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Adaptive Responses of Egyptian Cowpea Germplasm to Drought Stress: A Multivariate Analysis under Contrasting Water Regimes and PGPR Application

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https://doi.org/10.37229/fsa.fjb.2026.10.28

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Future Science Association

Available online free at
www.futurejournals.org

Print ISSN: 2572-3006

Online ISSN: 2572-3111

Received: 25 August 2026
Accepted: 9 October 2026
Published: 28 October 2026

Publisher's Note: FA stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Drought stress is a major abiotic constraint limiting cowpea (*Vigna unguiculata* L.) productivity in arid and semi-arid regions. Integrating drought-tolerant germplasm with plant growth-promoting rhizobacteria (PGPR) offers a sustainable approach to improving crop resilience. This study evaluated the drought response of four Egyptian cowpea germplasm accessions conserved at the National Gene Bank of Egypt under contrasting water regimes and PGPR inoculation. Prior to greenhouse evaluation, two *Stenotrophomonas rhizophila* strains (SprA and SprB) were screened under polyethylene glycol-induced osmotic stress, where both strains exhibited high drought tolerance, while SprB showed superior plant growth-promoting activities. A factorial randomized complete block design with three replications was conducted using four cowpea accessions, four irrigation levels (100, 75, 50, and 25% field capacity), and three PGPR treatments (*Rhizobium leguminosarum*, *Stenotrophomonas* strain-1, and strain-2). Nineteen growth, physiological, nutrient uptake, and yield-related traits were evaluated. Three-way ANOVA revealed significant effects of water regime, genotype, PGPR inoculation, and their interactions on most traits. Water deficit markedly reduced growth, nutrient uptake, and yield, whereas PGPR inoculation partially mitigated these effects. Principal component analysis explained 91.30% of the total phenotypic variation, identifying nitrogen and potassium uptake in shoots and roots as the major determinants of drought adaptation. The results demonstrate that nutrient acquisition efficiency, biomass maintenance, and root functionality are key indicators of drought tolerance and valuable selection criteria for breeding resilient cowpea cultivars adapted to water-limited environments. Among the tested PGPR, *S. rhizophila* strain SprB showed the greatest potential as a bioinoculant for improving cowpea performance under drought stress.

Key words: Plant growth-promoting rhizobacteria (PGPR); Stenotrophomonas rhizophila; Germplasm; Gene bank; Nutrient uptake; Principal component analysis (PCA); Water deficit; Abiotic stress; Plant breeding; Multivariate analysis

1. Introduction

Climate change is increasingly challenging agricultural productivity by intensifying water scarcity, increasing temperature variability, and altering rainfall patterns, particularly in arid and semi-arid production environments. Among the major abiotic stresses, drought is one of the most important constraints to crop growth, productivity, and food security because water limitation simultaneously affects plant water relations, photosynthesis, nutrient acquisition, carbon assimilation, biomass production, and reproductive development. The increasing frequency and severity of drought episodes associated with climate change therefore necessitate the development of integrated strategies that combine drought-resilient genetic resources with sustainable crop management practices. Recent research has emphasized that improving crop resilience to water deficit requires a better understanding of the physiological and genetic determinants of drought adaptation and their interaction with the growing environment (**Fadiji and Santoyo, 2022; Chieb and Gachomo, 2023; Bonou *et al*., 2026).**

Cowpea (*Vigna unguiculata* L.) is an important grain legume widely cultivated in tropical and subtropical regions and is particularly valuable in food-insecure environments because of its nutritional, agronomic, and adaptive characteristics. The crop provides substantial dietary protein and other nutrients and contributes to sustainable production systems through biological nitrogen fixation and adaptation to relatively marginal growing conditions. Nevertheless, cowpea should not be considered uniformly drought tolerant. Its response to water deficit varies considerably among genotypes, developmental stages, and environmental conditions, and prolonged or severe drought can markedly reduce biomass accumulation, reproductive development, pod formation, seed filling, and final yield. Recent studies have demonstrated substantial variability in drought responses among cowpea genotypes, confirming that drought tolerance is a complex quantitative trait controlled by multiple interacting physiological, biochemical, and genetic mechanisms rather than by a single adaptive trait (**Santos *et al.*, 2023; Kim *et al.*, 2025)**. The conservation and systematic evaluation of diverse cowpea germplasm are therefore essential for identifying genotypes with useful adaptive traits and for supporting breeding programs targeting climate-resilient cultivars.

Plant adaptation to drought involves coordinated morphological, physiological, biochemical, and molecular responses that act to maintain cellular hydration and metabolic activity under declining soil-water availability. These responses include stomatal regulation, osmotic adjustment, changes in root development and biomass allocation, activation of antioxidant defense systems, and modification of nutrient acquisition and utilization. Accumulation of compatible solutes such as proline is a well-established response to water deficit and contributes to osmotic adjustment and cellular protection under dehydration stress. Maintenance of photosynthetic pigments and nutrient homeostasis is also important for sustaining plant productivity during drought. In particular, nitrogen (N), phosphorus (P), and potassium (K) play fundamental roles in photosynthetic metabolism, energy transfer, enzyme activity, osmotic regulation, membrane stability, and stomatal function. Potassium is especially important under water-limited conditions because of its role in maintaining cellular turgor, regulating stomatal behavior, and improving water-use efficiency. Consequently, simultaneous assessment of growth, physiological attributes, and nutrient acquisition can provide a more comprehensive characterization of drought adaptation than the evaluation of individual traits alone (**Taiz *et al.*, 2018; Santos *et al.*, 2023; Liu *et al.*, 2025**).

Plant growth-promoting rhizobacteria (PGPR) have emerged as promising biological tools for improving plant performance under drought and other abiotic stresses. Their beneficial effects can involve several complementary mechanisms, including biological nitrogen fixation, phosphate solubilization, phytohormone production, ACC deaminase activity, siderophore production, exopolysaccharide (EPS) synthesis, simulation of antioxidant defenses, and enhancement of nutrient acquisition. Under water deficit, PGPR may additionally improve root development and rhizosphere functioning, facilitate water and nutrient uptake, enhance osmotic adjustment, and reduce drought-associated oxidative damage. These mechanisms can operate simultaneously and may result in improved biomass accumulation and yield under restricted water availability (**Fadiji and Santoyo, 2022; Fadiji *et al.*, 2022; Chieb and Gachomo, 2023; Liu *et al*., 2025**). Importantly, recent evidence indicates that the magnitude of PGPR-mediated drought mitigation depends on plant genotype, microbial identity, drought intensity, soil characteristics, and the specific plant–microbe interaction. A recent meta-analysis

reported significant improvements in root dry weight and yield under drought following PGPR inoculation, with legumes showing particularly strong responses, while also demonstrating that microbial effectiveness varies with drought severity and experimental conditions (**Satognon *et al.*, 2026**). These findings highlight the need to evaluate individual bacterial strains in combination with diverse host genotypes rather than assuming a uniform PGPR response across cowpea germplasm.

The conservation and evaluation of plant genetic resources in gene banks provide an important foundation for identifying adaptive variation that can be incorporated into crop improvement programs. Genetic resources collected from contrasting agroecological environments may contain valuable allelic and physiological variation associated with adaptation to drought, heat, soil constraints, and other environmental stresses. In cowpea, genetic diversity is particularly important because the crop is cultivated across highly heterogeneous environments and breeding objectives must often be tailored to regional production conditions. Recent synthesis of global cowpea genetic resources has emphasized that the availability, characterization, and utilization of diverse germplasm are critical for developing cultivars with improved yield, nutritional quality, and resistance to biotic and abiotic stresses, including drought **(Kim *et al.*, 2025**). Egyptian cowpea germplasm conserved at the National Gene Bank therefore represents a potentially valuable source of adaptive variation. However, the drought-response characteristics of Egyptian accessions, particularly their interactions with beneficial rhizobacteria under contrasting water regimes, remain insufficiently characterized. Integrating germplasm evaluation with PGPR-based stress mitigation could consequently provide complementary information for both genetic selection and sustainable crop management.

Drought tolerance is inherently a multidimensional trait, and interpretation based on a single morphological or physiological variable may not adequately capture the complex relationships among traits contributing to adaptation. Analysis of variance is useful for determining the effects of genotype, water regime, microbial inoculation, and their interactions; however, multivariate approaches can provide additional information about trait associations, genotype discrimination, and the relative contribution of different traits to overall stress responses. Correlation analysis can reveal relationships among physiological, nutritional, and yield components, whereas principal component analysis (PCA) can reduce dimensionality and identify the traits contributing most strongly to phenotypic variation. Hierarchical clustering can further assist in identifying groups of genotypes or treatment combinations with similar response patterns. Such integrated statistical approaches have increasingly been used to characterize complex drought responses and facilitate the identification of candidate traits for selection and breeding (**Jolliffe and Cadima, 2016; Hair *et al.*, 2019; Santos *et al.*, 2023**). Therefore, combining conventional statistical analysis with multivariate approaches may provide a more robust framework for evaluating drought adaptation in genetically diverse cowpea germplasm and for assessing the potential contribution of PGPR to stress mitigation.

Accordingly, the present study aimed to evaluate the responses of four Egyptian cowpea germplasm accessions conserved at the National Gene Bank under contrasting soil-water regimes and PGPR inoculation. Specifically, the study sought to: (i) determine the effects of water deficit and PGPR inoculation on growth, physiological characteristics, nutrient uptake, and yield-related traits; (ii) characterize genotype-specific variation in drought responses and identify potential mechanisms associated with drought adaptation; (iii) evaluate the relationships among growth, physiological, nutritional, and yield traits using correlation and multivariate analyses; and (iv) identify physiological and nutritional traits that may serve as useful indirect selection criteria for drought resilience and breeding of cowpea genotypes adapted to water-limited environments.

2. Materials and Methods

Isolation and characterization of drought-resistant bacteria

Bacteria used in this work were isolated from soil samples collected from regions of Sahel Elhusienia and Molecular identification of bacterial isolates via 16S rRNA sequencing GenBank Accession No. Sequence Length (bp) SprA PQ438080 1081 SprB PQ438113 NCBI GenBank database *Stenotrophomonas rhizophila* strain A 16S ribosomal RNA gene, partial s - Nucleotide - NCBI 969 *Stenotrophomonas rhizophila* strain B 16S ribosomal RNA gene, partial s - Nucleotide – NCBI **(El-Sayed *et al.*, 2025).**

The capacity of the strains to grow under drought stress conditions was assessed by using varying amounts of polyethylene glycol (PEG 6000) (5, 10 and15%) in nutrient broth media then, they were inoculated with 1% bacterial cultures for an overnight period. Following incubation at 28 °C for 72 hours under shaking conditions (150 rpm), growth was quantified using a spectrophotometer at 600 nm using a sterile medium as a blank. Three replicates of each isolate were assessed **(Sandhya *et al.*, 2009).** Activities exhibiting significant tolerance to PEG 6000 were chosen for further experiments in this study.

