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Evaluación de la tolerancia a la sequía en especies cultivadas y parientes silvestres del frijol (Phaseolus vulgaris L.) mediante caracteres morfológicos y pigmentos fotosintéticos

Evaluation of drought tolerance in cultivated species and wild relatives of beans (Phaseolus vulgaris L.) using morphological characters and photosynthetic pigments

Avaliação da tolerância à seca em espécies cultivadas e parentes silvestres de feijoeiro (Phaseolus vulgaris L.) utilizando caracteres morfológicos e pigmentos fotossintéticos

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Ciencias Técnicas y Aplicadas
Artículo de Investigación

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Resumen

La sequía es una limitación crítica para producir frijol (Phaseolus vulgaris L.). Este estudio evaluó la tolerancia a la sequía en genotipos de frijol cultivado y silvestre utilizando caracteres morfológicos y pigmentos fotosintéticos como biomarcadores. La longitud y diámetro del tallo (SL, SD), el número de hojas (NL), el peso fresco de hojas, tallo y raíces (FWL, FWS, FWR), el contenido de agua en el tejido (WCL, WCS, WCR) y la longitud de la raíz, así como los contenidos de clorofila a, clorofila b y carotenoides totales se analizaron bajo tres tratamientos de estrés hídrico: Control, estrés hídrico leve (WS 50%), estrés hídrico severo (WS 0%). Los resultados mostraron diferencias significativas entre genotipos para la mayoría de los caracteres morfológicos. SL y SD se mantuvieron estables bajo estrés, mientras que NL, FWL, FWS y FWR se redujeron significativamente bajo estrés severo (WS 0%), pero no bajo estrés leve (WS 50%), lo que indica un umbral de respuesta. Vigna unguiculata presentó un mayor contenido de agua en los tejidos, lo que sugiere una estrategia de mantenimiento de la turgencia, mientras que 'Frejol negro' mostró raíces más largas y mayor número de hojas, lo que indica una estrategia de evitación de la sequía. Si bien los pigmentos fotosintéticos no variaron significativamente bajo estrés moderado, el estrés severo (WS 0%) provocó una reducción drástica de la clorofila a (72,7%) y b (68,9%), pero un aumento considerable de los carotenoides, lo que sugiere la activación de mecanismos fotoprotectores. 'Frejol negro' presentó las mayores concentraciones de carotenoides. En conclusión, la tolerancia a la sequía en frijol es multifacética, con genotipos que exhiben estrategias diferenciadas de evitación de la sequía, mantenimiento de la turgencia y umbrales de respuesta a la intensidad del estrés. Estos hallazgos son valiosos para la selección de genotipos resilientes en programas de mejoramiento de frijol.

Palabras clave: Phaseolus vulgaris; sequía; tolerancia; caracteres morfológicos; pigmentos fotosintéticos; biomarcadores

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Abstract

Drought is a critical constraint to produce beans (Phaseolus vulgaris L.). This study evaluated drought tolerance in cultivated and wild bean genotypes using morphological characters and photosynthetic pigments as biomarkers. Stem length and diameter (SL, SD), number of leaves (NL), fresh weight of leaves, stem and roots (FWL, FWS, FWR), tissue water content (WCL, WCS, WCR) and root length, as well as chlorophyll a, chlorophyll b and total carotenoid contents were analyzed under three water stress treatments: Control, mild water stress (WS 50%), severe water stress (WS 0%). The results showed significant differences between genotypes for most morphological traits. SL and SD were stable under stress, while NL, FWL, FWS and FWR were significantly reduced under severe stress (WS 0%), but not under mild stress (WS 50%), indicating a response threshold. Vigna unguiculata exhibited higher water content in tissues, suggesting a turgor maintenance strategy, while 'Frejol negro' showed longer roots and a greater number of leaves, indicating a drought avoidance strategy. Although photosynthetic pigments did not vary significantly under moderate stress, severe stress (WS 0%) caused a drastic reduction in chlorophyll a (72.7%) and b (68.9%), but a considerable increase in carotenoids, suggesting the activation of photoprotective mechanisms. 'Frejol negro' had the highest concentrations of carotenoids. In conclusion, drought tolerance in beans is multifaceted, with genotypes exhibiting differentiated strategies of drought avoidance, turgor maintenance, and stress intensity response thresholds. These findings are valuable for the selection of resilient genotypes in bean breeding programs.

