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Physiological responses of interspecific-grafted *Phaseolus vulgaris* L. onto *Phaseolus acutifolius* A. Gray rootstock under water deficit

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Abstract
Background: Common bean (*Phaseolus vulgaris*) is a staple food in South America and North Africa. Conventional breeding has prioritized drought tolerance, and grafting has emerged as strategy to enhance stress tolerance.
Hypotheses: The grafting compatibility of *P. vulgaris* onto *P. acutifolius* influence the short-term adaptation to water deficit.
Studied species: Non-grafted *P. vulgaris* and *P. acutifolius* were used for to obtain grafted plants to evaluate physiological and biochemical responses to water deficit.
Study site and dates: The experiment was conducted in a greenhouse at Colegio de Postgraduados, Montecillo, Texcoco, México (19° 27′ 40″ N, 98° 54′ 19″ W and altitude of 2,353 m).
Methods: Plants were well watered until the pod-filling stage (R8). Then, plants were divided into two groups: one continued under well watered (control), while the other group was subjected to water suspension until physiological maturity stage (R9). Stomatal conductance was measured as an indicator of short-term water deficit, and proteomic analysis was performed. Statistical analysis was conducted using ANOVA followed by Tukey's test ($P \leq 0.05$).
Results: Grafted plants mitigated the water deficit in short term by increasing stomatal conductance (*gs*) at 24 h and declined after 48 y 72 h of water deficit. Plants reduced the seed number but stable seed weight distribution. Proteomic analysis from phloem exudates identified GDSL esterase/lipase, which is involved in catabolic processes, lipid catabolism, and transport.
Conclusions: This study provides further evidence for the role of the rootstock (*P. acutifolius*) affect short- term responses to water deficit.
Keywords: phloem proteins, rootstock, scion, stomatal conductance, water deficit.

Resumen
Antecedentes: El frijol (*Phaseolus vulgaris*) es un alimento básico en Sudamérica y el norte de África. El injerto es una estrategia a corto plazo para mejorar la tolerancia al estrés.
Hipótesis: El injerto de *P. vulgaris* unido a *P. acutifolius* influencia la adaptación al déficit de humedad.
Especies de estudio: Plantas de *P. vulgaris* y *P. acutifolius* se injertaron para evaluar respuestas fisiológicas y bioquímicas en plantas injertadas en déficit de humedad.
Sitio y años de estudio: El experimento se realizó en un invernadero del Colegio de Postgraduados, Campus Montecillo, Texcoco, México (19° 27′ 40″ N, 98° 54′ 19″ O y altitud de 2,353 m).
Métodos: Plantas mantenidas en riego hasta llenado del grano (R8) se dividieron en dos grupos: con riego (control) y déficit de humedad hasta madurez fisiológica (R9). Conductancia estomática se usó como indicador de estrés en periodo inicial, seguido de estudios de proteómica. Los datos se analizaron con ANOVA y pruebaTukey ($P \leq 0.05$).
Resultados: Plantas injertadas mitigaron el déficit de humedad con incremento de la conductancia estomática (*gs*) a las 24 h y disminución a 48 y 72 h después de la suspensión del riego. Se observó reducción en el número de semillas, pero distribución de peso individual estable. El análisis proteómico mostró que GDSL esterasa/lipasa presenta asociación con proteínas involucradas con procesos catabólicos, metabolismo de lípidos y de transporte.
Conclusión: El estudio provee evidencia del papel de portainjerto (*P. acutifolius*) en respuesta al déficit de humedad.
Palabras clave: conductancia estomática, déficit de humedad, injerto, portainjerto, proteínas del floema.

The common bean (*Phaseolus vulgaris* L.) is one of the most important legume crops worldwide, valued for nutritional content and socioeconomic significance (Uebersax *et al*. 2023). In Mexico, crops production occurs mostly under rainfed conditions, making it highly vulnerable to drought, which affects over 50 % of the territory and accounts for 80 % of annual yield losses (Ortega-Gaucin *et al*. 2021). However, with global climate change, Mexico experienced the year's drought more impactful in 2011 with the 85 percent of the country affected (Murray & Tortarolo, (2019) and according to Mexican Drought Monitor nearly 76 percent of the country has drought through the end of May 2024 (www.gob.mx/conagua). While biparental crosses have contributed the development of drought-tolerant cultivars, these approaches require extended time periods (Cruz *et al*. 2023). In contrast, grafting has emerged as a short-term strategy to mitigate the effects of water deficit (Padilla *et al*. 2023), with its success largely depending on the selection of the rootstock which normally is chosen for their resistance to certain adverse conditions. For instance, extensive evidence has demonstrated that rootstock is selected to face the drought stress (Li *et al*. 2020, Labarga *et al*. 2023) which the roots explore a larger soil volume for water thought root system traits as root diameter, volume, and length root biomass (Kou *et al*. 2022).

In this context, tepary bean (*Phaseolus acutifolius* A. Gray) is part of the tertiary gene pool and a promising rootstock to enhance the drought tolerance of *P. vulgaris* (Bornowski *et al*. 2023). Grafting offers a method to improve adaptive traits without genetic introgression, positively influencing yield and productivity (Vougeleka *et al*. 2023). Previous studies have shown that grafted plants can increase yield. Gurusamy *et al*. (2010) utilized cultivars of *P. vulgaris* and *P. coccineus* as rootstocks, while genotypes of *P. vulgaris*, *P. acutifolius*, and *P. angustissimus* were used as scions. Their results showed a 91 and 66 % increase in seed yield compared to non-grafted plants. Similarly, Vougeleka *et al*. (2023) reported higher fresh pod yield when a commercial cultivar was grafted onto six Greek landraces of *P. vulgaris*.

Several studies have identified thousands of vascular proteins in plants such as cucumber (*Cucumis sativus* L.), pumpkin (*Cucurbita* L.) (Xu *et al*. 2016), rice (*Oryza sativa* L.) (Aki *et al*. 2008), *Ricinus communis* L., and *Brassica* sp. (Balachandran *et al*. 1997). Rootstock-scion unions have been shown to enhance drought resistance and yield compared to non-grafted plants (Padilla *et al*. 2023). Proteomic analyses have revealed significant changes in phloem proteins in response to drought stress (Castañeda *et al*. 2021). These proteins are involved in lipid metabolism, chaperone-mediated protein folding, carboxylic acid metabolism, abscisic acid signaling, cytokinin biosynthesis, amino acid metabolism, and cell wall organization (Ogden *et al*. 2020).