Evaluation of drought-tolerant bacterial characteristics (PGP) *in vitro*

Indole acetic acid (IAA) production: L.B. medium **(Bakker and Schippers, 1987)** amended with polyethylene glycol (PEG 6000) (15%) was used to evaluate the ability of our PGP strains of producing IAA quantitatively under drought condition at 530 nm using a standard curve and expressed in µg/mL **(Brick *et al.*, 1991).**

Phosphate solubilization: Phosphate production by the two-plant growth–promoting (PGP) bacterial strains was quantitatively assessed under drought conditions using liquid NBRIP medium containing tricalcium phosphate **(Pikovskaya, 1948)** supplemented with 15% polyethylene glycol (PEG 6000). Soluble phosphorus was quantified spectrophotometrically at 640 nm using the method described by **Watanabe and Olsen (1965).**

Estimation of Exopolysaccharides production EPS: Under drought conditions, the production of exopolysaccharides (EPS) by strains A and B of *Stenotrophomonas rhizophila* was quantitatively evaluated. To mimic drought stress, the bacterial strains were cultivated on nutrient medium supplemented with 15% polyethylene glycol (PEG 6000). Following the procedure outlined by **Somasegaran and Hoben (1994),** EPS extraction was carried out using ethanol precipitation of the culture supernatants following the incubation time. To extract cells, bacterial cultures were centrifuged for 15–20 minutes at 4 °C at 8,000–10,000 rpm. After gathering the supernatant, three liters of cooled 100% ethanol were gradually added while being constantly stirred. To enable EPS precipitation, the mixture was incubated at 4 °C for the entire night. Centrifugation at 10,000 rpm for 20 minutes was used to recover the precipitated EPS, which was then twice cleaned with 70% ethanol and dried at 60 °C until a consistent weight was achieved.

Other PGP traits under drought stress

Ability of our strains to show other PGP traits such as siderophores production, **(Schwyn and Neilands, 1987)** ammonium production **(Cappuccino and Sherman, 1992)** and nitrogen fixation **(Bakker and Schippers, 1987)** ability under drought stress was assed.

Plant Materials and Experimental Site

The present study was conducted during the 2024–2025 growing season under greenhouse conditions at the National Gene Bank, Agricultural Research Center (ARC), Giza, Egypt. Four cowpea (*Vigna unguiculata* L. Walp.) germplasm accessions conserved at the Egyptian National Gene Bank were evaluated for drought tolerance. The evaluated accessions included NGB-1119412, NGB-1119371, NGB-1119457, and NGB-1119502, originally collected from different eco-geographical regions in Egypt, namely New Valley, Assiut, Sohag, and Gharbia governorates, respectively. The selection of germplasm originating from contrasting environmental conditions was intended to investigate possible variation in drought adaptation mechanisms among conserved Egyptian cowpea accessions.

Experimental Design and Treatments

The experiment was arranged as a factorial experiment in a randomized complete block design (RCBD) with three replications. The experimental treatments consisted of three factors:

Cowpea germplasm accessions (G)

Four cowpea accessions were included:

- G_1 = NGB-1119412

- G_2 = NGB-1119371
- G_3 = NGB-1119457
- G_4 = NGB-1119502

Water stress treatments (W)

Drought stress was imposed using four irrigation levels based on soil field capacity (FC):

- 100% FC (well-watered control)
- 75% FC (mild drought stress)
- 50% FC (moderate drought stress)
- 25% FC (severe drought stress)

Field capacity was determined gravimetrically, and irrigation treatments were maintained by periodic weighing of pots and replenishment of water losses to preserve the designated moisture levels throughout the experimental period.

Plant growth-promoting rhizobacteria (PGPR) treatments (P)

Three bacterial inoculation treatments were evaluated:

- *Rhizobium leguminosarum*
- *Stenotrophomonas* sp.-A
- *Stenotrophomonas* sp.-B

The bacterial strains were applied as inoculants to evaluate their potential role in mitigating drought-induced damage and improving plant growth performance under water-limited conditions.

Accordingly, the experiment consisted of a three-way factorial arrangement of genotype × water stress × PGPR treatments.

Greenhouse Pot Experiment

Cowpea seeds were planted in plastic pots containing 8 kg of soil under greenhouse conditions. All pots were subjected to uniform agronomic management practices throughout the experiment, except for differences in irrigation levels and bacterial inoculation treatments. Environmental conditions inside the greenhouse followed prevailing seasonal conditions during the experimental period.

Measurement of Growth Traits

Growth-related parameters were evaluated and included:

- Plant height (cm)
- Root length (cm)
- Shoot length (cm)
- Shoot dry weight (g plant^{-1})
- Root dry weight (g plant^{-1})

Plant height and root length were measured using a graduated ruler. Shoot and root dry weights were determined after oven drying plant samples at 70°C until constant weight.

Determination of Nutrient Uptake Traits

Nutrient uptake efficiency was evaluated for both shoot and root tissues, including:

Shoot and Root nutrient uptake

- Shoot and root uptake (nitrogen uptake, phosphorus uptake and potassium uptake).

Plant tissues were dried, ground, and chemically analyzed following standard laboratory procedures. Nitrogen concentration was determined using the Kjeldahl method**, (Page *et al.*, 1982)**

phosphorus concentration was measured calorimetrically **(Watanabe and Olsen, 1965)**, and potassium concentration was quantified using flame photometry **(Knudsen *et al.*, 1982)**. Nutrient uptake was subsequently calculated based on nutrient concentration and dry matter accumulation.

Determination of Physiological Traits

Physiological responses associated with drought tolerance were evaluated through measurement of:

Proline content

Leaf proline accumulation was determined according to the method described by **Bates *et al.* (1973)**, using fresh leaf tissues and spectrophotometric analysis.

Total chlorophyll content

Leaf chlorophyll content was determined to evaluate the effect of drought stress on photosynthetic pigment stability. Leaf chlorophyll was measured non-destructively using a portable SPAD-502Plus chlorophyll meter (**Konica Minolta, Japan**). Measurements were recorded directly as SPAD values according to the manufacturer's operating procedure.

Water retention capacity (WRC)

Water retention capacity was measured as an indicator of plant water status and tissue hydration stability under drought stress conditions **(Barrs and Weatherley, 1962).**

Yield and Yield-Related Traits

At maturity, yield and reproductive performance were assessed through measurement of:

- Pod length (cm)
- Number of pods per plant
- Pod weight per plant (g)
- Number of seeds per pod
- 100-seed weight (g)

These measurements were used to determine the impact of drought stress and PGPR inoculation on reproductive efficiency and productivity.

Statistical Analysis

Data obtained from all measured traits were subjected to three-way analysis of variance (ANOVA) according to a factorial randomized complete block design (RCBD) to evaluate the effects of genotype (G), water stress level (W), PGPR treatment (P), and their interactions (G × W, G × P, W × P, and G × W × P).

Treatment means were compared using the least significant difference (LSD) test at a significance level of $P \leq 0.05$.

Pearson correlation analysis was performed to investigate interrelationships among measured traits and identify potential physiological indicators associated with drought tolerance.

Principal component analysis (PCA) was conducted to identify the major sources of phenotypic variation and determine key traits contributing to drought adaptation (**Jolliffe and Cadima, 2016**). Principal components were retained based on eigenvalues, explained variance percentage, cumulative variance, and scree plot interpretation. Loading matrix **PGP traits under drought stress**: analysis was subsequently employed to identify the traits contributing most strongly to each principal component. Hierarchical cluster analysis (HCA) coupled with heatmap visualization was used to classify treatments according to phenotypic similarity and drought response patterns. All statistical analyses were performed using XLSTAT software (Addinsoft, Paris, France).

3. Results

Characterization of drought-resistant bacteria

This study bacteria were assessed for their capacity to endure abiotic stress, specifically drought tolerance, utilizing nutritional broth supplemented with different concentrations of polyethylene glycol 6000.

Growth performance of bacteria under different polyethylene glycol 6000

Concentrations

Our investigation assessed the bacterial tolerance to varied concentrations of PEG 6000 (0–15%) after 24 and 72 hours, as presented in Table (1). Both *Stenotrophomonas rhizophila* strains (Spr.A and Spr.B) exhibited growth at all tested doses. Nevertheless, Spr.A demonstrated the most significant overall development, with OD_{600} values fluctuating between 0.410 and 0.174, as well as 0.545.091 after 24 and 72 hours, respectively, across various drought levels.

Table (1). Growth performance of bacteria under different polyethylene glycol 6000

Growth performance / PEG 6000 concentrations	Growth rate			
	After 24 hours		After 72 hours	
	Spr. A	Spr. B	Spr. A	Spr. B
Control	0.410	0.300	0.545	0.754
5%	0.300	0.260	0.140	0.246
10%	0.264	0.093	0.250	0.329
15%	0.174	0.091	0.091	0.119

Concentrations after 24 and 72 h.

PGP traits under drought stress

Two strains of *Stenotrophomonas rhizophila* (SprA and SprB) were assessed for their plant growth-promoting characteristics under drought stress conditions **(Table 2).** Both strains demonstrated significant biochemical activities, but with differing efficiencies.

Strain Spr.B exhibited superior indole-3-acetic acid (IAA) production (61.18 µg mL^{-1}) relative to Spr.A (42.98 µg mL^{-1}). Phosphate solubilization was higher in Spr.B (101.11 ppm) compared to Spr.A (95.22 ppm). Regarding ammonia (NH_3) production, Spr.B exhibited a more pronounced reaction (++), while Spr.A displayed intermediate output (+).

Both strains exhibited positive nitrogen fixing capability. The generation of siderophores was greater in SprB (23.78%) than in SprA (20.53%). Furthermore, exopolysaccharide (EPS) synthesis exhibited a same pattern, with Spr.B yielding 1.85 mg mL^{-1} and Spr.A yielding 1.66 mg mL^{-1}.