Keywords: Phaseolus vulgaris; drought; tolerance; morphological characters; photosynthetic pigments; biomarkers.

Resumo

A seca é uma restrição crítica para a produção de feijão (Phaseolus vulgaris L.). Este estudo avaliou a tolerância à seca em genótipos de feijão cultivado e selvagem utilizando caracteres morfológicos e pigmentos fotossintéticos como biomarcadores. O comprimento e diâmetro do caule (SL, SD), o número de folhas (NL), o peso fresco das folhas, caule e raízes (FWL, FWS, FWR), o teor de água no tecido (WCL, WCS, WCR) e o comprimento da raiz, bem como os teores de clorofila a, clorofila b e carotenóides totais foram analisados sob três tratamentos de stress hídrico: Controlo, stress hídrico ligeiro (WS 50%), stress hídrico severo (WS 0%). Os resultados mostraram diferenças significativas entre genótipos para a maioria das características morfológicas. O SL e o SD foram estáveis sob stress, enquanto o NL, FWL, FWS e FWR foram significativamente reduzidos sob stress severo (WS 0%), mas não sob stress moderado (WS 50%), indicando um limiar de resposta. A Vigna unguiculata apresentou um maior teor de água nos tecidos, sugerindo uma estratégia de manutenção do turgor, enquanto o ‘Frejol negro’ apresentou raízes mais compridas e um maior número de folhas, indicando uma estratégia de prevenção da seca. Embora os pigmentos fotossintéticos não tenham variado significativamente sob stress moderado, o stress severo (WS 0%) provocou uma redução drástica da clorofila a (72,7%) e b (68,9%), mas um aumento considerável dos carotenóides, sugerindo a ativação de mecanismos fotoprotetores. O 'Frejol negro' apresentou as concentrações mais elevadas de carotenóides. Em conclusão, a tolerância à seca em feijoeiros é multifacetada, com os genótipos a exibirem estratégias diferenciadas de prevenção da seca, manutenção do turgor e limiares de resposta à intensidade do stress. Estes achados são valiosos para a seleção de genótipos resilientes em programas de melhoramento genético do feijoeiro.

Palavras-chave: Phaseolus vulgaris; seca; tolerância; caracteres morfológicos; pigmentos fotossintéticos; biomarcadores.

Introduction

The common bean (*Phaseolus vulgaris* L.) is a legume of vital global importance, constituting a fundamental source of protein, carbohydrates, fiber and micronutrients for millions of people, especially in developing countries (Celmeli et al., 2018). However, the productivity of this crop is seriously compromised by various abiotic factors, with drought being one of the most limiting factors worldwide (Ray et al., 2020). Water stress negatively affects the growth and development of bean plants, altering key physiological and biochemical processes, resulting in significant reductions in biomass and grain yield (Emam et al., 2012).

The response of plants to drought stress is complex and involves a series of adaptation mechanisms at the morphological, physiological and biochemical level (Nour et al., 2024). In *Phaseolus vulgaris*, drought tolerance is a polygenic trait that involves the interaction of multiple genes and metabolic pathways (Celmeli et al., 2018). The selection of tolerant genotypes is crucial to ensure food security in climate change scenarios, which predict a higher frequency and intensity of drought events (Yadav et al., 2018).

In this context, the evaluation of drought tolerance in beans has focused on the identification of reliable biomarkers that allow a rapid and efficient differentiation between sensitive and tolerant genotypes. Morphological traits, such as plant height, leaf area, root biomass, and root-to-stem ratio, are direct indicators of the impact of drought on plant growth (Javornik et al., 2025). In addition, photosynthetic pigments, such as chlorophylls and carotenoids, are biomarkers sensitive to water stress, since photosynthetic machinery is one of the main targets of drought damage

(Mohammadkh & ., 2007) Photosystem II (PSII) efficiency, assessed by chlorophyll fluorescence, is a powerful tool for detecting the early impact of drought on the photosynthetic process (Guidi et al., 2019).

