as well as the selection of 70 seedlings (the minimum germination standard required for the species), followed the guidelines of BRASIL (2009), as established by the Brazilian Ministry of Agriculture (MAPA).

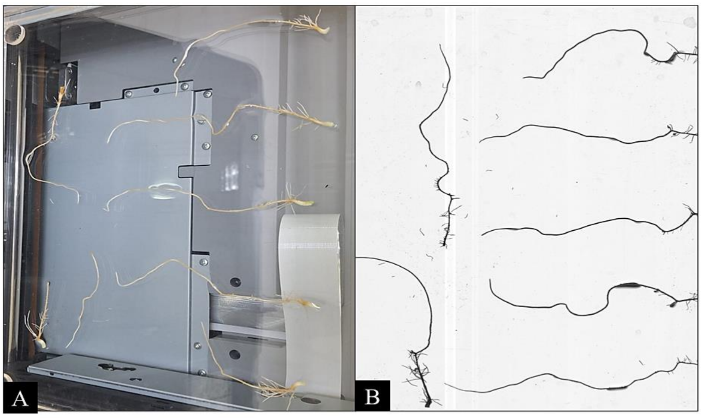
Figure 1. Representation of the production site and visual characteristics of the seeds of each cowpea variety used in this experiment.



Source: Authors (2025).

The root systems were then scanned at 300 dpi resolution using an Epson XL 10000 scanner. During this process, both the root and shoot systems remained submerged in water within an acrylic container (Figure 2). The images were processed and analyzed using WinRhizo® software, version 4.1c (Regent Instruments Inc., Sainte-Foy, QC, Canada), following the methodology described by Bouma et al. (2000), with adaptations by Umburanas et al. (2019). Root structure identification was based on the thresholding technique, in which dark pixels corresponding to the roots are distinguished from a white background. Through this segmentation, WinRhizo® quantifies root volume by integrating morphological parameters such as diameter and length, measured continuously along each root segment. Similarly, for the shoot system, the software analyzes plant structures as image objects, extracting two-dimensional parameters such as height, width, and area. These parameters are then used to derive or estimate stem volume, often through the application of standard geometric models.

Figure 2. Roots prepared to be digitized into images (A) and analysis of the images by WinRhizo® software version 4.1c (B).



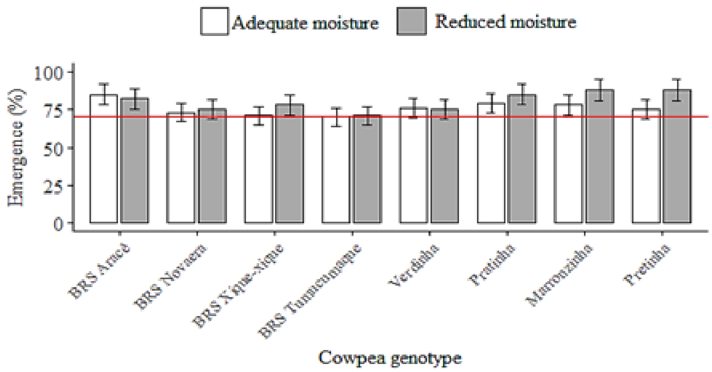
Source: Authors (2025).

Accordingly, the following root attributes were evaluated: primary root length, surface area, volume, and diameter. For the shoot system, aboveground stem length, diameter, and volume were measured. After these assessments, the cotyledons were discarded, and the remaining plant material was oven-dried at 65 °C for 72 hours in a forced-air circulation chamber to determine the dry biomass of the root, shoot, and total seedling. Based on these measurements, the biomass partitioning between root and shoot was also estimated. Regarding statistical analyses, the dataset normality was assessed using the Lilliefors test at 5% ($p \leq 0.05$), recommended for large sample sizes as in the present study ($n = 1120$). To achieve normal distribution, the Box-Cox transformation was applied to the variables primary root length, projected root area, root surface area, and aboveground stem length. After that, the homogeneity of variances was tested using Bartlett's test, also at a 5% significance level. Once the assumptions of normality and homoscedasticity were met, an analysis of variance (ANOVA) was performed, likewise adopting a 5% significance threshold. When ANOVA indicated significant treatment effects, means were compared using Tukey's test at a 5% ($\alpha = 0.05$). All statistical analyses were conducted using "Agicolae package" from RStudio® software (4.3.2).