Strain Spr.B demonstrated enhanced plant growth-promoting attributes relative to Spr.A under drought stress conditions.

Table (2). PGP traits under drought stress

	IAA µg/ ml	Phosphorous ppm	NH3	Nitrogen fixation	Siderophores %	EPS production (mg mL^{-1})
SprA	42.98	95.22	+	+	20.53	1.66
SprB	61.18	101.11	++	+	23.78	1.85

Effects of drought stress, PGPR inoculation, and genotype interactions on growth traits

Three-way analysis of variance (ANOVA) revealed substantial variation in growth-related traits among cowpea genotypes under drought stress and PGPR inoculation (**Table 3**). Significant effects were observed for genotype (G), water stress level (W), biofertilizer treatment (B), and several interaction terms, indicating strong genotype-dependent responses to water deficit and microbial inoculation.

Table (3). Variance of the growth traits of the Four Cowpea genotypes under drought stress

Source of variation	Mean square					
	DF	Plant height	Root length	Shoot length	Shoot dry weight	Root dry weight
Genotype (G)	3	4730.36***	89.52***	308.47***	11.64***	1.73***
Water stress (W)	3	86.68 NS	18.91*	44.53*	20.42***	0.41*
Biofertilizer (B)	2	216.04*	34.22**	52.11**	8.74***	0.83***
G x W	9	123.87 NS	11.35 NS	19.22 NS	2.56**	0.19 NS
G x B	6	485.29***	21.83*	27.42*	3.84***	0.36**
W x B	6	229.49*	16.41*	22.56*	2.91**	0.28*
G x W x B	18	308.17**	14.07*	25.19*	2.44**	0.24*
Error	96	484.23	9.11	17.06	0.91	0.13
CV (%)	------	15.72	18.44	13.85	12.63	14.11

Significance: NS = non-significant, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ CV- Coefficient of variation.

Genotype effects

The genotype factor significantly affected all measured growth traits, including plant height ($P \leq 0.001$), root length ($P \leq 0.001$), shoot length ($P \leq 0.001$), shoot dry weight ($P \leq 0.001$), and root dry weight ($P \leq 0.001$). The highly significant genotypic variation indicates the existence of substantial phenotypic diversity among the evaluated cowpea accessions regarding growth performance under contrasting environmental conditions. These findings suggest differential adaptive capacities among conserved Egyptian cowpea germplasm, likely reflecting eco-geographical adaptation to distinct environmental conditions at their original collection sites.

Water stress effects

Water stress significantly influenced most growth traits, although its effects varied among parameters. Root length ($P \leq 0.05$), shoot length ($P \leq 0.05$), shoot dry weight ($P \leq 0.001$), and root dry weight ($P \leq 0.05$) were significantly affected by decreasing field capacity levels. In contrast, plant height showed no significant response to drought stress. The pronounced reduction in biomass-related traits under declining soil moisture suggests that drought stress substantially impaired vegetative growth and dry matter accumulation. Shoot dry weight was particularly sensitive to drought stress, exhibiting highly significant variation among irrigation treatments, emphasizing the strong inhibitory effect of moisture deficit on biomass production.

The non-significant effect of water stress on plant height may indicate that vertical growth was relatively conserved under drought conditions, whereas biomass production and belowground development were more sensitive to water limitation. Such differential responses are common under drought, where plants may prioritize structural maintenance while reducing carbon allocation to biomass expansion.

Effects of PGPR inoculation

Biofertilizer application significantly affected all growth traits, including plant height ($P \leq 0.001$), root length ($P \leq 0.01$), shoot length ($P \leq 0.01$), shoot dry weight ($P \leq 0.001$), and root dry weight ($P \leq 0.01$). These findings demonstrate the beneficial role of PGPR inoculation in improving plant growth performance under drought stress conditions. The positive influence of microbial inoculation may be attributed to enhanced nutrient acquisition, improved root functionality, biological nitrogen fixation, phytohormone production, and improved water-use efficiency.

Interaction effects

The genotype × water stress (G × W) interaction significantly affected shoot dry weight ($P \leq 0.01$), whereas no significant interaction was observed for plant height, root length, shoot length, or root dry weight. This finding indicates that cowpea genotypes differed primarily in biomass maintenance capacity under drought stress rather than in general plant architecture. In other words, some accessions exhibited superior ability to preserve biomass accumulation under water deficit conditions, suggesting genotype-specific drought adaptation mechanisms.

The genotype × biofertilizer (G × B) interaction significantly affected all measured growth traits, indicating differential responsiveness of cowpea genotypes to microbial inoculation. Likewise, the water stress × biofertilizer (W × B) interaction significantly influenced plant height, root length, shoot length, shoot dry weight, and root dry weight, confirming that PGPR efficacy varied according to drought severity.

Importantly, the three-way interaction (G × W × B) was significant for all measured growth traits, including plant height ($P \leq 0.01$), root length ($P \leq 0.05$), shoot length ($P \leq 0.05$), shoot dry weight ($P \leq 0.001$), and root dry weight ($P \leq 0.05$). The significance of the triple interaction strongly suggests that drought responses among cowpea genotypes were not uniform but rather depended on both genetic background and bacterial inoculation. This finding highlights the complexity of drought adaptation and emphasizes the importance of considering genotype-specific microbial interactions when evaluating drought resilience.

Integration with correlation analysis and PCA

The significant reduction in shoot and root biomass traits under drought stress, as confirmed by ANOVA, was strongly supported by correlation analysis, which revealed positive associations between biomass accumulation and nutrient uptake traits, particularly nitrogen and potassium acquisition. These relationships suggest that maintenance of plant growth under drought conditions was closely linked to nutrient acquisition efficiency.

Furthermore, PCA results reinforced these findings by demonstrating that shoot potassium uptake and shoot nitrogen uptake were major contributors to the dominant principal component (PC1), while root nutrient acquisition traits strongly influenced PC2. The strong contribution of nutrient uptake variables to PCA structure suggests that maintenance of growth under drought stress was largely mediated through efficient nutrient acquisition and metabolic adjustment mechanisms. Therefore, the ANOVA, correlation matrix, and PCA collectively support the conclusion that biomass preservation under drought stress in cowpea is closely associated with nutrient uptake efficiency and genotype-specific physiological adaptation.

Effects of drought stress, PGPR inoculation, and genotype interactions on Physiological traits; Nutrient uptake and Yield and yield-related traits

Table (4). Variance of the Nutrient uptake and Physiological traits of the Four Cowpea genotypes under drought stress

Source of variation	Mean square						
	DF	Shoot N uptake	Shoot P uptake	Shoot K uptake	Root N uptake	Root P uptake	Root K uptake
Genotype (G)	**3**	0.084***	0.006**	0.052***	0.012***	0.004**	0.009***
Water stress (W)	3	0.112***	0.008***	0.073***	0.007*	0.003*	0.006**
Biofertilizer (B)	2	0.095***	0.007***	0.061***	0.010***	0.005**	0.008***
G x W	9	0.029*	0.002 NS	0.017*	0.004 NS	0.001 NS	0.003 NS
G x B	6	0.047**	0.003*	0.024**	0.006*	0.002 NS	0.004*
W x B	6	0.053**	0.004*	0.031**	0.005*	0.002*	0.005*
G x W x B	18	0.041**	0.003*	0.026**	0.005*	0.002*	0.004*
Error	96	0.011	0.001	0.008	0.002	0.001	0.002
CV(%)	-----	14.63	12.71	13.84	16.07	18.92	15.44

Significance: NS = non-significant, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ **CV- Coefficient of variation.**

Effects of drought stress, PGPR inoculation, and genotype interactions on nutrient uptake traits

Three-way ANOVA revealed highly significant effects of genotype (G), water stress level (W), and biofertilizer treatment (B) on most nutrient uptake traits, indicating strong variability in nutrient acquisition efficiency among cowpea accessions under drought stress (**Table 4**). Significant genotypic differences were observed for shoot nitrogen uptake ($P \leq 0.001$), shoot phosphorus uptake ($P \leq 0.01$), shoot potassium uptake ($P \leq 0.001$), root nitrogen uptake ($P \leq 0.001$), root phosphorus uptake ($P \leq 0.01$), and root potassium uptake ($P \leq 0.001$), demonstrating substantial phenotypic diversity among the evaluated germplasm.

Water stress significantly influenced all nutrient uptake traits, particularly shoot N uptake, shoot P uptake, and shoot K uptake, which exhibited highly significant reductions under decreasing field capacity levels ($P \leq 0.001$). Similarly, root nutrient uptake traits were significantly affected by drought stress, although with comparatively lower significance levels. These findings indicate that nutrient acquisition efficiency was highly sensitive to soil moisture depletion, likely due to reduced nutrient mobility, impaired root absorption capacity, and restricted translocation processes under water deficit conditions.

PGPR inoculation significantly enhanced nutrient uptake performance across all measured parameters, with highly significant effects observed for shoot and root nutrient uptake traits. This finding suggests an important role of microbial inoculation in improving nutrient availability and uptake efficiency under drought stress conditions, potentially through biological nitrogen fixation, nutrient solubilization, improved rhizosphere activity, and enhanced root growth.

Regarding interaction effects, the genotype × water stress (G × W) interaction significantly affected shoot nitrogen uptake and shoot potassium uptake, whereas most root nutrient traits exhibited non-significant responses. This suggests that genotype-specific drought responses were more pronounced in shoot nutrient allocation than in root nutrient accumulation. Conversely, genotype × biofertilizer (G × B), water stress × biofertilizer (W × B), and the three-way interaction (G × W × B) significantly influenced most nutrient uptake traits, indicating that PGPR-mediated nutrient acquisition differed according to both genotype and drought intensity.

Importantly, the strong responsiveness of nitrogen and potassium uptake traits to drought and PGPR treatments was consistent with PCA results, where shoot potassium uptake (0.903), root potassium uptake (0.785), shoot nitrogen uptake (0.414), and root nitrogen uptake (0.590) emerged

among the strongest contributors to principal components explaining drought-related phenotypic variation. These findings strongly suggest that nutrient acquisition efficiency, particularly K and N uptake, constituted a central physiological mechanism governing drought adaptation among evaluated cowpea accessions.