Ecuador, as a center of genetic diversity of the genus *Phaseolus*, is home to both cultivated species and wild populations of *Phaseolus vulgaris* (Sathe, 2016). These wild populations, often adapted to challenging environmental conditions, represent a valuable gene source for genetic improvement of drought tolerance in cultivated beans (Ray et al., 2020). Understanding the tolerance mechanisms present in these wild forms, comparing them with those of cultivars, may offer new perspectives for the development of bean varieties that are more resilient to water stress.

The objective of this study was to evaluate drought tolerance in cultivated and wild bean (*Phaseolus vulgaris* L.) species, using morphological characters and photosynthetic pigments as biomarkers. In this way, it was sought to identify genotypes with an outstanding capacity to adapt to conditions of water stress, which will be crucial for future genetic improvement programs aimed at bean production in areas impacted by drought.

Materials and methods

Plant Material

The plant material used in this study consisted of two local cultivars 'Frejol de campo' and 'Frejol negro' of the species (*Phaseolus vulgaris* L.) and a related wild relative of bean (*Vigna unguiculata*) from the researcher's personal collection. The seeds of the cultivars and the wild species were germinated under controlled conditions of humidity and temperature. Once the seedlings reached a suitable size, they were transplanted individually into pots. Subsequently, these pots were moved to a greenhouse, where they were kept under controlled environmental conditions to ensure uniformity of growth before the application of the treatments. Three irrigation regimes were applied to induce different levels of water stress over a period of 30 days, Control (C) plants were watered with 200 ml of water per pot three times per week. Mild water stress (WS 50%), plants received 100 ml of water per pot three times a week, which represents 50% of the irrigation volume of the control group. Severe water stress (WS 0%), irrigation was completely suspended throughout the treatment period.

Experimental Design

The experiment was conducted in a greenhouse and organized under a completely randomized design. Five replicates were used for each genotype and treatment, thus ensuring the statistical robustness of the results.

Morphological characters

At the end of the treatments, the following morphological characters were measured: number of leaves (NL), stem length (SL), stem diameter (SD) and root length (RL).

Immediately after the experiment, plant material from different tissue types was collected separately to determine the fresh weight of leaf (FWL), stem (FWS), and root (WFR). A portion of this fresh material was stored at -20 °C for use in the extraction of photosynthetic pigments. Samples of the three fabrics were also dried in an oven at 65 °C for 72 hours. Finally, they were weighed to calculate the dry weight of the leaf, stem and root.

Photosynthetic Pigment Content

Chlorophyll *a*, chlorophyll *b*, and total carotenoid concentrations were determined according to the protocols of Lichtenthaler and Welburn (1983).

For the extraction of these pigments, 0.1 g of fresh leaf tissue was macerated in 1 mL of 80% acetone (v/v) at low temperature. The mixture was centrifuged at 13,300 *g* for 15 minutes at 4 °C. The resulting supernatant was collected, and its absorbance was measured at 663, 646 and 470 nm. Concentrations of Chl *a*, Chl *b*, and Caro were calculated using the equations of Lichtenthaler and Welburn (1983) and expressed in mg $^{g-1}$ dry weight (WD).

All UV visible spectrophotometric measurements, including pigment quantification, were performed with a DR1900 handheld spectrophotometer.

Data Analysis

The statistical analysis was carried out using a bivariate ANOVA. In this, genotype and water stress treatments were considered as the main factors of variation for all the parameters evaluated. The significance of the mean differences ($p<0.05$) was determined using Duncan's multiple range test.

Results

Morphological characters

The results of the analysis of the mean values and coeficiente de variación in the growth traits of the factors genotype and treatment are shown in Table 1. Significant differences were observed between genotypes for stem length and diameter traits (SL and SD), with *Vigna unguiculata* and 'Frejol negro' being the genotypes that recorded the longest and shortest stems, respectively; on the other hand, 'Frejol de monte' and *Vigna unguiculata* had the widest stem diameter and 'Black bean' the longest stem. thinner (Table 1; Figure 1). Both traits, stem length (SL) and stem diameter (SD), did not show significant differences between water stress treatments.