3. Results

All evaluated varieties presented germination rates above 70%, thus complying with the minimum standards established by the Seeds analysis rules (BRASIL, 2009) (Figure 3). In this manner, the analysis of variance (ANOVA) indicated statistically significant effects of water availability (W) on seven out of twelve traits: root diameter (RD), root volume (RV), shoot length (SL), shoot volume (SV), root dry matter (RDM), shoot dry matter (SDM), and total dry matter (TDM). Additionally, significant effects were observed for variety (V) and for the interaction between water availability and variety (W × V) across all assessed variables (Table 1). Water supply alone influenced root system development, shoot architecture, and biomass accumulation. The variety effect and its interaction with hydric conditions could suggest distinct physiological responses to water deficit, suggesting variability in drought tolerance among the materials evaluated.

Figure 3. Percentage of cowpea seedling emergence as a function of water availability. The seeds germination percentage meets the 70% minimum recommended for the species according to the Seed Analysis Rules (BRASIL, 2009). Bars mean average deviation.



Source: Research data (2025).

Under adequate moisture conditions (60% capacity), the landraces "Pratinha" (35.7 cm), "Pretinha" (28.5 cm), and "Marronzinha" (25.8 cm) exhibited the greatest primary root lengths (Figure 4A). When exposed to water restriction (30%), the values for "Marronzinha", "Pretinha", BRS Xique-xique, and BRS Tumucumaque remained statistically equivalent to those under ideal conditions. In contrast, "Pratinha" experienced a 30.8% reduction. On the other hand, BRS Aracê, BRS Novaera, and "Verdinha" recorded increases of 47.6%, 26.5%, and 17.8%, respectively. Only three varieties presented significant root elongation under drought, while "Pratinha" was the most sensitive. Rroot surface area followed a similar pattern. Under optimal water availability, "Pratinha" recorded the highest value (8.2 cm²), followed by "Marronzinha" (7.4 cm²) and "Pretinha" (6.6 cm²) (Figure 4B). Under drought, "Marronzinha" retained a high surface area (6.7 cm²), while "Pratinha" decreased by 32.4%.

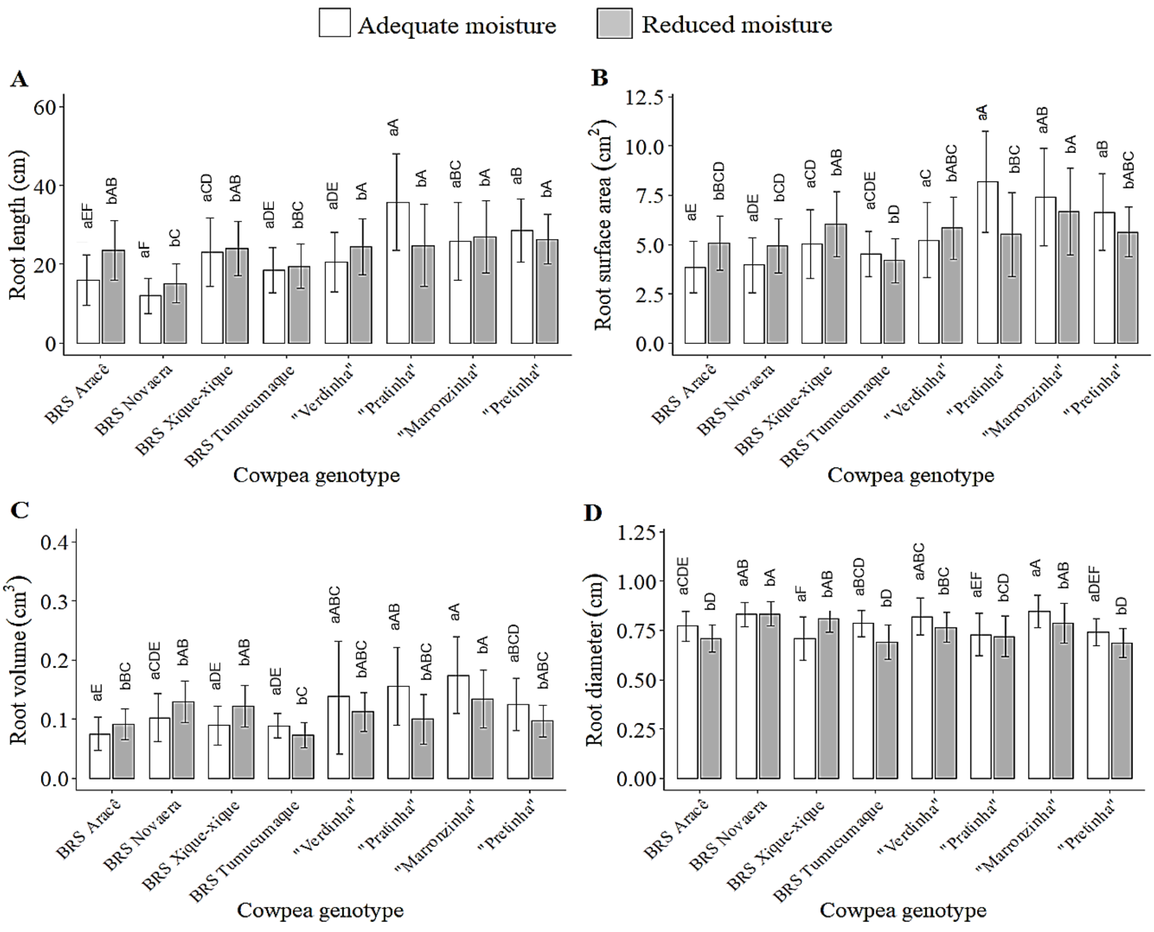
BRS Novaera, BRS Xique-xique, BRS Tumucumaque, and "Verdinha" maintained or increased surface area under stress.