Effects of drought stress, PGPR inoculation, and genotype interactions on physiological traits

The evaluated physiological traits exhibited contrasting responses to drought stress and microbial inoculation (**Table 5**). Proline accumulation was strongly influenced by genotype, water stress, and PGPR treatment, all exhibiting highly significant effects ($P \leq 0.001$). Moreover, all interaction terms, including G × W, G × B, W × B, and G × W × B, were highly significant for proline content, indicating substantial genotype-specific variation in osmotic adjustment responses under drought stress.

Table (5). Variance of the Physiological traits of the Four Cowpea genotypes under drought stress

Source of variation	Mean square			
	DF	Proline	chlorophyll	WRC
Genotype (G)	**3**	150.38***	0.018 NS	9.41*
Water stress (W)	3	1251.92***	0.063 NS	18.26***
Biofertilizer (B)	2	1093.45***	0.081*	11.72**
G x W	9	461.10***	0.022 NS	4.83 NS
G x B	6	1191.20***	0.031 NS	6.55*
W x B	6	1066.11***	0.028 NS	7.12*
G x W x B	18	448.05***	0.019 NS	5.94*
Error	96	7.24	0.015	2.17
CV(%)	-----	8.43	10.92	7.86

Significance: NS = non-significant, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ CV- Coefficient of variation.

The pronounced increase in proline accumulation under drought conditions reflects an adaptive osmoprotective response aimed at maintaining cellular osmotic balance, protecting membrane integrity, and reducing oxidative stress damage. The highly significant three-way interaction further indicates that proline accumulation was jointly regulated by genetic background, drought severity, and microbial inoculation, suggesting complex physiological adaptation patterns among accessions.

In contrast, total chlorophyll content showed no significant response to genotype, drought stress, or interaction effects, except for a weak but significant effect of biofertilizer application ($P \leq 0.05$). The relative stability of chlorophyll content under drought stress may indicate preservation of photosynthetic pigments despite moisture limitation, suggesting partial physiological tolerance mechanisms among evaluated genotypes.

Water retention capacity (WRC) was significantly affected by genotype ($P \leq 0.05$), water stress ($P \leq 0.001$), and PGPR treatment ($P \leq 0.01$). Significant G × B, W × B, and G × W × B interactions further confirmed differential genotype-specific water conservation responses under microbial inoculation and drought stress. These findings suggest that drought tolerance among cowpea accessions was partially mediated through improved tissue hydration maintenance and water conservation efficiency.

The physiological responses observed through ANOVA were further supported by PCA interpretation, where water-related adaptation mechanisms likely contributed to secondary variation patterns represented in PC2 and PC3, emphasizing the multidimensional nature of drought adaptation in cowpea.

Effects of drought stress, PGPR inoculation, and genotype interactions on yield traits

Yield and yield-related traits were significantly influenced by genotype, water stress, and PGPR inoculation (**Table 6**). Significant genotypic differences were observed for number of pods per plant ($P \leq 0.001$), pod length ($P \leq 0.001$), pod weight per plant ($P \leq 0.001$), number of seeds per pod ($P \leq 0.01$), and 100-seed weight ($P \leq 0.001$), indicating strong variability in reproductive performance among the evaluated germplasm.

Table (6). Variance of the Yield traits of the Four Cowpea genotypes under drought stress

Source of variation	Mean square					
	DF	Number of pods/plants	Pod length	Pods weight/plant	Number of seeds/pod	100-seed weight
Genotype (G)	3	24.17***	3.84***	42.66***	5.22**	18.91***
Water stress (W)	3	38.94***	2.61**	56.71***	7.11***	9.83**
Biofertilizer (B)	2	16.33**	1.92*	28.44***	3.58*	7.24**
G x W	9	6.11 NS	0.83 NS	8.57*	1.44 NS	2.17 NS
G x B	6	8.94*	1.11 NS	11.42**	1.87*	3.55*
W x B	6	7.26*	1.29*	10.16**	1.95*	3.08*
G x W x B	18	5.73*	0.91 NS	9.34*	1.62*	2.71*
Error	96	2.41	0.54	4.18	0.77	1.34
CV(%)	------	11.28	8.93	12.17	9.54	7.82

Significance: NS = non-significant, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001 CV- Coefficient of variation.

Water stress exerted a pronounced negative effect on reproductive performance, significantly affecting all yield traits, particularly pod weight per plant and number of pods per plant, which exhibited highly significant responses ($P \leq 0.001$). This result demonstrates the severe impact of drought stress on reproductive development and yield formation in cowpea.

Biofertilizer application significantly improved reproductive performance, suggesting a beneficial role of PGPR in alleviating drought-induced reproductive penalties. Significant G × B, W × B, and G × W × B interactions for several yield traits further indicate that yield stability under drought depended strongly on genotype-specific microbial responsiveness.

Interestingly, pod weight per plant exhibited significant responses across nearly all interaction terms, suggesting that yield productivity was highly dependent on the combined effects of genotype, drought intensity, and microbial inoculation. This pattern is biologically meaningful because yield formation under drought is strongly influenced by integrated physiological performance, including nutrient uptake, biomass retention, and osmotic adjustment.

The observed reductions in yield traits under drought stress closely corresponded with correlation and PCA findings. Strong positive relationships between biomass traits and nutrient uptake indicate that maintenance of reproductive performance was closely associated with efficient nutrient acquisition and vegetative growth preservation. Likewise, PCA results demonstrated that nutrient uptake-related variables contributed strongly to the principal axes explaining phenotypic variation, emphasizing their importance in sustaining productivity under drought conditions.

Integrated interpretation of ANOVA, correlation, and PCA

Collectively, ANOVA, correlation analysis, and PCA provided highly consistent evidence that drought tolerance in the evaluated cowpea germplasm was governed by interconnected physiological mechanisms involving nutrient acquisition efficiency, biomass maintenance, osmotic adjustment, and water conservation capacity. While ANOVA confirmed significant treatment effects and genotype-specific responses, correlation analysis revealed functional relationships among growth, nutrient, and yield traits. PCA further identified shoot and root nitrogen and potassium uptake as dominant contributors to phenotypic differentiation under drought stress.

These findings strongly suggest that maintenance of nutrient uptake efficiency, particularly nitrogen and potassium acquisition, constitutes a major adaptive mechanism underlying drought tolerance in Egyptian cowpea germplasm and may therefore serve as reliable indirect selection criteria in cowpea breeding programs targeting water-limited environments.

Hierarchical Clustering and Heat map Analysis Discussion

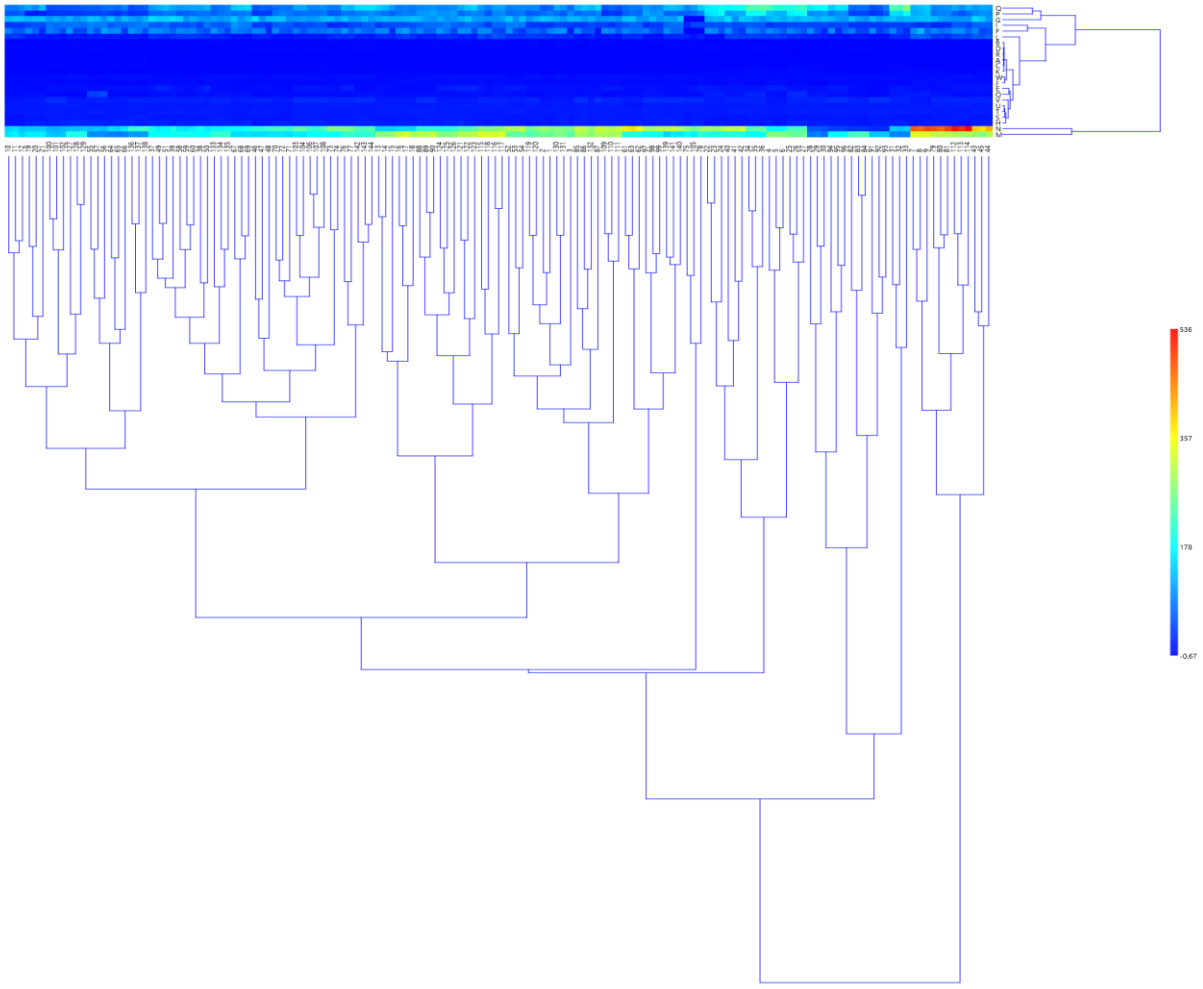


Fig. (1). Hierarchical clustering analysis (HCA) coupled with heatmap visualization provided an integrated multivariate overview of phenotypic variation among cowpea genotypes, water stress treatments, and biofertilizer applications

Unlike univariate statistical approaches, hierarchical clustering enables simultaneous examination of relationships among multiple traits and experimental units, thereby facilitating identification of similarity patterns and adaptive responses under drought stress.