The number of leaves (NL) at the end of the experiment varied considerably in the three selected genotypes, ranging from 6.3 leaves in *Vigna unguiculata* to 18.6 in 'Frejol negro' (Figure 1). On the other hand, significant effects induced by severe water stress (WS 0%) were observed, while no significant differences were observed between the Control and the treatment of mild water stress (WS 50%) (Table 1).

The fresh weight of leaves, stem, and roots showed some significant differences between genotypes, as well as between treatments (Table 1). For most genotypes, generally significant differences were observed in the fresh weights of the three tissues, for example, the fresh weight of the leaves (FWL) was significantly higher in 'Frejol negro' 19.29, while *Vigna unguiculata* showed the lowest values 6.48. On the other hand, no significant differences were observed between genotypes for fresh root weight (FWR), while fresh stem weight (FWS) varied significantly, with the *Vigna unguiculata* genotype showing the highest values 8.53 (Figure 1). On the other hand, considerable effects induced by severe water stress (WS 0%) were observed, which led to an average reduction in fresh weight (FW) of 57.5% in leaves and 57.9% in stems, compared to the corresponding controls; however, no significant differences were observed between the control and mild water stress treatments (WS 50%).

Table 1. *Mean value and coefficient of variation (VC) resulting from the analysis of genotype variance and treatment on growth traits in two local cultivars: beans and the wild species Vigna unguiculata. These cultivars were subjected to three water stress treatments: Control, Mild Water Stress (WS 50%), Severe Water Stress (WS 0%). The various capital letters indicate significant differences between the means within each cultivar or treatment, as determined by Duncan's multiple-rank test (p<0.05).*

Factor	SL (cm)	SD (cm)	LN (n°)	FWL (g)	FWR (g)	FWS (g)	WCL (%)	WCR (%)	WCS (%)	RL (cm)
Genotype										
'Frejol de monte'	93.8 A	5.13 C	9.4 B	8.91 A	1.34	5.61 A	78.90 A	52.24 A	74.15 B	12.72 A
'Frejol negro'	195 C	2.7 A	18.6 C	19.29 B	1.64	5.02 A	80.66 A	67.7 A	81.67 C	44.88 B
Vigna unguiculata	130.47 B	3.93 B	6.3 A	6.48 A	1.12	8.53 B	88.58 B	78.57 B	92.73 A	8.67 A
VC	36.66	31.00	55.95	58.86	19.10	29.43	6.23	20.01	11.28	89.82
Treatment										
WS 0%	122.87	3.76	9.2 A	8.2 A	1.05	4.72 A	83.54 A	51.43 A	81.14	21.4
WS 50%	142.95	3.86	12.1 B	11.67 B	1.19	6.3 B	85.35 A	67.23 B	85.40	21.77
Control	153.45	4.14	12.9 B	14.82 B	1.86	8.14 B	77.80 B	72.04 B	85.50	23.09
VC	11.12	5.02	17.08	28.64	31.68	26.80	4.79	16.96	2.96	4.02

Water content in leaves, roots, and stems differed significantly between the three genotypes (Table 1). For the water content in the three tissues: leaf (WCL), root (WCR), stem (WCS), the genotype *Vigna unguiculata* obtained the highest values of 88.58, 78.57, 92.73 respectively, while 'Frejol del monte' reached the lowest values of 78.90, 52.24 and 74.15 respectively (Table 1). On the other hand, significant differences were observed between treatments for the water content in leaves and roots, but not in stem.

Significant differences were found for root length between genotypes, but not between treatments. The 'Frejol negro' genotype had, on average, longer roots 44.88 and *Vigna unguiculata* the shortest 8.67. The treatment (WS 0%) resulted in shorter roots than the control plants and WS 50%, with no differences between treatments (Table 1).