Table 1. Summary of the analysis of variance (ANOVA) for the effects of water availability (W), cowpea varieties (V), and their interaction (W × V) on root traits, shoot traits and biomass allocation. Values correspond to *p*-values for each source of variation associated with the evaluated traits.

Variable	Water avaibility (W)	Varieties (V)	W x V	CV (%)
RL	0.2918 ns	< 0.0001**	< 0.0001**	34.30
RPA	0.0908 ns	< 0.0001**	< 0.0001**	30.68
RSA	0.3682 ns	< 0.0001**	< 0.0001**	31.62
RD	< 0.0001**	< 0.0001**	< 0.0001**	10.90
RV	0.0157*	< 0.0001**	< 0.0001**	38.53
SL	< 0.0001**	< 0.0001**	< 0.0001**	14.52
SSA	0.8701 ns	< 0.0001**	< 0.0001**	14.56
SV	0.0459*	< 0.0001**	< 0.0001**	16.84
RDM	0.0188*	< 0.0001**	< 0.0001**	36.42
SDM	0.0103*	< 0.0001**	< 0.0001**	19.33
TDM	0.0069**	< 0.0001**	< 0.0001**	19.94
R/S	0.1462 ns	< 0.0001**	< 0.0001**	27.11

Note: ns: not significant ($p > 0.05$); *: significant at the 5% level ($p \leq 0.05$); **: significant at the 1% level ($p \leq 0.01$). RL: root length; RPA: root projected area; RSA: root surface area; RD: root diameter; RV: root volume; SL: shoot length; SSA: shoot surface area; SV: shoot volume; RDM: root dry matter; SDM: shoot dry matter; TDM: total dry matter; R/S: root-to-shoot dry matter ratio.
Source: Research data (2025).

Figure 4. Mean root traits of cowpea seedlings as a function of varieties and water availability: root length (A), root surface area (B), root volume (C), and root diameter (D). Lowercase letters indicate comparisons among varieties within each water condition, whereas uppercase letters denote comparisons between water conditions within the same variety. The lowercase symbols preceding Tukey's significance letters represent moisture levels: "a" corresponds to adequate moisture (60%) and "b" to reduced moisture (30%). Means followed by the same letter do not differ statistically by Tukey's test ($p < 0.05$). Bars represent mean deviation.



Source: Research data (2025).