The heatmap revealed substantial heterogeneity in trait expression patterns across experimental treatments, indicating marked phenotypic plasticity among cowpea genotypes under varying drought conditions. Variation in color intensity across clusters suggests differential responses in growth, nutrient uptake, physiological, and yield-related traits, confirming that drought stress induced complex multidimensional changes rather than isolated effects on individual traits. Such multidimensional variation agrees with the ANOVA findings, which demonstrated significant effects of water stress, genotype, and their interactions for many studied traits.

The dendrogram structure showed clear hierarchical grouping among experimental units, suggesting the existence of distinct response profiles under drought stress. Samples clustered together

likely shared similar physiological and agronomic performance, whereas distant clusters indicate contrasting adaptation strategies. The presence of distinct clustering patterns strongly supports the significant genotype × environment interaction observed in the ANOVA analysis, confirming that cowpea genotypes responded differently to reduced field capacity.

A particularly important observation is the apparent formation of separate clusters associated with differential trait expression intensity. Clusters characterized by higher signal intensity in growth, nutrient uptake, and physiological stability traits likely represent drought-tolerant response groups, whereas clusters exhibiting lower trait expression may correspond to drought-sensitive treatments. Physiologically, drought-tolerant clusters are expected to maintain superior water relations, nutrient acquisition efficiency, osmotic adjustment, and biomass retention under stress conditions.

The clustering pattern further suggests that drought adaptation in cowpea may rely on coordinated regulation of multiple physiological mechanisms rather than single-trait responses. Traits involved in nutrient acquisition, biomass accumulation, and water status likely acted synergistically to determine adaptation efficiency. This interpretation is strongly supported by the correlation matrix, which revealed close associations between shoot biomass and nutrient uptake efficiency, as well as strong relationships between root biomass and root nutrient acquisition. Therefore, the clustering results reinforce the hypothesis that integrated root-shoot functioning plays a central role in drought tolerance.

From a physiological perspective, genotypes grouped within high-performance clusters may possess superior mechanisms for maintaining hydraulic conductivity, stomatal regulation, osmotic balance, and nutrient transport under limited water availability. Efficient potassium uptake may contribute to osmotic adjustment and stomatal control, while enhanced nitrogen acquisition likely supports sustained protein synthesis and metabolic activity under stress conditions. Simultaneously, stronger root systems may facilitate access to residual soil moisture and nutrient reserves, thereby supporting improved resilience under drought.

The hierarchical clustering results also complement PCA findings by confirming the existence of structured phenotypic divergence among treatments and genotypes. While PCA identified the principal sources of variation, particularly nutrient uptake and biomass-related traits, hierarchical clustering provided visual evidence of similarity and divergence among experimental units. The consistency between PCA and HCA strengthens the robustness of the multivariate interpretation and confirms that drought adaptation in the evaluated cowpea materials is governed by integrated physiological and nutritional mechanisms.

Importantly, the observed clustering structure provides practical implications for breeding programs. Genotypes consistently grouped within superior-performing clusters under drought conditions may represent valuable parental materials for drought tolerance breeding. Conversely, genotypes positioned in low-performance clusters may possess greater drought sensitivity and therefore lower breeding value under water-limited environments. Thus, hierarchical clustering represents an effective complementary approach for genotype classification and selection under abiotic stress conditions.

Collectively, the integration of ANOVA, correlation analysis, PCA, and hierarchical clustering provides strong evidence that drought tolerance in cowpea is a multicomponent trait governed by coordinated interactions among biomass maintenance, nutrient acquisition efficiency, osmotic adjustment, and water conservation mechanisms. The convergence among these statistical approaches further supports the suitability of shoot dry weight, root dry weight, nutrient uptake efficiency, and water relation traits as reliable indicators for indirect selection of drought tolerance in cowpea breeding programs.

Correlation Analysis among Morph physiological and Nutrient Traits under Drought Stress in Cowpea Genotypes

The correlation matrix revealed substantial variation in the degree and direction of relationships among morph physiological, biochemical, and nutrient uptake traits under drought stress conditions in the evaluated cowpea genotypes **(Table 7).** The obtained relationships provide valuable insights into the adaptive mechanisms involved in drought tolerance and nutrient acquisition.

Table (7). Correlation Matrix among studied traits under overall treatments and types

Variables	Cvs	Treatment	water level	proline	Total Chlorophyll	root length	shoot length	shoot d.w	root d.w	shoot P uptake	shoot N. uptake	shoot K. uptake	root P uptake	root N. uptake	root K. uptake	WRC
Cvs	**1**															
Treatment	0.000	**1**														
water level	0.000	0.000	**1**													
prolien	0.058	-0.227	-0.288	**1**												
Total Chlorophyll	0.008	0.014	0.047	-0.003	**1**											
root length	0.244	0.502	-0.222	-0.056	0.024	**1**										
shoot leng	0.194	0.428	-0.039	0.104	0.071	0.432	**1**									
shoot d.w	0.239	-0.554	-0.203	0.293	0.089	-0.160	-0.150	**1**								
root d.w	-0.448	0.215	0.039	0.179	-0.002	-0.111	-0.095	0.147	**1**							
sh.P up	-0.184	-0.534	-0.001	0.176	0.164	-0.393	-0.111	0.633	0.311	**1**						
sh N. up	0.195	-0.514	-0.197	0.170	0.121	-0.145	-0.180	0.895	0.140	0.532	**1**					
sh K. upta	0.153	-0.284	-0.151	0.118	0.104	-0.250	-0.099	0.612	0.220	0.543	0.523	**1**				
root P up	-0.369	0.099	0.050	0.083	0.057	-0.001	0.136	-0.064	0.525	0.115	-0.102	0.039	**1**			
root N. up	-0.414	0.150	0.017	0.259	-0.047	-0.158	-0.109	0.182	0.929	0.277	0.168	0.197	0.502	**1**		
root K. up	-0.408	0.159	0.071	0.289	-0.074	-0.141	-0.122	0.032	0.835	0.194	-0.011	0.042	0.420	0.831	**1**	
WRC	0.015	-0.250	0.078	-0.038	0.007	-0.137	-0.107	0.210	-0.176	0.223	0.099	-0.021	-0.469	-0.202	-0.110	**1**

A strong positive association was observed between shoot dry weight and shoot nutrient uptake traits, particularly nitrogen uptake (r = 0.895), phosphorus uptake (r = 0.633), and potassium uptake (r = 0.612), indicating that increased biomass accumulation was closely linked with enhanced nutrient acquisition efficiency under drought conditions. These findings suggest that genotypes capable of maintaining higher shoot biomass under water deficit are likely to exhibit superior nutrient absorption and assimilation capacity. Nitrogen, phosphorus, and potassium are essential macronutrients involved in photosynthesis, osmotic regulation, protein synthesis, and energy metabolism; therefore, their improved uptake may contribute directly to drought resilience.

Similarly, root dry weight exhibited strong positive correlations with root nutrient uptake traits, particularly root nitrogen uptake (r = 0.929) and root potassium uptake (r = 0.835), while a strong association was also observed between root nitrogen and potassium uptake (r = 0.831). These findings emphasize the critical role of root system development in sustaining nutrient uptake under drought stress. A vigorous root system may improve soil exploration capacity, facilitating nutrient acquisition from deeper soil layers when water availability becomes limiting. Such responses are considered fundamental adaptive mechanisms enhancing drought tolerance in legumes.

Moderate positive relationships were observed between root length and shoot length (r = 0.432), suggesting coordinated development between belowground and aboveground plant organs. This coordination may represent an adaptive growth strategy that allows plants to optimize water uptake and maintain vegetative growth under moisture-deficit conditions. Furthermore, root length showed a moderate positive correlation with treatment effects (r = 0.502), indicating that the applied treatments may have contributed positively to root elongation, thereby potentially improving drought adaptation.

Proline accumulation exhibited a negative relationship with water level (r = −0.288), indicating increased proline accumulation as water availability decreased. This result is biologically expected because proline is a well-established osmoprotectant that accumulates in plants exposed to drought stress. The positive correlations of proline with shoot dry weight (r = 0.293), root nitrogen uptake (r = 0.259), and root potassium uptake (r = 0.289) further suggest that proline accumulation may contribute to maintaining metabolic activity and cellular osmotic balance under water-limited conditions. The accumulation of compatible solutes such as proline plays a crucial role in protecting cellular structures, scavenging reactive oxygen species, and preserving membrane stability during drought stress.

Interestingly, total chlorophyll content showed generally weak associations with most measured traits, except for a pronounced negative relationship with replication (r = −0.708), whereas its relationships with nutrient uptake and growth parameters remained weak. This may suggest that chlorophyll content was relatively stable across treatments or that drought-induced physiological adjustments affected photosynthetic pigments independently from biomass production and nutrient accumulation. Such findings indicate that chlorophyll content alone may not represent a sufficiently sensitive indicator of drought tolerance in cowpea under the tested conditions.

Shoot nutrient uptake traits exhibited moderate to strong interrelationships. Shoot phosphorus uptake showed positive correlations with shoot nitrogen uptake (r = 0.532) and shoot potassium uptake (r = 0.543), while shoot nitrogen uptake was positively associated with shoot potassium uptake (r = 0.523). These findings indicate a coordinated nutrient acquisition system in the shoot, where balanced uptake of essential macronutrients may contribute synergistically to sustaining plant growth under drought stress.

Water retention capacity (WRC) exhibited weak positive associations with shoot dry weight (r = 0.210) and shoot phosphorus uptake (r = 0.223), suggesting a potential contribution of improved water retention to sustaining plant productivity under drought conditions. However, a moderate negative relationship was observed between WRC and root phosphorus uptake (r = −0.469), which may indicate a compensatory physiological adjustment in nutrient allocation under water deficit conditions. Such differential responses may reflect genotype-dependent mechanisms governing water conservation and nutrient partitioning.

Overall, the correlation analysis demonstrated that drought tolerance in cowpea was strongly associated with biomass production, root development, nutrient uptake efficiency, and osmotic adjustment through proline accumulation. In particular, the strong coordination between dry matter accumulation and nutrient acquisition highlights the importance of integrated physiological responses in enhancing drought resilience. Therefore, traits such as shoot dry weight, root dry weight, nitrogen and potassium uptake, root architecture, and proline accumulation may serve as reliable selection criteria for identifying drought-tolerant cowpea genotypes in breeding programs.