In legumes, Castañeda *et al*. (2021) investigated the relative distribution of proteins across different tissues in *Medicago truncatula* Graertn and their response to moderate drought stress using shotgun proteomics. They identified a core stress-responsive proteome (CSRP), involving 92 protein isoforms shared among roots, phloem sap, and petioles in response to moderate drought stress. In okra (*Abelmoschus esculentus* [L.] Moench), drought-tolerant rootstocks mitigated the deleterious effects of drought stress by increasing physiochemical parameters and lowering reactive oxygen species. Additionally, the authors identified proteins related to photosynthesis, energy metabolism, defense responses, and nucleic acid biosynthesis (Razi & Muneer 2023). However, knowledge of the ubiquitous proteins involved in drought responses is still limited and requires further investigation. Based on this background, the present study aimed to generate grafted common bean plants using the commercial cultivar Pinto Saltillo as the scion and the desert-adapted Tepary bean, native to Sonora, Mexico, named Tepari Café as the rootstock. This is a comparative study of high drought-tolerant bean rootstocks to evaluate the anatomical, phenotypic, physiological, and phloem sap protein profiles in non-grafted and grafted plants and provides an opportunity to investigate the drought-tolerance mechanisms in grafted interspecific legume species and open new strategies for improving common bean performance under limited water conditions.

Materials and methods

Plant material and experimental site. The experiments were conducted between March and June 2023 in a greenhouse at the Colegio de Postgraduados, Campus Montecillo, Texcoco, Estado de México (19° 27′ 40″ N, 98° 54′

19″ W and altitude of 2,353 m). The cv. Pinto Saltillo of *P. vulgaris* was selected as the scion, and Tepari Café of *P. acutifolius* was chosen as the rootstock based on their high drought tolerance.

Grafting procedure. The plants used for grafting were individually grown in plastic tube containers (120 mL) filled with peat moss as substrate. Grafting was performed when the plants reached the first fully expanded compound leaf stage (V3; CIAT 1982). A transverse cut was made on the stem of the *P. acutifolius* rootstock (2 cm above the cotyledons) to remove the apical part, and a longitudinal cut (1.5 cm deep) at the center of the stem to create a slot for scion insertion (Lee *et al*. 2010). The stem of *P. vulgaris* was transversely cut (2 cm above the cotyledons) to obtain a 3 cm scion, with a pair of true leaves, the apical bud, and axillary buds. At the basal end, two diagonal cuts were made to form a wedge for insertion into the rootstock. Grafted plants were acclimatized for seven days at 23 /18.8 °C (day/night). All plants were transplanted 15 days after grafting into 2 L pots filled into a substrate (volcanic stone cinder with particles ≤ 5 mm diameter). Graft survival rate was determined four weeks after grafting (two weeks after transplanting). The substrate water content was periodically monitored and adjusted with Steiner nutrient solution (Steiner 1961) by weighing each pot, as described by Padilla-Chacón *et al*. (2017).

Experimental design. The experimental design was completely randomized with a factorial arrangement of four treatments (grafted and non-grafted, well-watered and terminal drought). The experimental unit was one plant, with five replications per treatment. During the pod-filling stage (R8; CIAT 1982), the plants were randomly divided into two groups: one group was maintained with regular well watered to keep the soil at 100 % of field capacity (control treatment), while well watered was suspended for the other group until physiological maturity (R9; CIAT 1982).

Graft union formation. Fresh stem segments (1 cm in length) were transversely sectioned using a manual microtome. The sections (100 µm thick) were carefully collected with a fine brush and placed in Petri dishes (100 × 20 mm) containing distilled water at room temperature to prevent dehydration. Subsequently, the sections were mounted directly onto glass slides with a drop of water and covered with a coverslip for further observation under an optical microscope (ZEISS SteREO Discovery V20, Germany) equipped with a digital camera (Canon SD, Japan).

Phenotype of leaflet. Trifoliate leaves from the middle stratum of three-month-old grafted and non-grafted plants were collected and photographed using a Huawei YS Prime smartphone (Huawei Technologies Co., Ltd., China) equipped with a triple camera system: 16 megapixels (main), 8 megapixels (wide-angle), and 2 megapixels (depth sensor).

Stomatal length and width. The central leaflets of trifoliate leaves located in the middle stratum of grafted and non-grafted *P. vulgaris* plants, at four months of age, were fixed in 5 × 5 mm sections under low vacuum in 3 % glutaraldehyde in Sorensen phosphate buffer solution (0.1 M, pH 7.2) for 24 h. Subsequently, the samples were rinsed twice with the same buffer for 10 min each. The samples were then post-fixed in 2 % osmium tetroxide for 15 h, followed by two 30 min rinses with deionized water. Next, they were dehydrated in a graded ethanol series (30, 40, 50, 60, 70, 80, 90, and 100 % [two changes]), for 40 min at each concentration. The samples were dried to the critical point at 31 °C using a Samdri-780A dryer and coated with a thin layer of gold-palladium (80:20) using a metal ionizer (FINE COAT ION SPUTTER JFC-1100). Observations were made using a scanning electron microscope (SEM-JEOL-JSM-6390), operated at 10 kV. Stomatal length and width were measured from micrographs using ImageJ software (v. 1.51k, NIH, USA). Measurements were taken from the adaxial (upper) and abaxial (lower) surfaces, referencing the central vein, from four central leaflets, with eight repetitions per leaflet.

Stomatal conductance (gs). To assess water deficit, stomatal conductance (*gs*) was measured on the central leaflet in intact plants of non-grafted and grafted plants under well-watered and water deficit condition between 8:30 and 9:00 am, at 24, 48, and 72 h. Measurements were using a portable porometer (AP4, Delta-T Devices, United Kingdom). The experiment included five independent biological replicates during the pod-filling stage (R8).

Seed weight distribution. At physiological maturity (R9), pods were harvested and the total number of seeds per treatment was determined and weighed individually to evaluate seed weight distribution. Top-view images of the seeds were acquired using the Scanalyzer PL phenotyping platform (LemnaTec GmbH, Aachen, Germany). Image acquisition was performed with a digital camera (Basler AG, Ahrensburg, Germany) at a resolution of 1,628 × 1,236 pixels, operating under visible RGB illumination (400-700 nm), with a pixel size of 4.4 × 4.4 µm.