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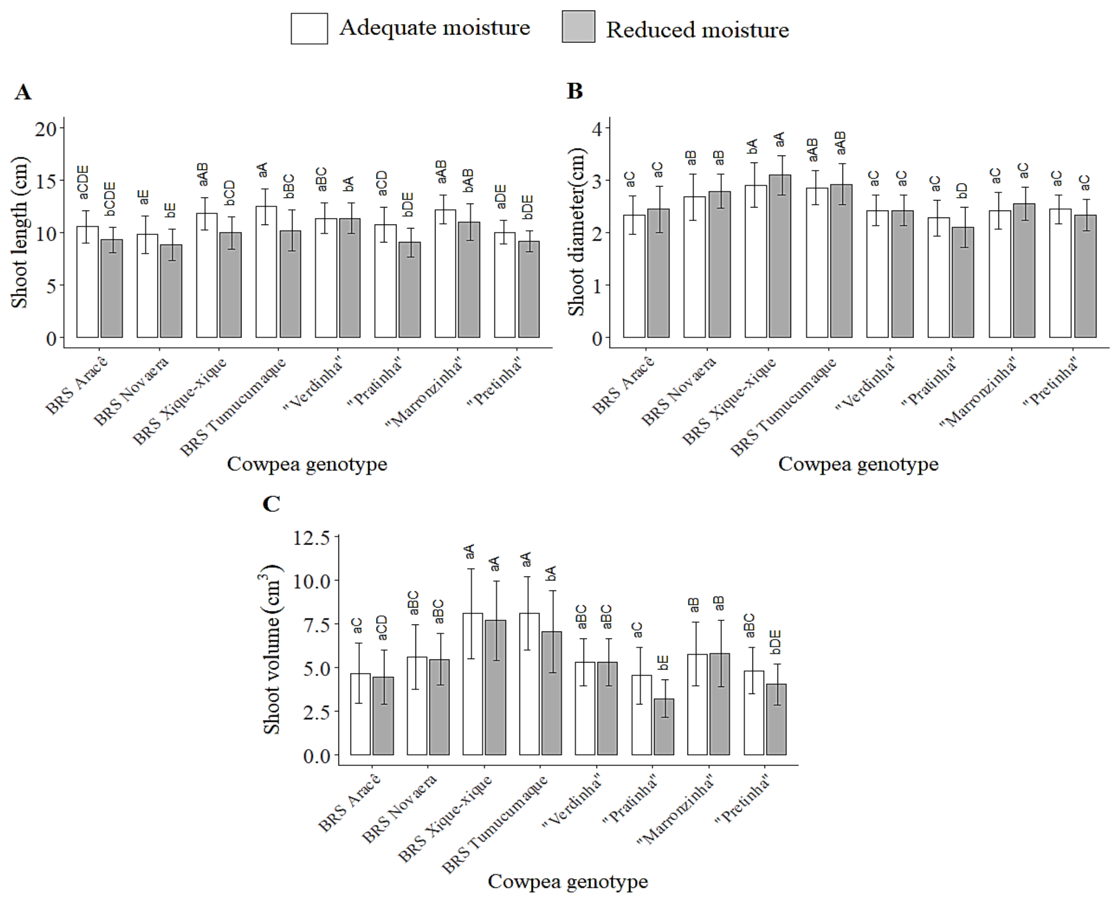
Thereafter, under 60% moisture, “Marronzinha” (0.173 cm³) and “Pratinha” (0.155 cm³) registered the highest’s root volumes. Under water deficit, “Marronzinha” and BRS Novaera maintained relatively high volumes (0.133 and 0.129 cm³), whereas “Pratinha” declined to 0.100 cm³. The lowest volumes were associated with BRS Xique-xique (0.073 cm³) and BRS Aracê (0.077 cm³). While reductions were observed in “Marronzinha” and “Pratinha”, BRS Novaera showed an increase of approximately 26%. Root diameter also varied significantly according to the interaction between water availability and variety (Figure 4D). Under well-watered conditions, “Marronzinha” (0.85 cm), BRS Novaera (0.83 cm), and “Verdinha” (0.82 cm) recorded the largest diameters. Under drought, BRS Novaera and BRS Tumucumaque maintained relatively high values, while BRS Xique-xique and “Pretinha” recorded the lowest (0.69 cm). Other varieties, such as “Marronzinha”, “Verdinha”, and “Pratinha”, occupied intermediate positions. Some varieties showed reductions under water deficit, while BRS Tumucumaque presented an increase. In terms of shoot length (Figure 5A), under adequate moisture, BRS Tumucumaque recorded the highest average (12.5 cm), followed by BRS Xique-xique and “Marronzinha”. BRS Novaera consistently presented the lowest values under both conditions. Under drought, “Verdinha” reached the greatest height (11.4 cm), followed by “Marronzinha”. In general, shoot elongation was reduced in most varieties under limited water supply. Stem diameter remained statistically similar among varieties under optimal conditions, with values ranging from 2.5 to 3.0 cm. However, under water restriction, the interaction effect was evident (Figure 5B). BRS Xique-xique and BRS Tumucumaque registered the highest averages (~3.3 cm), while “Pratinha” recorded the smallest (2.1 cm). The remaining varieties showed intermediate values without significant differences.