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Principal component analysis

Principal component analysis (PCA) was performed to summarize the multivariate structure of the 19 measured morphological, physiological, nutritional, and yield-related traits. PCA is particularly useful for agronomic datasets containing multiple correlated traits because it reduces the dimensionality of the dataset while retaining the major patterns of trait covariation and allowing the multivariate positioning of observations to be visualized through principal-component scores and biplots (**Matthew, 2025**). The analysis was performed on standardized variables using the correlation matrix, thereby minimizing the influence of differences in measurement scales among traits (**Matthew, 2025)**.

Table (8). Principal component loadings of the 19 measured traits in cowpea

Trait	**PC1 (4.228; 22.09%)**	**PC2 (3.338; 17.45%)**	**PC3 (2.543; 13.29%)**	**PC4 (1.793; 9.37%)**	**PC5 (1.155; 6.04%)**	**PC6 (1.064; 5.56%)**	**PC7 (1.013; 5.29%)**
Proline	0.291	0.241	0.098	0.285	**−0.422**	0.104	0.333
Total chlorophyll	0.006	0.186	−0.147	0.302	**0.509**	−0.419	**0.568**
Plant height	0.447	−0.358	−0.347	−0.034	0.192	−0.166	0.368
Root length	−0.402	−0.168	0.269	**0.430**	0.062	0.399	0.269
Shoot length	−0.241	−0.157	0.071	**0.686**	−0.044	0.430	0.084
Shoot dry weight	0.329	**0.833**	−0.177	0.004	0.073	0.284	0.033
Root dry weight	**0.805**	0.011	**0.471**	0.077	−0.075	−0.070	0.039
Shoot P uptake	**0.568**	**0.538**	−0.309	0.003	0.079	0.070	−0.001
Shoot N uptake	0.276	**0.803**	−0.142	−0.030	0.140	0.256	−0.022
Shoot K uptake	0.312	**0.667**	−0.107	0.156	0.242	−0.056	−0.280
Root P uptake	**0.560**	−0.296	0.324	0.329	0.318	0.086	−0.176
Root N uptake	**0.811**	0.021	**0.454**	0.092	−0.147	−0.062	0.001
Root K uptake	**0.724**	−0.087	**0.462**	0.038	−0.310	−0.164	0.117
WRC	−0.070	0.190	**−0.489**	−0.354	**−0.425**	0.126	0.367
Number of pods/plant	**−0.589**	0.430	**0.543**	−0.008	0.093	−0.078	0.158
Pod length	0.315	−0.199	0.256	**−0.598**	0.289	**0.457**	0.254
Number of seeds/pod	−0.257	**0.557**	**0.552**	−0.246	−0.126	−0.184	0.106
Pods weight/plant	−0.345	0.058	**0.678**	**−0.427**	0.235	0.104	0.045
100-seed weight	**−0.583**	**0.567**	0.211	0.300	−0.127	−0.184	−0.017

Note: Values are standardized PCA loadings based on the correlation matrix. Eigenvalues and explained variance are shown in parentheses in each PC heading. Bold values indicate relatively strong loadings (approximately |loading| ≥ 0.50).

The first seven principal components (PCs) had eigenvalues >1 and collectively accounted for 79.09% of the total standardized variance (Table 1). PC1 explained 22.09% of the total variance, followed by PC2 (17.45%) and PC3 (13.29%), resulting in a cumulative contribution of 52.83%. PC4, PC5, PC6, and PC7 explained an additional 9.37%, 6.04%, 5.56%, and 5.29%, respectively, resulting in 79.09% cumulative explained variance. The predominance of the first three components indicates that a substantial proportion of the phenotypic variation among the evaluated cowpea observations could be represented by three major multivariate dimensions. Similar use of PCA to identify major trait associations under drought stress has been reported in legume germplasm, where the principal components separated morphological, physiological, and yield-associated variation among genotypes and water regimes (**Gutiérrez et al., 2023**).

The loading structure revealed distinct biological dimensions among the principal components (**Table 8**). PC1 was predominantly associated with root biomass and nutrient acquisition, showing strong positive loadings for root N uptake (0.811), root dry weight (0.805), and root K uptake (0.724), followed by shoot P uptake (0.568) and root P uptake (0.560). These traits also showed the largest contributions to PC1, accounting for 15.57%, 15.33%, 12.39%, 7.62%, and 7.41%, respectively (**Table 9**). In contrast, number of pods plant^{-1} (−0.589) and 100-seed weight (−0.583) were negatively associated with PC1 and contributed 8.21% and 8.05%, respectively. Accordingly, PC1 can be interpreted as a **root biomass and nutrient-acquisition axis**, reflecting coordinated variation in below-ground development and mineral acquisition. Root development and nutrient uptake are recognized as important components of plant adaptation to water limitation because changes in root growth can influence the plant's ability to acquire water and nutrients under heterogeneous soil moisture conditions (**Chieb and Gachomo, 2023; El-Saadony *et al.*, 2024**).

Table (9). Contribution (%) of the 19 traits to the first seven principal components

Trait	PC1 (%)	PC2 (%)	PC3 (%)	PC4 (%)	PC5 (%)	PC6 (%)	PC7 (%)
Proline	2.01	1.74	0.38	4.54	15.42	1.02	10.99
Total chlorophyll	0.00	1.04	0.85	5.75	22.41	16.51	**31.79**
Plant height	4.73	3.84	4.74	0.06	3.19	2.59	13.35
Root length	3.81	0.84	2.85	10.29	0.33	15.00	7.12
Shoot length	1.37	0.74	0.20	**26.28**	0.17	**17.45**	0.69
Shoot dry weight	2.57	**20.78**	1.23	0.00	0.46	7.45	0.01
Root dry weight	**15.33**	0.00	8.72	1.05	0.48	0.53	0.15
Shoot P uptake	7.62	8.69	3.75	0.00	0.57	0.46	0.00
Shoot N uptake	1.80	**19.31**	0.79	0.05	1.76	6.15	0.01
Shoot K uptake	2.30	**13.32**	0.45	1.36	5.26	0.29	7.65
Root P uptake	7.41	2.63	4.49	5.96	9.03	0.73	3.02
Root N uptake	**15.57**	0.01	8.20	0.47	1.94	0.37	0.00
Root K uptake	**12.39**	0.23	8.49	0.08	8.62	2.69	1.32
WRC	0.12	1.09	**9.41**	3.93	**16.01**	1.59	13.23
Number of pods/plant	**8.21**	5.55	**11.60**	0.00	0.77	0.60	2.45
Pod length	2.35	1.18	2.58	**20.52**	6.90	**18.00**	6.22
Number of seeds/pod	1.56	**9.28**	**11.99**	3.76	1.31	2.92	1.08
Pods weight/plant	2.81	0.10	**18.09**	10.28	4.77	0.94	0.20
100-seed weight	**8.05**	**9.61**	1.39	6.76	1.18	3.72	0.02
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Contribution (%) = squared loading / sum of squared loadings for the corresponding PC × 100.

PC2 represented a contrasting **shoot biomass and nutrient-acquisition axis**. Shoot dry weight showed the strongest loading (0.833) and the largest contribution (20.78%), followed by shoot N uptake (0.803; 19.31%) and shoot K uptake (0.667; 13.32%). Shoot P uptake (0.538), 100-seed weight (0.567), and number of seeds pod^{-1} (0.557) also contributed appreciably, accounting for 8.69%, 9.61%, and 9.28%, respectively (**Tables 8 and 9**). The simultaneous contribution of shoot biomass and N and K uptake suggests that variation in above-ground productivity was closely associated with nutrient acquisition. This interpretation is consistent with experimental evidence showing that beneficial rhizobacteria can enhance shoot biomass and nutrient acquisition under water limitation, although the magnitude of these effects depends on bacterial strain, host genotype, and environmental conditions **(Jabborova *et al.*, 2021; Chieb and Gachomo, 2023)**.

PC3 was primarily characterized by reproductive and yield-related traits. Pods weight plant^{-1} exhibited the highest loading (0.678) and contribution (18.09%), followed by number of seeds pod^{-1} (0.552; 11.99%) and number of pods plant^{-1} (0.543; 11.60%). Root dry weight, root K uptake, and root N uptake also contributed substantially, whereas WRC showed a negative loading (−0.489) and contributed 9.41% to this component (Tables 1 and 2). Therefore, PC3 is more appropriately interpreted as a yield-component and water-response axis rather than as a direct drought-tolerance axis. This distinction is important because PCA identifies patterns of covariation among measured traits but does not by itself establish a causal drought-tolerance mechanism (**Matthew, 2025**). The association between yield components and physiological water-related traits is nevertheless biologically meaningful because reproductive development is particularly sensitive to water limitation in grain legumes (**Gutiérrez *et al.*, 2023; Mansour *et al.*, 2021**).

PC4 was dominated by plant architectural traits, particularly shoot length (0.686; 26.28%) and pod length (−0.598; 20.52%), with additional contributions from root length (10.29%) and pods weight plant^{-1} (10.28%). PC5 was characterized primarily by physiological variables, particularly total chlorophyll (0.509; 22.41%), WRC (−0.425; 16.01%), and Proline (−0.422; 15.42%). PC6 was mainly associated with pod length, shoot length, total chlorophyll, and root length, whereas PC7 was strongly influenced by total chlorophyll (0.568; 31.79%), plant height (0.368; 13.35%), WRC (0.367; 13.23%), and Proline (0.333; 10.99%). These later components indicate that physiological status, water relations, and plant architecture contributed additional dimensions to the overall phenotypic variation that were not completely represented by the first three PCs. In drought-stressed legumes, changes in photosynthetic pigments, plant water relations, osmoprotectant accumulation, root characteristics, and yield components are recognized as interconnected components of stress adaptation (**Mansour *et al.*, 2021; Chieb and Gachomo, 2023**).

Multivariate positioning of cowpea cultivars under PGPR and water regimes

The PCA score–loading biplots provided an integrated view of how cultivar, microbial treatment, and water regime jointly shaped the multivariate phenotype of cowpea (**Fig. 2 and 3**). The PC1–PC2 biplot accounted for 39.54% of the total standardized variation and revealed distinct multivariate positioning among cultivar × PGPR × water-regime combinations. Cultivar 1 showed a pronounced displacement toward positive PC1 scores under several treatment combinations, particularly T1 at 50% FC (PC1 = 4.47; PC2 = 2.45) and T3 at 100% and 25% FC (PC1 = 4.19 and 4.33, respectively). This positioning coincided with the positive loading direction of root N uptake, root dry weight, and root K uptake on PC1, indicating that these combinations were characterized by a multivariate phenotype strongly associated with below-ground biomass and nutrient acquisition.