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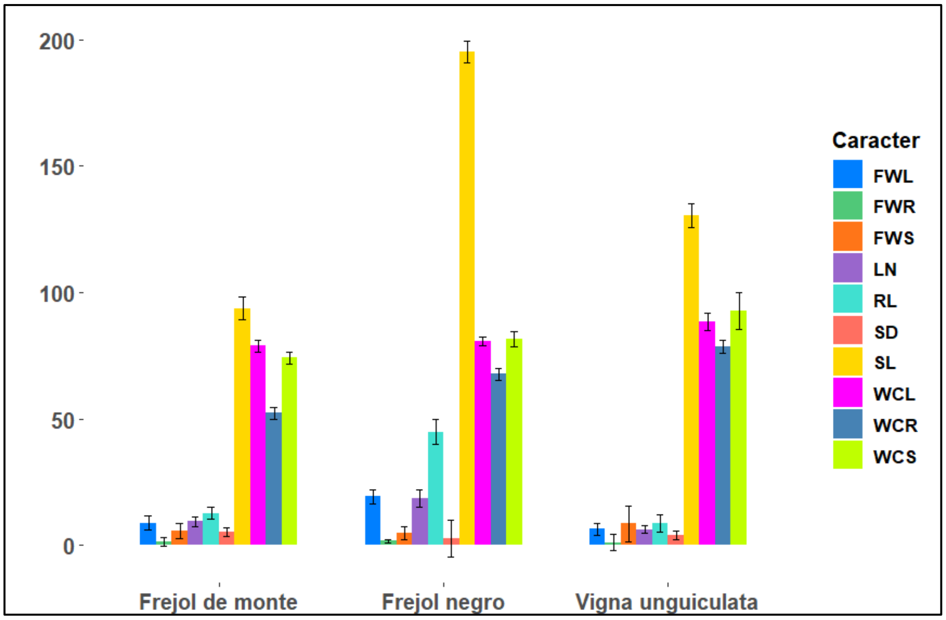

Figure 1. *Morphological characters that exhibited significant differences between genotypes. Fresh Leaf Weight (FWL), Fresh Root Weight (FWR), Fresh Stem Weight (FWS), Leaf Number (NL), Root Length (RL), Stem Diameter (SD), Stem Length (SL), Leaf Water Content (WCL), Root Water Content (WCR), Stem Water Content (WCS).*

Photosynthetic pigments

Regarding photosynthetic pigments (chlorophyll a, chlorophyll b and total carotenoids), no significant differences were observed between cultivars and treatments (Table 2). The mean chlorophyll a and b contents were the highest in 'Frejol negro' and *Vigna unguiculata* (26.82 and 42.61 mg $^{g-1}$ DW, respectively) and the lowest in *Vigna unguiculata and* ‘Frejol negro' (26.82 and 17.78 mg $^{g-1}$ DW) (Figure 2). The concentrations of carotenoids in 'Frejol negro' (0.64 mg $^{g-1}$ DW) were significantly higher than in the rest of the cultivars.

Table 2. *Mean value and coefficient of variation (VC) resulting from the analysis of variance of the cultivar and the treatment on the characters of photosynthetic pigments: chlorophyll a, b, and carotenoids in two local cultivars bean and the wild species Vigna unguiculata. These cultivars were subjected to three water stress treatments: Control (100%), Mild Water Stress (WS 50%), Severe Water Stress (0%). The various capital letters indicate significant differences between the means within each cultivar or treatment, as determined by Duncan's multiple-rank test (p<0.05).*

Factor	Chlorophyll a (mg g^{-1} DW)	Chlorophyll b (mg g^{-1} DW)	Carotenoids (mg g^{-1} DW)
Genotype			
'Frejol de monte'	27.44	31.73	0.18
'Frejol negro'	27.73	17.78	0.64
Vigna unguiculata	26.82	42.61	0.34
VC	24.48	27.87	37.67
Treatment			
WS 0%	11.15	14.35	0.23
WS 50%	24.10	30.41	0.01
Control	40.94	46.22	0.10
VC	49.58	82.34	21.28

Regarding the water stress treatments, no significant differences were observed in the content of the three pigments between the control and the treatment wild water stress (WS 50%). However, the severe water stress treatment (WS 0%) resulted in significant reductions in its concentrations: 72.7%, 68.9% with respect to its corresponding controls, for Chlorophyll a and b, however, the Carotenoids were considerably increased (Table 2).