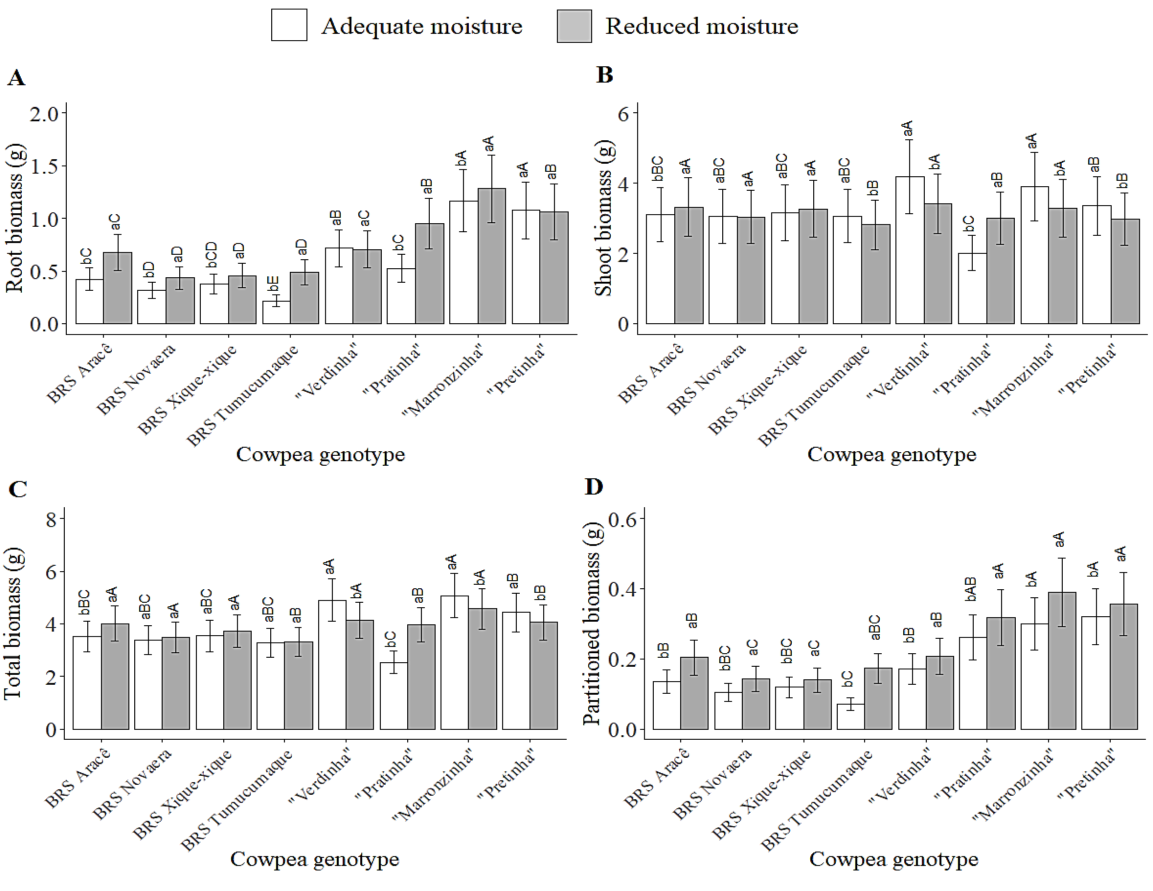
Figure 5. Mean stem traits of cowpea seedlings according to varieties and water availability: stem length (A), stem diameter (B), and stem volume (C). Means followed by the same uppercase letters for water conditions and lowercase letters for varieties do not differ according to Tukey’s test ($p < 0.05$). Bars mean average deviation.



Source: Research data (2025).