Phloem sap collection. Phloem sap exudate isolation was performed using the EDTA-facilitated method described by Tetyuk *et al*. (2013). A composite sample was obtained from three central leaflets of five grafted and non-grafted plants under well-watered and well-watered suspension conditions. A total of 15 leaflets were evaluated for each treatment. Exudates were collected into Eppendorf tubes containing 20 mM K_2EDTA solution. After discarding the EDTA solution, sterile water was added, and sap collection was continued under the same conditions for 6 h in darkness. The exudates were preserved in liquid nitrogen and stored at -80 °C for subsequent analysis.

Identification of proteins by shotgun mass spectrometry. Protein identification by mass spectrometry was conducted as described by López-Toledo *et al*. (2022). Sample preparation prior to identification followed the protocol developed in the Research and Industry Support Services Unit (USAII) of the Faculty of Chemistry at the National Autonomous University of Mexico (UNAM). Data processing and protein identification were performed using the Protein Lynx Global Server version 2.4 (PLGS; Waters Corporation). PLGS scores with a confidence level > 95 % were accepted as correct. Searches were performed in the UniProt database, and peptides were compared with the theoretical peptides of the proteins reported for the samples. To explore the underlying mechanisms of moisture deficit in phloem-transported proteins, changes in protein profiles were analyzed using Shotgun proteomics, with identification confirmed by the UniProt database, represented by an "OK" value equal to 2.

Protein interactions. Protein interactomes were constructed using the Multiple Proteins by Name section of the STRING database, Version 12. The interaction of the GDLS esterase/lipase from *P. vulgaris* was investigated.

Statistical analysis. The data obtained were subjected to analysis of variance (ANOVA), and mean comparisons were performed using Tukey's test ($P \leq 0.05$). Statistical analyses were conducted with SAS software v. 9.4 (SAS Institute Inc., Cary, NC, USA). Graphs were created using GraphPad Prism v. 8.0.2.

Results

Graft union formation. The graft union exhibited no signs of incompatibility between scion and rootstock tissues (Figure 1), and a continuous, functional vascular connection was established between them (Figure 2). In this study, the survival rate of grafted *P. vulgaris* scions was 33 %. Anatomical analysis showed well-defined vascular differentiation, with evident connectivity between the xylem and phloem, indicating the successful establishment of the graft union.

Phenotype of leaflet. The leaflet morphology of non-grafted *P. acutifolius* (rootstock) and *P. vulgaris* (non-grafted and grafted) plants exhibited species-specific characteristics (Figure 3 A, B, C). The trifoliate leaves of *P. vulgaris* were ovate rounded, with well-defined veins and smooth margins (Figure 3A), while the leaves of *P. acutifolius* displayed a lanceolate shape (Figure 3B). Interestingly, the trifoliate leaves of grafted plants exhibited a morphology similar to that of the rootstock (*P. acutifolius*), suggesting that the rootstock exerts an influence on scion leaf development (Figure 3C).

Stomatal length and width. To assess the impact of drought on grafted plants with the focus on cultivar Pinto Saltillo, we comparing grafted plants with non-grafted. The grafted plants exhibited a significant increase in stomatal length, with increments of 34.11 % on the adaxial surface and 12.98 % on the abaxial surface of the leaves, compared to

non-grafted *P. vulgaris* plants (Table 1). Similarly, grafted plants exhibited an increase in stomatal width, with increments of 13.51 and 9.97 % on the adaxial and abaxial surfaces, respectively. These results suggest that the rootstock directly influences stomatal structure, which may be associated with enhanced gas exchange capacity and adaptability to environmental conditions. Figure 4 A, B illustrates the stomatal frequency and distribution, revealing variations in stomatal density.

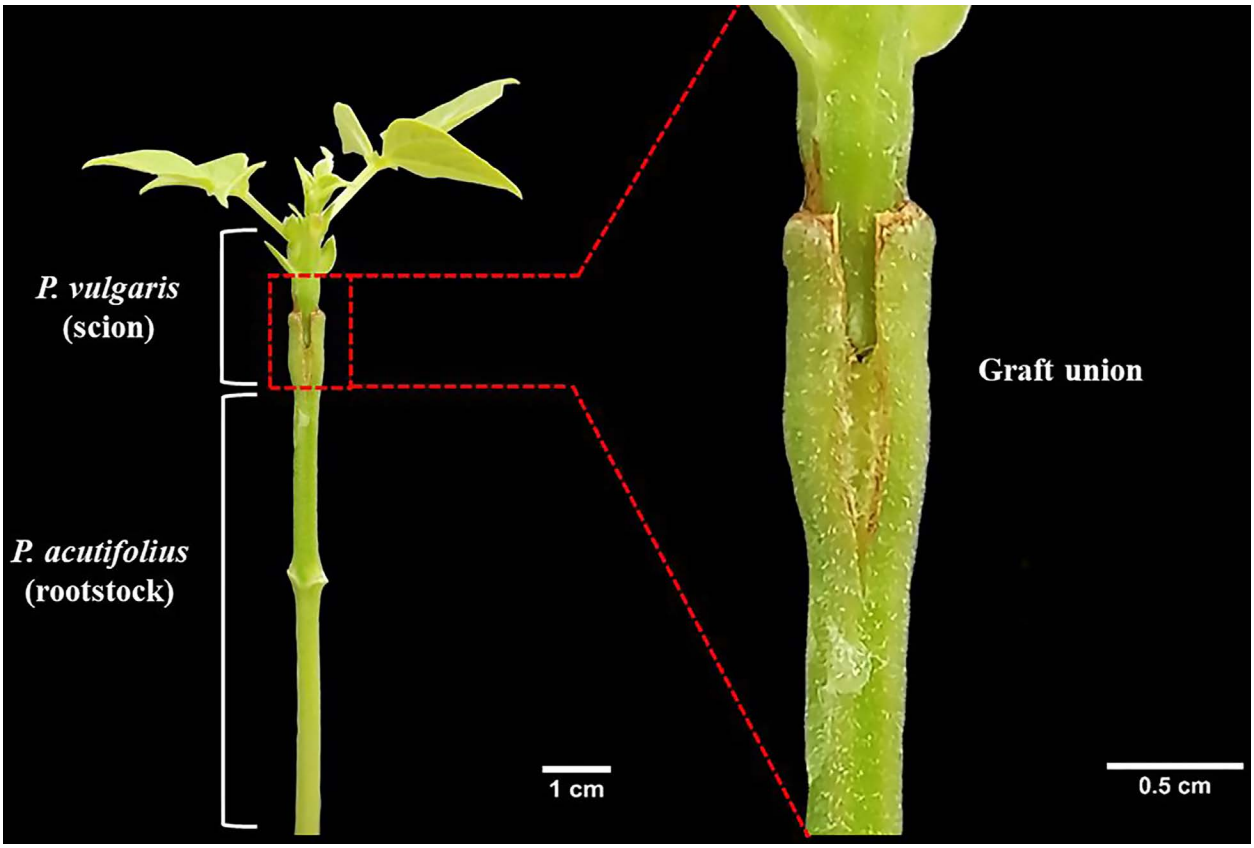

Figure 1. Grafting between *P. vulgaris* (scion) and *P. acutifolius* (rootstock), and approach to the graft union.