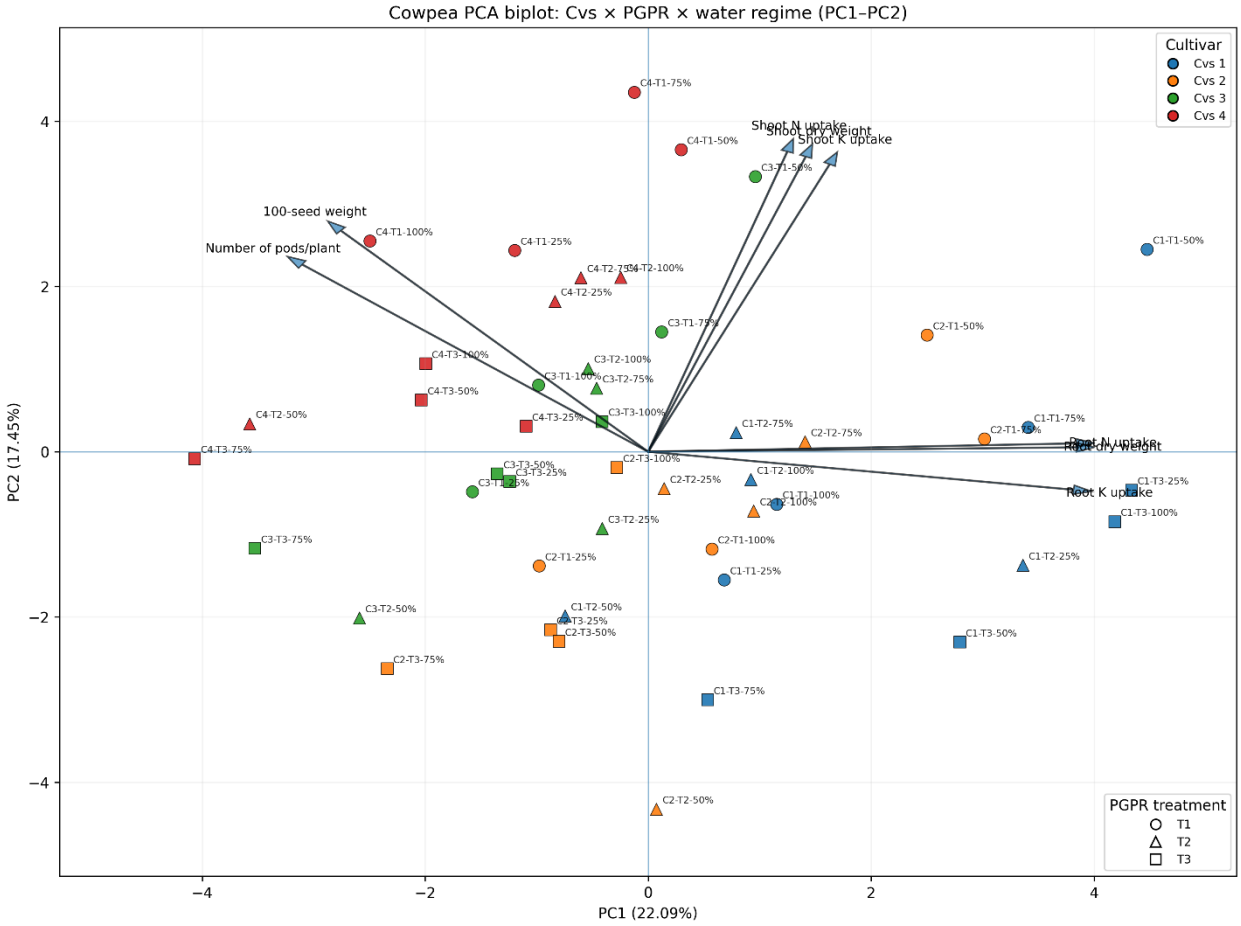


Fig. (2). Cowpea PCA biplot: Cvs × PGPR × water regime (PC1–PC2)

In contrast, cultivar 4 was consistently positioned toward positive PC2 scores under several T1 combinations, including 75% FC (PC1 = −0.13; PC2 = 4.35), 50% FC (0.29; 3.66), and 100% FC (−2.50; 2.55). These positions were aligned with the strong positive loadings of shoot dry weight, shoot N uptake, and shoot K uptake on PC2, suggesting a distinct shoot biomass and nutrient-acquisition phenotype. Cultivars 2 and 3 exhibited greater treatment-dependent shifts across the PCA space, indicating that their multivariate responses were less consistently oriented along a single principal axis.

The PC1–PC3 biplot provided additional resolution of the reproductive dimension of the response, with the two axes jointly accounting for 35.38% of the total variance. The positive direction of PC3 was strongly associated with pods weight plant^{-1}, number of pods plant^{-1}, and number of seeds pod^{-1}, together with root biomass and nutrient-acquisition traits. Notably, cultivar 1 under T3 remained strongly positive on both PC1 and PC3 at 100% FC (4.19 and 3.60, respectively) and 25% FC (4.33 and 3.04, respectively), placing these combinations close to the joint loading directions of root nutrient acquisition and reproductive performance. In contrast, several cultivars × treatment combinations of cultivars 3 and 4 shifted toward negative PC1 values while remaining positive on PC3, indicating a different multivariate organization in which reproductive traits were relatively more prominent than the PC1-associated root nutrient-acquisition syndrome.

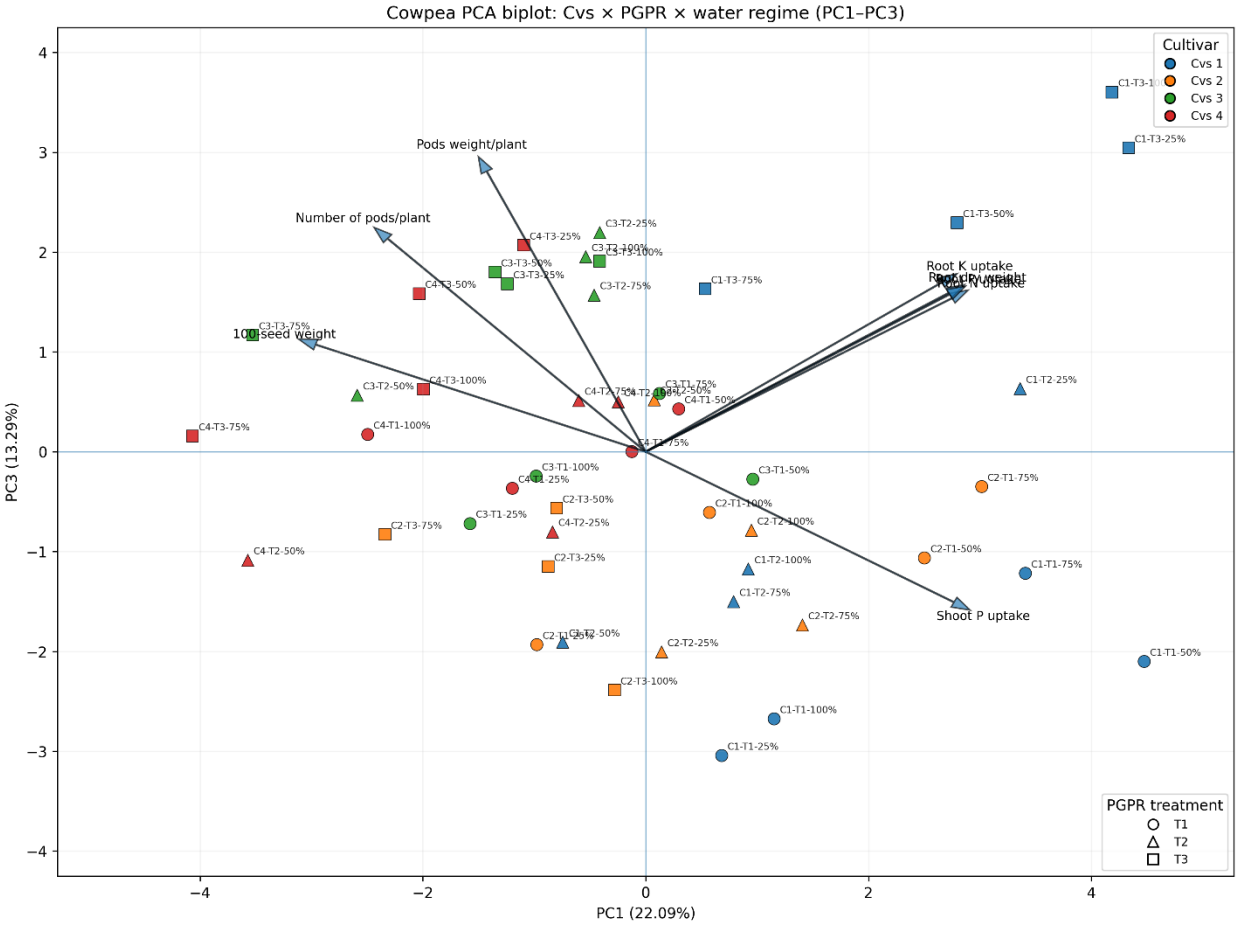


Fig. (3). Cowpea PCA biplot: Cvs × PGPR × water regime (PC1–PC3)

Importantly, the overall mean scores calculated across all cultivars and microbial treatments showed only small differences among water regimes, with PC1 scores ranging from −0.163 to 0.099 and PC3 scores from −0.034 to 0.084 across the four water levels. This pattern indicates that water regime alone did not produce a uniform displacement of all observations in PCA space. Instead, the direction and magnitude of multivariate shifts depended strongly on cultivar and microbial treatment. Thus, the PCA suggests a context-dependent response to water limitation in which the phenotypic expression of drought-associated traits varies according to the genetic background and microbial treatment.

Taken together, the biplots indicate that the response to water limitation was multidimensional and treatment dependent. Positive PC1 positioning was primarily associated with root biomass and nutrient acquisition, positive PC2 positioning with shoot biomass and nutrient acquisition, and positive PC3 positioning with reproductive performance and associated water-response traits. The displacement of individual cultivar × PGPR × water-regime combinations across these axes therefore provides a multivariate framework for identifying treatment combinations associated with contrasting drought-response phenotypes. However, these PCA-derived patterns represent multivariate associations rather than statistical tests of interaction; confirmation of cultivar × PGPR × water-regime effects require the corresponding factorial ANOVA or mixed-model analyses for the individual traits.