Regarding shoot volume (Figure 5C), BRS Tumucumaque (~9.8 cm³) and BRS Xique-xique (~9.5 cm³) recorded the highest mean in both water conditions, significantly surpassing the other varieties. "Pratinha" consistently presented the lowest volume (~4.0 cm³), while BRS Aracê remained among the least vigorous (~5.0 cm³) under adequate moisture. Subsequently, considering root dry matter (Figure 6A), "Marronzinha" accumulated the highest values under both conditions (~1.6 g and ~1.5 g), followed by "Pretinha" under adequate moisture (~1.3 g). "Pratinha" presented intermediate values (~1.0 g), while BRS Tumucumaque recorded the lowest (~0.3 g). Under drought, BRS Novaera and BRS Xique-xique also showed reduced root biomass. In this regard, Shoot dry mass (Figure 6B) was higher in BRS Aracê, BRS Novaera, BRS Xique-xique, "Verdinha", and "Marronzinha" (4.0–5.0 g) under water restriction. Conversely, BRS Tumucumaque (~3.0 g), "Pratinha" (~3.5 g), and "Pretinha" (~3.7 g) presented lower values.

Figure 6. Mean biomass production of cowpea seedlings according to varieties and water availability: root biomass (A), shoot biomass (B), total biomass (C), and root-to-shoot biomass partitioning (D). Means followed by the same uppercase letters for water conditions and lowercase letters for varieties do not differ according to Tukey's test ($p < 0.05$). Bars mean average deviation.



Source: Research data (2025).

Thus, the landraces "Marronzinha", "Verdinha", and "Pretinha" accumulated the highest vaulues for total dry matter (~6.0 g), regardless of water regime. These results suggests strong maintenance of growth and high adaptive capacity under drought. "Pratinha" recorded intermediate values but experienced a significant reduction under stress. BRS Tumucumaque obtained the lowest totals. The commercial varieties BRS Aracê, BRS Novaera, and BRS Xique-xique presented intermediate performance (~4.0 g), without significant differences. In sequence, regarding the root-to-shoot ratio, the highest values under drought (~0.40) were observed in the landraces "Marronzinha", "Pretinha", and "Pratinha", statistically differing from most

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commercial cultivars. This result suggests greater functional plasticity, with preferential biomass allocation to the root system under hydric stress. In contrast, BRS Novaera, BRS Xique-xique, and BRS Tumucumaque recorded the lowest ratios (<0.20), independent of moisture conditions. On the other hand, BRS Aracê and "Verdinha" exhibited intermediate values.

4. Discussion

The results of this study highlight significant variability among cowpea genotypes in their morphological and biomass allocation responses to early water deficit, particularly regarding root system traits. Landraces such as "Marronzinha" and "Verdinha" demonstrated greater resilience through the maintenance or even enhancement of root length, surface area, and total biomass under drought, whereas commercial cultivars such as BRS Tumucumaque and BRS Aracê showed marked sensitivity, especially in root volume and shoot development. The enhanced primary root length observed in BRS Aracê, BRS Novaera, and "Verdinha" under stress may reflect a greater morphological plasticity associated with drought tolerance, enabling deeper substrate exploration. This adaptive response aligns with previous findings in soybean by Bukan et al. (2024), where drought stress more severely affected shoot growth than root development, thereby increasing the root-to-shoot ratio as a functional adaptation. Conversely, the reduction in primary root length in "Pratinha" suggests lower drought tolerance, contrasting with the stability observed in "Marronzinha" and "Pretinha". Similar patterns have been reported in common bean (*Phaseolus vulgaris* L.) genotypes exhibiting drought-resilient traits (Javornik et al., 2025; Zheng et al., 2023).

However, it is important to recognize that our findings diverge from those of Kashiwagi et al. (2015), who observed limited variability in root elongation among chickpea genotypes subjected to early drought stress, suggesting a more conservative growth strategy. Likewise, Matsui and Singh (2003) reported that in cowpea, shoot attributes were more reliable indicators of drought tolerance than root traits during early growth stages. These discrepancies highlight the complex and species-dependent nature of drought responses, as well as the influence of experimental conditions and genotypic background.

In our study, root surface area and volume showed high plasticity under drought in some genotypes (e.g., BRS Novaera and "Marronzinha"), suggesting efficient resource reallocation to maintain root exploration under restrictive conditions. The observed increase in root volume under drought in BRS Novaera contrasts with the decline in "Pratinha", reinforcing the importance of genotype-specific strategies. These patterns align with observations in soybean and common bean, where plastic root systems are often linked to greater drought resilience (Silveira et al., 2024; Afonso et al., 2025).