Stomatal conductance (*gs*). Water suspension caused a drastic reduction in substrate water content in non-grafted (*P.vulgaris* and *P. acutifolius*) and grafted plants, decreasing available water by approximately 30-35 % at 48 h and progressively declining to 0.15 mL g^{-1}. After it was confirmed that reduced substrate water content (Padilla-Chacón *et al*. 2017) induces stress conditions, the impact on *gs* and its potential influence on seed production were evaluated. Grafted plants exhibited an increase in *gs*, 24 h after well watered was suspended; however, it gradually declined between 48 and 72 h to same levels to control, following a pattern similar to that observed in non-grafted plants (*P. vulgaris* and *P. acutifolius*) (Figure 5). This finding suggests that the rootstock may help to maintain *gs*.

Seed weight distribution. The previously described physiological adjustments may directly enhance photosynthetic efficiency and, consequently, contribute to biomass accumulation and seed filling. In this study, we evaluated the weight distribution of seeds produced in non-grafted and grafted plants under well-watered (Figure 6 A) and water deficit conditions (Figure 6 C). Representative seed images from plants under well-watered (Figure 6 C) and water deficit conditions (Figure 6 D). Notably, the rootstock *P. acutifolius* exhibited high seed production, although with smaller and lighter seeds compared to those of *P. vulgaris*. Under well-watered (Figure 6A), non-grafted *P. acutifolius*

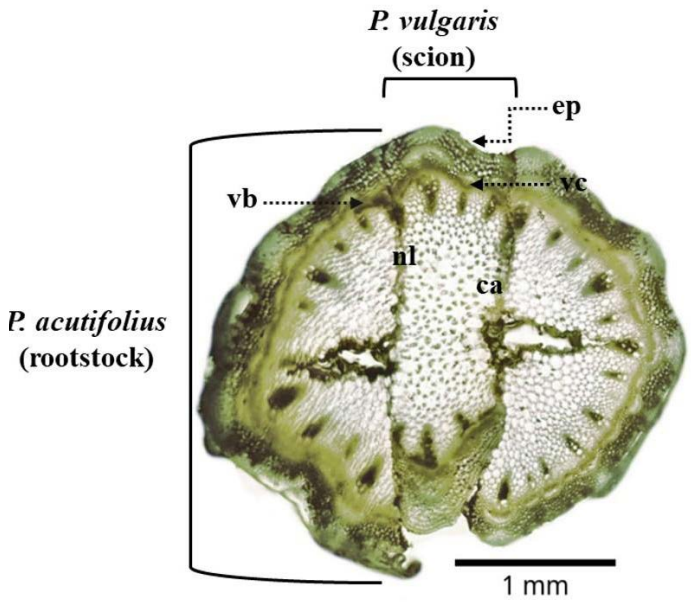

Figure 2. Fresh transverse section of stem of grafted plants of *P. vulgaris* (scion) and *P. acutifolius* (rootstock). Callus (ca), epidermis (ep), necrotic layer (nl) vascular bundle (vb), vascular cambium (vc).

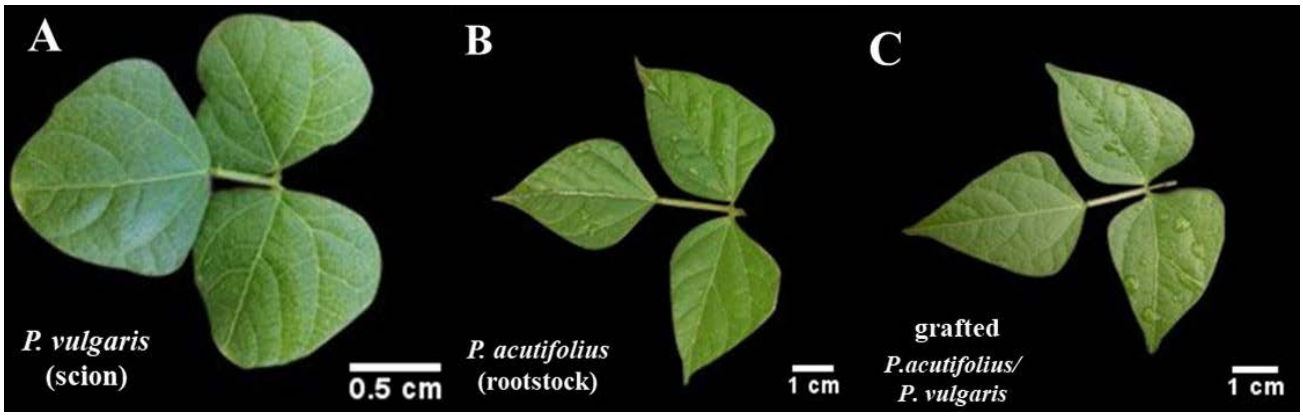

Figure 3. Effect of *P. acutifolius* (rootstock) on the leaf morphology of *P. vulgaris* (scion) under well-watered conditions. Central leaflets of trifoliate leaves in the middle canopy from non-grafted plants, A) *P. vulgaris,* B) *P. acutifolius* and grafted plants, C) *P. acutifolius/P. vulgaris*.

produced 614 seeds, within a weight range of 0.005 to 0.25 g. In contrast, non-grafted *P. vulgaris* produced 218 seeds, primarily distributed within the 0.11 to 0.35 g range. Grafted *P. vulgaris* plants yielded 120 seeds, mostly within the 0.11-0.35 g weight interval.

Under water deficit (Figure 6C), seed production decreased across all treatments. *P. acutifolius* exhibited a 13.8 % reduction in seed number, while *P. vulgaris* showed an 18.3 % decrease and grafted *P. vulgaris* a 14.2 % reduction. The seed weight distribution of *P. acutifolius* showed greater heterogeneity compared to non-grafted and grafted *P. vulgaris* under both water regimes. Under water deficit, most seeds from non-grafted and grafted *P. vulgaris* were consistently distributed within the 0.11 to 0.35 g range, suggesting greater weight stability, possibly associated with the rootstock's influence on carbon partitioning during seed filling.