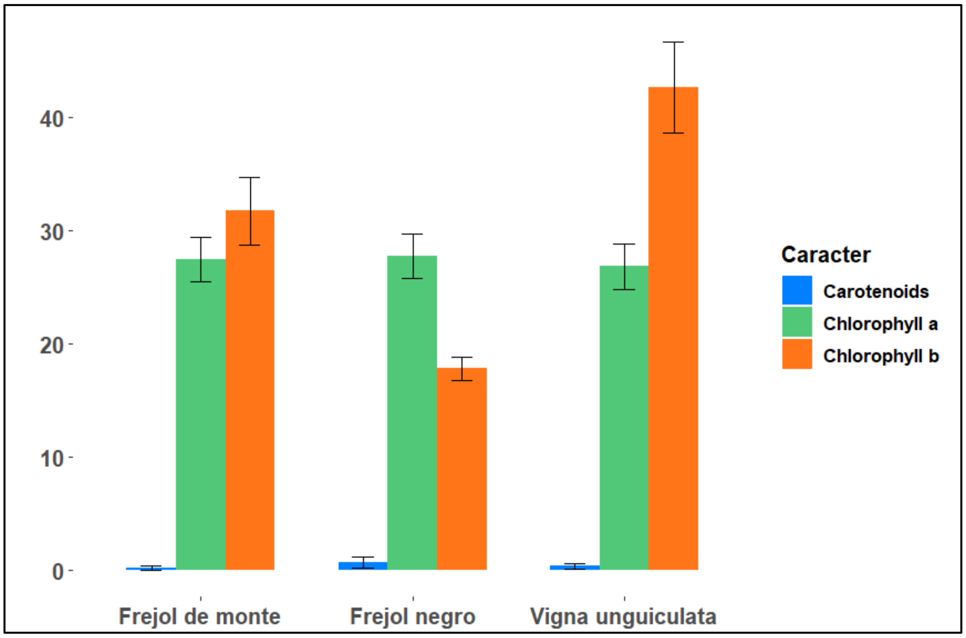

Figure 2. *Photosynthetic pigments Chlorophyll a, Chlorophyll b and Carotenoids in genotypes evaluated.*

Discussion

The present research aimed to evaluate drought tolerance in cultivated and wild relatives' genotypes of beans (*Phaseolus vulgaris* L.) by analyzing morphological characters and photosynthetic pigments. The results obtained reveal differential responses between genotypes and water stress treatments, providing valuable information on the mechanisms of adaptation to drought in this species.

The significant variations observed in stem length and diameter between genotypes, with *Vigna unguiculata* showing the longest and widest stems and 'Frejol negro' the shortest and thinnest, suggest inherent differences in the growth pattern and architecture of the plant. It is interesting to note that these traits did not show significant differences between water stress treatments, which could indicate that stem length and diameter are genetically stable traits and less influenced by water stress in the evaluated stages, or that adjustment mechanisms occur in other structures. This contrasts with studies where severe drought does affect stem growth (Smith et al., 2023), suggesting that the response may depend on the genotype and duration of stress.

The number of leaves showed a marked genotypic variation, with 'Frejol negro' maintaining a higher number of leaves than *Vigna unguiculata*. Significant reduction in leaf numbers under severe water stress treatment is a common adaptive response to reduce transpiratory surface area and conserve water, a well-documented mechanism in plants under drought (Johnson & Lee, 2022). The absence of significant differences between control and moderate stress suggests that genotypes can maintain a similar leaf number under mild or moderate water stress, indicating a drought response threshold for this trait.

The fresh weight of leaves, stem, and roots is a direct indicator of biomass growth and accumulation. The genotypic differences in fresh leaf and stem weight, with 'Frejol negro' showing higher fresh leaf weight and *Vigna unguiculata* higher fresh stem weight, reflect different biomass allocation strategies. The lack of significant differences in fresh root weight between genotypes is a notable finding, suggesting a priority in root development regardless of genotype, which is crucial for water absorption under stress. The drastic reduction in fresh weight (57.5% in leaves and 57.9% in stems) under severe water stress is consistent with decreased growth and cell turgor due to water scarcity (Wang et al., 2021). The absence of significant differences between the Control and mild water stress for most fresh weights reiterates the idea of a tolerance threshold, where moderate stress does not significantly compromise the accumulated biomass compared to optimal conditions.