Root diameter responses were also genotype-dependent. BRS Novaera maintained diameter under both conditions, while BRS Xique-xique increased this trait under drought, possibly as a mechanism to strengthen vascular tissues and enhance water transport, as suggested by Taiz et al. (2017) and corroborated in legumes by Javornik et al. (2025). Although root diameter alone is not a decisive trait, its stability or increment under stress may represent an important secondary indicator of adaptation.

Regarding shoot traits, BRS Tumucumaque exhibited superior stem elongation under well-watered conditions, while "Verdinha" maintained shoot growth even under drought, suggesting morphological flexibility. In contrast, genotypes such as BRS Novaera showed limited shoot development under both conditions. The general reduction in shoot length under drought may be linked to decreased turgor pressure and inhibited auxin synthesis, limiting meristematic activity (Wang et al., 2022). This trend aligns with Bukan et al. (2024), who also reported greater shoot sensitivity in soybean under osmotic stress.

Stem diameter responses also reflected adaptive strategies. BRS Xique-xique and BRS Tumucumaque maintained or increased diameter under drought, suggesting structural reinforcement for vascular conductivity. Conversely, "Pratinha" exhibited significant reductions, indicating higher stress susceptibility. These contrasting responses support the use of stem diameter as a complementary trait in drought resilience screening (Afonso et al., 2025; Silva et al., 2021).

Biomass allocation patterns further underscore the adaptive potential of landraces. "Marronzinha" demonstrated the highest root biomass in both treatments, indicating a preferential investment in belowground structures under drought, a trait previously identified as critical for legume drought adaptation (Bukan et al., 2024; Afonso et al., 2025). In contrast, BRS Tumucumaque showed the lowest values, evidencing its limited ability to allocate biomass to the root system under stress. Shoot biomass was more stable in genotypes such as BRS Aracê, BRS Novaera, and "Verdinha", supporting their ability to sustain vegetative growth under adverse conditions.

The root-to-shoot ratio proved to be a reliable indicator of drought adaptation. Landraces such as "Marronzinha", "Pretinha", and "Pratinha" showed higher ratios under drought, indicating functional plasticity and preferential biomass allocation to roots. Conversely, the commercial cultivars BRS Novaera, BRS Xique-xique, and BRS Tumucumaque showed lower ratios, suggesting reduced adaptive capacity. These findings are consistent with those reported by Bukan et al. (2024), who observed increased root-to-shoot ratios in drought-tolerant soybean genotypes. Despite these insights, this study presents some limitations. First, the duration of exposure to water deficit was relatively short (10 days), which may not fully capture the long-term physiological adjustments and recovery potential of the genotypes. Second, although image-based analysis provides high-throughput and non-destructive assessments of morphological traits, it is limited to two-dimensional projections and may underestimate complex root architecture features, such as branching patterns or deep vertical growth. Future research should consider integrating three-dimensional imaging techniques, longer drought cycles, and physiological measurements (e.g., stomatal conductance, water use efficiency) to provide a more comprehensive evaluation of drought adaptation strategies (Zegaoui et al., 2017).

Taken together, the results support our initial hypothesis that integrating standardized experimental protocols, high replication, and image-based morphological analysis enables the development of robust and scalable phenotyping platforms for cowpea. These methodologies proved effective in detecting intra-specific variability under early drought stress and allowed precise quantification of morphological traits and biomass allocation patterns. By facilitating the rapid identification of drought-resilient genotypes (especially landraces with adaptive root traits) this approach offers a promising tool for accelerating selection cycles in breeding programs. Hence, digital phenotyping applied at early growth stages provides both the resolution and efficiency required to advance genetic gains toward climate-resilient cultivars.

5. Conclusion

This study suggests that combining standardized protocols, high replication, and image-based analysis can contribute to early-stage identification of drought-resilient cowpea genotypes. Landraces such as "Marronzinha" and "Verdinha" appeared to exhibit greater morphological plasticity and biomass allocation under water deficit, which could indicate adaptive potential. However, some methodological limitations must be considered. The short duration of drought exposure may not fully capture long-term adaptive responses, and the use of two-dimensional imaging restricts the assessment of complex root architecture, such as depth and branching patterns. These factors may limit extrapolations to field conditions and later growth stages. Future studies should incorporate longer stress periods, field-based trials, and advanced three-dimensional imaging techniques to improve the understanding and selection of drought-resilient genotypes in cowpea.

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