Identification of proteins by shotgun mass spectrometry. The protein concentration in the phloem sap of non-grafted and grafted plants was determined in the range of 0.138 and 0.508 µg μL^{-1}. Shotgun proteomics identified 12 proteins

Table 1. Effect of *P. acutifolius* (rootstock) on the stomatal length and width of *P. vulgaris* (scion) under well-watered conditions.

	Stomatal length (µm)								Stomatal width (µm)							
	Adaxial				Abaxial				Adaxial				Abaxial			
Non-grafted	19.00	±	0.30	a	17.41	±	0.19	a	11.03	±	0.19	a	12.14	±	0.16	a
Grafted	25.48	±	0.56	b	19.67	±	0.28	b	12.52	±	0.18	b	13.35	±	0.17	b

The data are means ± SE, different letters in the columns indicate statistical differences (Tukey, $P \leq 0.05$), n = 8.

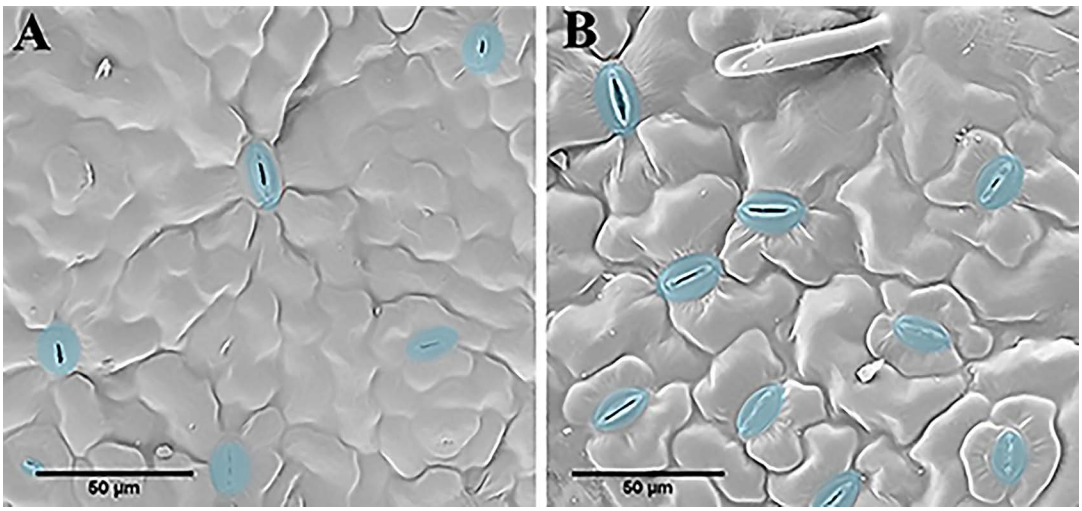

Figure 4. Scanning electron micrographs of the abaxial superface of the central leaflet of trifoliate leaves in the middle canopy, showing the stomatal shape. A) non-grafted plants (*P. vulgaris*) and B) grafted plants (*P. acutifolius/P. vulgaris*) Stomatal were indicated in blue for clarity.

based on peptide hits that passed validation filters, with confidence levels ranging from 2 to 29 peptide matches. In non-grafted plants were identified stress-induced protein KIN2 and WRC domain protein while under water deficit no proteins were detected. In contrast, phloem exudates from *P. acutifolius* plants we identified large and small subunit of ribulose bisphosphate carboxylase (Rubisco) isoforms from exudates in both water regimes used in this study and not detected in the phloem of *P. vulgaris*. Detailed information is summarized in the Table S1.

In grafted plants under well watered conditions, five proteins were identified in phloem exudates: two proteins with Cupin type 1 domains, the SNF1 complex (ASC), RWP-RK domain-containing protein and uncharacterized protein. Interestingly, in grafted plants under water deficit only one protein GDSL esterase/lipase were identified.

The protein interaction analysis was conducted using the GDSL esterase/lipase as the query protein in the STRING database (string-db.org), which resulted in the identification of 23 proteins organized into three interconnected networks associated with catabolic processes, lipid catabolism, and transport (Figure 7). Furthermore, network analysis of the interactome revealed an indirect functional association between GDSL esterases/lipases and aquaporins, a class of intrinsic membrane proteins (MIPs) through an uncharacterized protein (V7C9U3_PHAVU).

Direct interaction was identified with a protein containing the cytochrome b561 domain (observed gene count = 7, interaction strength = 3.03, false discovery rate = 7.95E-16). Additionally, this protein interacted with two peroxidase-type proteins: V7CCK5_PHAVU (observed gene count = 8, interaction strength = 0.72, false discovery rate = 0.0435) and V7CJV6_PHAVU (observed gene count = 9, interaction strength = 0.89, false discovery rate = 1.23E-19). The detailed information is summarized in Table S2.

Discussion

Graft union formation. The close phylogenetic relationship between *P.acutifolius* and *P. vulgaris* suggests that genes from *P. acutifolius* could serve as a valuable resource for enhancing the drought tolerance of *P. vulgaris* (Bornowski *et al*. 2023). However, interspecific hybridization between these species is limited by genomic barriers that may lead to embryo abortion, necessitating techniques such as *in vitro* embryo rescue, which often result in low survival rates (Rogo *et al*. 2023). Given these constraints, grafting emerges as a viable alternative. This technique facilitates the union of plant tissues (stem segments), combining desirable traits of the scion and rootstock while preserving the beneficial characteristics of both plants (Yang *et al*. 2022). In this study, the graft union between the scion and rootstock showed no evidence of tissue incompatibility, and the formation of a continuous and functional vascular connection between both components was confirmed (Figures 1, Figure 2). The graft union is essential for the exchange of water, nutrients, and photoassimilates, allowing the scion to benefit from the physiological traits of the rootstock. In recent years, grafting has attracted increasing attention for its potential to enhance stress tolerance in horticultural crops, particularly in cucurbits and solanaceous species. However, the physiological and molecular mechanisms involved in grafting junctions remain unknown, becoming a significant area of research, focusing on molecular exchanges between rootstocks and scions (Feng *et al*. 2024). The key process underlying the successful establishment of grafted plants is the formation of a functional vascular connection. These observations are consistent with previous studies in Arabidopsis, where a well-established vascular connection has been documented in the stems (Melnyk *et al*. 2015). In tomato, it has been observed that xylem connection occurs between 4 and 5 days post-grafting, while phloem connection is established between 5 and 6 days (Cui *et al*. 2021). In rice, the grafting of embryonic hypocotyls follows a similar sequence of events, with phloem connection occurring at 5-7 days and xylem connection at 6-10 days (Feng *et al*. 2024). Previous studies have described the developmental stages of graft union formation and demonstrated that grafting can improve survival rates, although not necessarily focused on seed production. For example, grafting between *Lotus japonicus* and *Medicago truncatula* has been used to promote host specificity in the legume-rhizobium symbiosis (Lohar & VanderBosh 2005).