The water content in leaves, roots and stems is a key morphological marker of the turgor and water status of the plant. *Vigna unguiculata* exhibited the highest values of water content in the three tissues, while 'Frejol del monte' showed the lowest. This could indicate an increased ability of *Vigna unguiculata* to maintain cellular turgor under stress conditions, a desirable characteristic in drought-tolerant genotypes (Chen et al., 2020). Significant differences in leaf water and root water content between treatments, but not in stem water content, suggest that leaves and roots are more sensitive to changes in water availability, while the stem might have more robust water homeostasis mechanisms or serve as a more stable water reservoir.

Root length is a critical trait for drought tolerance, as a longer root allows the plant to explore a larger volume of soil in search of water (Lynch, 2019). Black bean showed significantly longer roots than *Vigna unguiculata*, suggesting a drought evasion strategy by accessing deeper water reserves. The reduction in root length under severe water stress is a paradoxical response, as an increase would be expected to search for water. However, under conditions of severe and prolonged stress, the plant may prioritize short-term survival over root growth, or cell damage may inhibit growth (Kim et al., 2020). The lack of differences between control and mild water stress again points to a response threshold.

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Photosynthetic pigments, such as chlorophyll a, chlorophyll b, and total carotenoids, are sensitive indicators of the physiological state and photosynthetic efficiency of plants under stress (Taiz et al., 2015). The absence of significant differences in chlorophyll and carotenoid content between cultivars and treatments except in severe water stress is an important finding. This could indicate that, under moderate stress conditions, genotypes keep their photosynthetic machinery relatively intact, suggesting an acclimatization capacity or tolerance to non-severe stress levels.

However, severe water stress treatment induced significant reductions in chlorophyll a (72.7%) and chlorophyll b (68.9%) concentrations. This decrease is a typical response to severe water stress, reflecting damage to photosynthetic complexes, pigment degradation, and inhibition of chlorophyll biosynthesis, leading to a reduction in photosynthetic capacity (Flexas et al., 2004). The chlorosis observed under extreme drought conditions is a visual symptom of this degradation.

On the other hand, the considerable increase in carotenoids under severe water stress treatment is a crucial adaptive response. Carotenoids act as accessory pigments in photosynthesis and, more importantly, as potent antioxidants that dissipate excess light energy and protect the photosynthetic apparatus from oxidative damage caused by stress-generated reactive oxygen species (ROS) (Demmig-Adams & Adams, 2002; Kranner et al., 2010). This increase suggests that, in the face of severe water stress, genotypes activate photoprotection mechanisms to mitigate damage, which could be an indicator of tolerance. The higher concentration of carotenoids in 'Frejol negro' under severe stress conditions could contribute to its resilience.

Conclusions

The results suggest that drought tolerance in Phaseolus vulgaris is a multifaceted trait involving a combination of morphological and physiological responses. The genotypes show differentiated strategies 'Frejol negro' seems to employ a drought avoidance strategy through a more extensive root system and a greater ability to maintain leaf count, in addition to a robust carotenoid-mediated photoprotective response under severe stress. Vigna unguiculata, on the other hand, shows a remarkable ability to maintain water content in tissues, which could indicate a strategy of maintaining turgor. 'Frejol del monte' seems to be the most susceptible in terms of water content.

The presence of response thresholds, where mild water stress (WS 50%) does not induce significant changes in many traits compared to control, but severe water stress (WS 0%) does, is a consistent finding in both types of biomarkers. This underscores the importance of stress intensity in the manifestation of tolerance phenotypes. The identification of genotypes such as 'Frejol negro' with a more developed root system and improved antioxidant response, and Vigna unguiculata with high water retention, provides valuable starting points for future genetic improvement programs aimed at developing bean varieties that are more resilient to climate change.

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