Phenotype of leaflet. Grafting is a widely used vegetative propagation technique in various horticultural systems worldwide, applicable to both open field and greenhouse cultivation (Fullana-Pericàs *et al*. 2020). The modifications induced by the rootstock on the scion's phenotype are well-documented across numerous horticultural crops, as rootstocks have a substantial impact on the physiological characteristics of grafted plants, influencing their growth and development. Notably, one of the most significant effects is the alteration of scion morphology (Faria-Silva *et al*. 2020, Frioni *et al*. 2020, Hayat *et al*. 2021).

In the grafting of *P. acutifolius* (rootstock) with *P. vulgaris* (scion), it was observed that grafted plants exhibited a reduced scion height compared to non-grafted plants, providing the advantage of increased planting density and potentially enhanced yield. Furthermore, changes in leaf morphology and anatomy were observed, with the rootstock's leaf shape prevailing over that of the scion.

A prominent mechanism underlying the effects of rootstock on scion growth is the differential ability of rootstocks to absorb water and nutrients. Yan (2022) propose that translocated mRNA triggers changes in scion leaf morphology. Both coding and non-coding RNAs, such as small interfering RNAs (siRNA), have been identified as mobile molecules that traverse the graft union, mediating gene expression. Moreover, alterations in DNA methylation patterns have been reported, which can lead to transcriptional reprogramming. If these changes are heritable, they may result in stable altered phenotypes, positioning grafting as a potential tool for plant improvement aimed at enhanced traits (Kapazoglou *et al*. 2021).

Coban & Öztürk (2020) demonstrated that rootstocks exert a significant influence on the lateral branches and leaf characteristics of grafted pear cultivars. Similarly, Liu *et al*. (2023) found that nutrient concentrations in leaves varied according to the rootstock, potentially due to modifications in root morphology and hydraulic conductance. The interactions between the rootstock and scion also resulted in changes to mineral element composition and antioxidant enzyme activity in the scion, as well as transcriptional shifts (Liu *et al*. 2023).

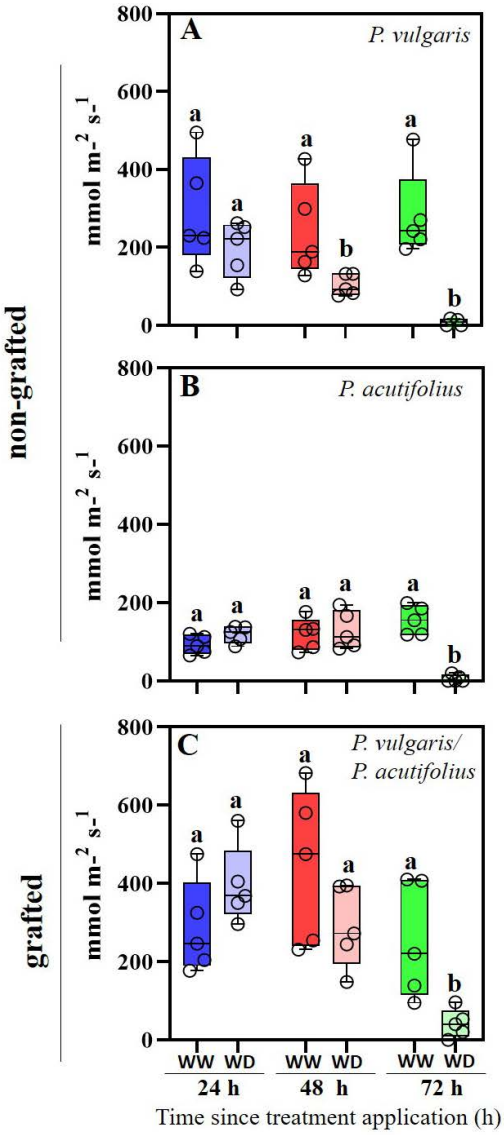

Figure 5. Box-and-whisker plots show the effect of water deficit on stomatal conductance (*gs*) of the central leaflet of non-grafted plants A. *P. vulgaris,* B. *P. acutifolius* and C. grafted plants *P. vulgaris/P. acutifolius* in pod filling stage or R8 during 24, 48 and 72 h after water deficit imposition. Bars represent standard errors of the mean for 5 replicates. (n = 5). Different letters were significantly different ($P \leq 0.05$) according to Tukey test. WW= well-watered, WD = water deficit.

Stomatal length and width. Grafted plants exhibited an increase in stomatal length and width on both the adaxial and abaxial leaf surfaces respect to non-grafted *P. vulgaris* (Table 1). This finding suggests that the interaction between the scion *P. vulgaris* and the rootstock *P. acutifolius* occur signals associated with stomatal architecture. Although this analysis was made by scanning electron microscope in leaflets of trifoliate leaves under well watering condition (Figure 4 A, B), previous studies have demonstrated that *P. acutifolius* tends to maintain stomatal opening under arid conditions while *P. vulgaris* conserves water by closing stomata early, potentially inhibiting stomatal development (Polania *et al*. 2022). Thus, grafted combination may enhance drought tolerance through short-term mechanisms where ABA possibly have pivotal role to determining the balance between productivity and water loss to induce stomatal transpiration through a branch signal cascade (Li *et al*. 2021). In addition, we do not rule out the possibility that the stomatal regulation observed in grafted plants (Figure 3B), the rootstock modulated the photosynthetic parameters derived from stomatal conductance (Figure 5).

Stomatal conductance (gs). To obtain a valuable indicator of short-term water deficit stress, stomatal conductance was measured in non-grafted and grafted plants under water deficit (Figure 5). The results show a clear and significate increment of *gs* in grafted plants compared to non-grafted plants at 24 and 48 h and reduced to 72 h (Figure 5). It reflects the rate of gas exchange through the leaf stomata, which are pores that regulate transpiration is a valuable indicator of plant stress, particularly water deficit. Previous studies have reported similar findings, suggesting that rootstocks can mitigate stress-induced damage to photosynthetic functions (Fullana-Paricàs *et al*. 2020). Liu *et al*. (2011) demonstrated that interspecific grafting enhances photosynthesis, emphasizing the complex interactions between graft combinations and stress conditions. It is suggested that initial water deficit may have triggered a response from the drought-tolerant rootstock towards the scion (Ďurkovič *et al*. 2016, Davoudi *et al*. 2022). Furthermore, it has been documented that grafted plants increase the size of mesophyll structures, which may have contributed to reducing CO_2 diffusion resistance (Camposeco-Montejo *et al*. 2018). *P. acutifolius* has been demonstrated to exhibit high transpiration and photosynthetic efficiency under water deficit, possibly associated with prolonged stomatal opening and increased carbon fixation (Polania *et al*. 2022). These findings support the conclusion that the *P. acutifolius* rootstock may contribute to adaptability only in the early stages of drought (24 - 48 h) under low water availability stress.

Seed weight distribution. Seed weight distribution is a critical trait that influences germination, seedling establishment, and overall crop productivity. This parameter is highly variable and can be influenced by genetic background, environmental conditions, and agronomic factors, which can ultimately affect seed quality and yield. Morales-Elias *et al*. (2022) reported that plants can modify carbon allocation in response to water deficit. In grafted pepper, the rootstock contributed to maintaining carbon and nitrogen balance, which in turn supported biomass accumulation under water-deficit conditions (López-Serrano *et al*. 2019). Seed filling can occur through the translocation of photoassimilates to developing seeds and the remobilization of stored reserves from source tissues (Berny Mier & Teran *et al*. 2019). In rice and wheat (*Triticum aestivum* L.), under optimal conditions, stored reserves contribute approximately 10-40 % of the final seed weight (Prasad *et al*. 2011). The remobilization of these reserves toward the seed determines its final size and is negatively affected by plant exposure to unfavorable environmental conditions (Sehgal *et al*. 2018). In sorghum (*Sorghum bicolor* L. Moench), the remobilization of photoassimilates stored in the stem is a substantial factor in the development of seeds under drought stress (Ghate *et al*. 2017). Nevertheless, these reserves are frequently insufficient to complete seed filling, resulting in a substantial reduction in seed size and number (Morales-Elias *et al*. 2022). Future studies could explore the maximum seed production potential in grafted scions by integrating various photosynthetic parameters under different levels of drought stress.

Identification of proteins by shotgun mass spectrometry. To identify proteins from phloem, the EDTA-facilitated method was employed to obtain exudates from grafted and non-grafted plants under both water regimes. However, it is important to note that the collection technique may result in contamination of adjacent tissues (Killiny 2019). This could help explain the presence of photosynthetic proteins, such as ribulose bisphosphate carboxylase (Rubisco), in the phloem exudates of *P. acutifolius*, despite the absence of photosynthetic activity in sieve elements (Doering-Saad *et al*. 2006). Numerous studies have reported the presence of Rubisco and other proteins associated with photosynthesis in the phloem, suggesting that some of these proteins may result from contamination (Knoblauch *et al*. 2018). For example, smaller Rubisco proteins have also been observed in *Arabidopsis thaliana* (Guelette *et al*. 2012), *Ricinus communis* (Doering-Saad *et al.* 2006), *Brassica napus* (Giavalisco *et al*. 2006), and pumpkin (*Cucurbita maxima*) (Lin *et al*. 2009). However, we also identified proteins previously reported in the vascular tissue of plants under stress conditions. For instance, in this study, we identify a Cupin-type 1 domain-containing protein, which, in accordance with previous reports, plays an important role in the xylem sap of maize subjected to drought stress, contributing to signaling and adaptation to environmental stress (Álvarez *et al*. 2008). Under water-restricted conditions, only one GDSL esterase/lipase protein was identified in grafted plants. This protein has been previously localized in the phloem of plants exposed to drought and is noted for its role in lipid

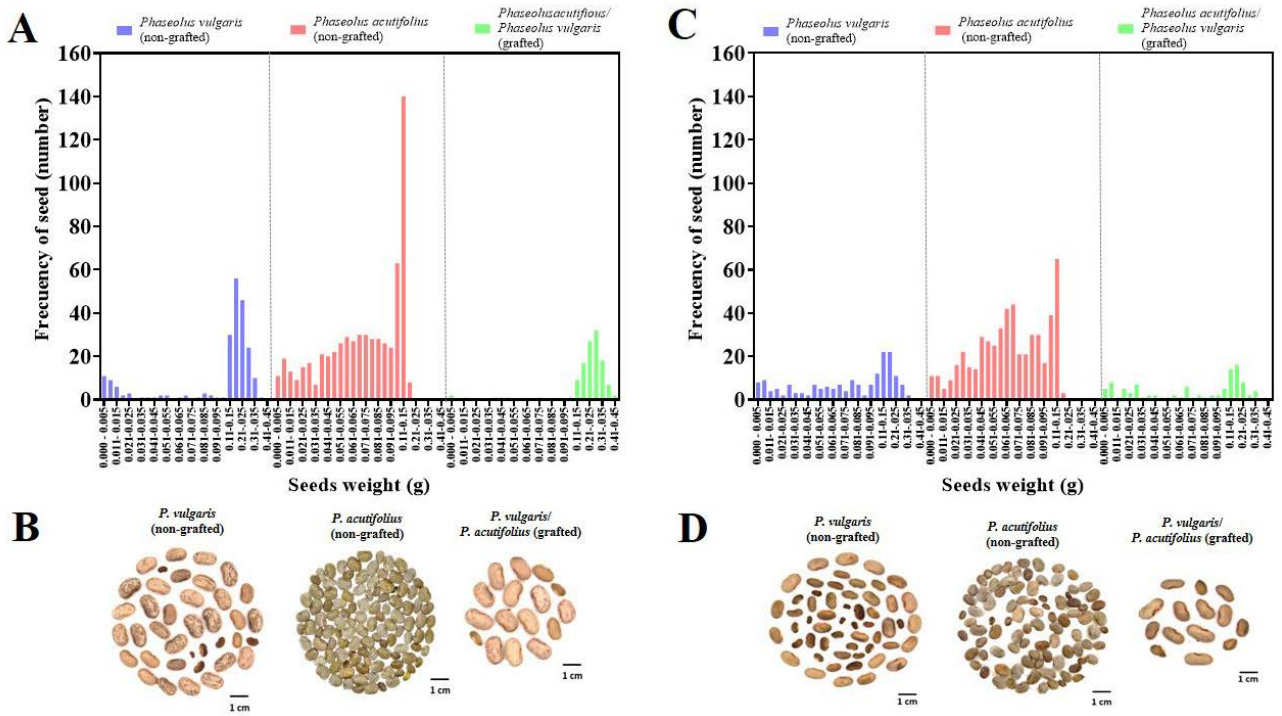

Figure 6. Effect of water deficit on seed weight distribution in non-grafted and grafted plants. Frequency distribution of seed weight under A) well-watered condition, C) water deficit condition. Representative images of seeds from B) well-watered plants, D) water deficit plants. (n = total number of seeds from five replicates).

metabolism and adaptation to adverse environmental conditions (Ogden *et al*. 2020). Lipids and their derivatives have also been implicated as signaling molecules that could modulate the plant defense response (Reina-Pinto & Yephremov 2009, Benning *et al*. 2012). Jasmonic acid (JA), a lipid-derived molecule, is a key phytohormone involved in regulating physiological processes at the graft union site, alongside auxins, cytokinins, ethylene, and gibberellins (Nanda & Melnyk 2018). Ursache *et al*. (2021) identified two sets of GDSL lipases regulated by auxins, suggesting a role in hormone signaling and drought response. A comparative proteomic analysis revealed that scions grafted onto okra (*Abelmoschus esculentus* L.) rootstocks showed increased abundance of photosynthesis-related proteins under drought stress, including GDSL esterase/lipase (Razi & Muneer 2023). In *Oryza sativa*, these enzymes are involved in lipid hydrolysis, cell wall structure and function, and xylem development (Zhang *et al*. 2019).

Proteomic interaction analysis revealed an indirect functional interaction between GDSL esterase/lipase and aquaporins, specifically membrane intrinsic proteins (MIPs), mediated by an uncharacterized protein. This interaction is particularly relevant given the well-documented roles of aquaporins in water transport and cellular homeostasis (Irisarri *et al*. 2024), suggesting that they may have played a key role in regulating water balance in grafted plants. Direct interaction was also identified with a protein containing the cytochrome b561 domain. Asard *et al*. (2013) reported that the tonoplast membrane of *P. vulgaris* contains at least one member of the di-heme cytochrome b561 family, which typically catalyzes electron transport across membranes. Recent studies have emphasized that proteins containing the cytochrome b561 domain are key regulators of plant responses to various stress factors. These proteins modulate the redox states of different cellular compartments, thereby influencing plant development, stress responses, and iron homeostasis (Clúa *et al*. 2025). In *Citrullus lanatus*, a cytochrome b561 protein accumulates in response to drought and light stress, suggesting its involvement in energy dissipation mechanisms (Nanasato *et al*. 2005). In *Nicotiana tabacum*, two cytochrome b561 proteins have been identified in sieve tube elements, indicating

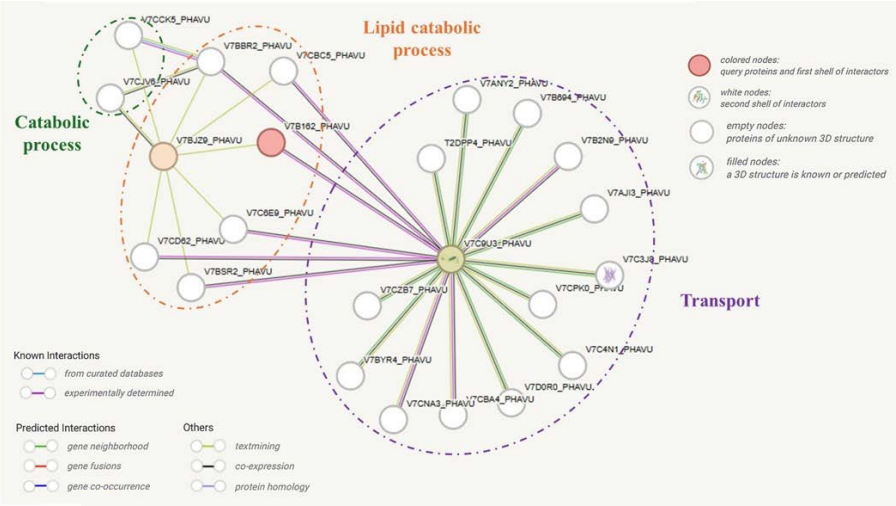

Figure 7. Functional network analysis using STRING 12.0 (string-db.org) of the GDSL esterase/lipase protein identified by shotgun proteomics in phloem exudates of grafted plants under water deficit. 23 clusters of highly interacting protein nodes are marked with circles and include proteins involved in the network. The line colors represent the types of evidence used to predict protein-protein interactions.

a possible role in cell-to-cell communication (Liu *et al*. 2021). The interactome analysis further suggests that the *P. vulgaris* GDSL esterase/lipase is involved in functional networks with other phloem proteins, reinforcing its role in lipid metabolism and the response to environmental stress. Genes encoding these enzymes are essential for abiotic stress adaptation and cellular homeostasis (de Almeida *et al*. 2024). These findings highlight the importance of lipid signaling mechanisms in plant responses to water deficit and underscore the need for further studies to elucidate the roles of these proteins in scion-rootstock communication.

The investigation of grafting in *Phaseolus* genus in response to stress tolerance holds significant implications for improving agricultural yield. It is important to understand the molecular mechanisms against drought stress is to improve plant adaptation and positively influence water use efficiency (WUE). In a previous study, it was noted that Abscisic Acid (ABA) influences stomatal regulation, while IAA sup ports and enhances root system development, making these phytohormones crucial (Muhammad Aslam *et al*. 2022). For example, IAA produced in the leaves of scions can be transferred to rootstocks, promoting lateral root formation, while ABA can be transported from the rootstock to the scion to regulate stomatal conductance during drought ex posure (Nie & Wen 2023). This provides a possible explanation for the comparatively inferior performance of stomatal physiological indicators in grafted plants compared non-grafted plants. Our results showed that *P. acutifolius* potential rootstock source for drought tolerance in common bean.

In conclusion, the development of new strategies for enhancing drought tolerance in common bean is crucial to mitigate the effects of climate change. This study provides evidence of strong compatibility between *P. vulgaris* (scion) and *P. acutifolius* (rootstock) through interspecific grafting to obtain plants with more water deficit tolerance. This was confirmed by a clear short-term increase in stomatal conductance (*gs*) under drought conditions, likely influenced by GDSL esterase/lipase activity and other related proteins. For future research, combining different scion and rootstock genotypes may offer a promising strategy to improve water use efficiency, contributing to breeding programs aimed at developing drought-resilient crops.

Supplementary material

Supplemental data for this article can be accessed here: https://doi.org/10.17129/botsci.3727

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