4. Discussion

Growth performance of bacteria under different polyethylene glycol 6000 concentrations

The decrease in growth under osmotic stress caused by polyethylene glycol (PEG) is in line with earlier research on *Stenotrophomonas rhizophila*. These bacteria demonstrate adaptive tolerance by accumulating compatible solutes including trehalose and glucosylglycerol, which support cellular homeostasis, despite the fact that osmotic stress normally lengthens the lag phase and slows growth rate **(Alavi *et al.*, 2013)**. Additionally, better survival and long-term development are facilitated by increased biofilm production under stress. These results demonstrate that *S. rhizophila* may continue to develop in spite of decreased water supply and is well suited to drought-like conditions.

PGPR traits under drought stress

Although both strains exhibited plant growth-promoting characteristics under stress circumstances, SprB demonstrated superiority over SprA.

SprB's superior performance across all evaluated parameters (IAA generation, phosphate solubilization, ammonia production, siderophore production, and EPS synthesis) indicates that it is a more effective drought-tolerant PGPR compared to SprA. The enhanced performance may be due to higher metabolic flexibility and stress tolerance mechanisms, suggestive of efficient rhizobacterial strains. **Ryan *et al*. (2009)** demonstrated analogous strain-dependent alterations in *Stenotrophomonas spp*., highlighting the ecological versatility of this genus.

4.1. Drought-induced variation in growth traits and genotype-specific adaptation

The present study demonstrated that drought stress significantly altered most growth-related traits in cowpea, confirming the pronounced inhibitory effect of soil moisture deficit on plant development. Three-way ANOVA revealed significant reductions in root length, shoot length, shoot dry weight, and root dry weight under decreasing field capacity levels, whereas plant height showed relatively limited responsiveness to water stress. These findings indicate that drought primarily constrained biomass accumulation rather than structural elongation, suggesting differential allocation of assimilates under stress conditions.

The observed reduction in vegetative biomass under drought conditions may be attributed to impaired cell expansion, loss of turgor pressure, reduced stomatal conductance, and suppression of photosynthetic carbon assimilation. Under water deficit, plants frequently prioritize survival-related processes over vegetative expansion, thereby limiting dry matter accumulation to reduce metabolic demand (**Fadiji & Santoyo, 2022; Chieb & Gachomo, 2023**). Similar reductions in shoot and root biomass under drought stress have recently been reported in cowpea and other grain legumes, where reduced water availability markedly limited vegetative growth and carbon partitioning efficiency **(Wayal *et al.*, 2026; Bonou *et al.*, 2026**).

Among the studied growth traits, shoot dry weight appeared particularly sensitive to drought stress, exhibiting highly significant responses across both main and interaction effects. This observation suggests that shoot biomass maintenance may represent an effective indicator of drought tolerance in cowpea. The sensitivity of biomass accumulation to water deficit has previously been linked to impaired nutrient translocation, oxidative stress, and reduced photosynthetic activity under limited soil moisture (**Ahmad *et al.*, 2022**).

Interestingly, plant height exhibited comparatively limited variation under drought stress despite significant reductions in biomass-related traits. This pattern suggests that the evaluated cowpea accessions may maintain shoot elongation while reducing tissue density and biomass accumulation as an adaptive response to water deficit. Similar observations have been documented in drought-adapted legumes, where morphological elongation remained relatively stable despite substantial reductions in physiological performance (**Wayal *et al.*, 2026**).

The highly significant genotype effects observed across all growth traits further indicate substantial phenotypic diversity among the evaluated Egyptian cowpea accessions. Such variation strongly supports the existence of exploitable genetic variability for drought adaptation among germplasm conserved in the Egyptian National Gene Bank. Since the evaluated accessions originated from distinct agroecological regions, including arid and semi-arid environments, their differential responses may partially reflect historical adaptation to local climatic conditions. Germplasm originating from water-limited habitats frequently exhibits superior root development, enhanced water conservation, and improved biomass maintenance under drought conditions (**Bonou *et al.*, 2026**).

More importantly, the significant genotype × water stress interaction for shoot dry weight and the highly significant genotype × water stress × biofertilizer interaction across growth traits suggest that drought responses varied substantially among genotypes and were strongly influenced by rhizobacterial inoculation. This finding highlights the complexity of drought adaptation and emphasizes that genotype performance under stress conditions cannot be evaluated independently from rhizosphere interactions. Rather, drought resilience appears to result from dynamic interactions among genetic background, environmental severity, and microbial activity.

4.2. PGPR-mediated alleviation of drought stress

One of the most important findings of the present study was the significant improvement in plant performance following PGPR inoculation under drought stress conditions. Biofertilizer treatments significantly improved most growth, physiological, nutrient uptake, and yield-related traits, demonstrating the beneficial role of rhizobacterial inoculation in mitigating drought-induced damage.

The beneficial effects of *Rhizobium leguminosarum* and *Stenotrophomonas* strains may be attributed to multiple physiological and biochemical mechanisms. Plant growth-promoting rhizobacteria (PGPR) are known to stimulate root system architecture, enhance nutrient acquisition, regulate phytohormone production, and improve plant water relations under stress conditions **(Ahmad *et al.*, 2022; Fadiji *et al.*, 2022).** Rhizobacterial inoculation can further enhance drought resilience through stimulation of auxins, gibberellins, and cytokinins, thereby promoting root elongation and improving access to soil water reserves.

Another important mechanism involves the modulation of stress ethylene production through ACC deaminase activity. Under drought stress, elevated ethylene accumulation commonly inhibits root growth and accelerates senescence. PGPR-producing ACC deaminase can reduce stress ethylene concentration, thereby sustaining root development and biomass accumulation during water deficit **(Fadiji & Santoyo, 2022)**. Such mechanisms may explain the improved growth performance observed in inoculated treatments under severe moisture limitation.

Furthermore, PGPR-mediated enhancement of drought tolerance may involve improved osmotic adjustment and antioxidant defense systems. Beneficial rhizobacteria have been shown to stimulate osmolyte accumulation, increase antioxidant enzyme activity, and reduce oxidative damage caused by reactive oxygen species (ROS) under drought stress **(Gowtham et al., 2022; Chieb & Gachomo, 2023)**. These physiological improvements likely contributed to the superior growth performance observed in inoculated plants.

The highly significant water stress × biofertilizer and genotype × water stress × biofertilizer interactions indicate that microbial effectiveness varied according to drought severity and host genotype. This observation is biologically meaningful because rhizobacterial performance is often conditioned by root exudate composition, genotype compatibility, and rhizosphere dynamics. Recent meta-analytical evidence suggests that PGPR effectiveness tends to increase under moderate to severe drought stress but may differ substantially among genotypes and environmental conditions **(Satognon *et al.*, 2026).**

Therefore, the significant interactions observed in the present study strongly suggest that PGPR-mediated drought alleviation should not be considered a universal response but rather a genotype-dependent phenomenon requiring careful optimization for specific germplasm and environmental conditions.

4.3. Nutrient acquisition efficiency as a determinant of drought tolerance

A major outcome of the present study was the strong responsiveness of nutrient uptake traits to drought stress and microbial inoculation. ANOVA revealed highly significant reductions in shoot and root nitrogen, phosphorus, and potassium uptake under decreasing field capacity levels, indicating substantial impairment of nutrient acquisition under water deficit conditions.

Drought stress commonly restricts nutrient availability and uptake through several mechanisms, including reduced nutrient mobility in dry soils, impaired membrane transport, decreased root hydraulic conductivity, and restricted transpiration-driven mass flow **(Marschner, 2012)**. Under declining soil moisture, nitrogen uptake becomes particularly vulnerable because nitrate transport largely depends on water movement toward the root surface. Similarly, potassium diffusion decreases substantially in dry soils, limiting plant acquisition efficiency **(Taiz *et al.*, 2018).**

The strong reduction observed in nutrient uptake traits under severe drought therefore likely reflects both physical limitations in nutrient transport and physiological restrictions in root absorption capacity. Similar findings have recently been reported in cowpea and other legumes exposed to water deficit stress, where nutrient acquisition efficiency was substantially reduced due to impaired root function and restricted translocation processes **(Wayal *et al.*, 2026).**

Importantly, PGPR inoculation significantly improved nutrient uptake efficiency under drought conditions, suggesting that microbial inoculation partially compensated for drought-induced nutrient limitation. Rhizobacteria may improve nutrient acquisition through biological nitrogen fixation, phosphate solubilization, improved rhizosphere nutrient mobilization, and enhanced root growth **(Ahmad *et al.*, 2022; Fadiji *et al.*, 2022).** These mechanisms may explain the significant increases observed in shoot and root nutrient uptake following bacterial inoculation.

The importance of nutrient acquisition efficiency was further reinforced by correlation and PCA analyses. Strong positive correlations between biomass traits and nutrient uptake suggest that maintenance of vegetative growth under drought was closely linked to efficient nutrient acquisition. Moreover, PCA identified shoot potassium uptake, root potassium uptake, shoot nitrogen uptake, and root nitrogen uptake among the strongest contributors to phenotypic differentiation, indicating their major role in drought adaptation.

Physiologically, potassium is essential for stomatal regulation, osmotic balance, enzyme activation, and maintenance of cellular turgor under drought conditions. Efficient potassium uptake enables improved water-use efficiency and stabilization of metabolic processes during dehydration stress **(Marschner, 2012)**. Similarly, nitrogen plays a critical role in chlorophyll biosynthesis, protein synthesis, and maintenance of photosynthetic activity, thereby contributing directly to biomass preservation and drought resilience **(Taiz *et al.*, 2018).**

Taken together, the convergence of ANOVA, correlation analysis, and PCA strongly suggests that nutrient acquisition efficiency—particularly nitrogen and potassium uptake—constitutes one of the principal physiological mechanisms governing drought tolerance in Egyptian cowpea germplasm and may therefore serve as a reliable indirect selection criterion in breeding programs targeting water-limited environments.

4.4. Physiological adaptation mechanisms: proline accumulation, chlorophyll stability, and water retention capacity

The physiological responses observed in the present study provided important insights into the adaptive mechanisms underlying drought tolerance among the evaluated cowpea germplasm. Proline accumulation, chlorophyll content, and water retention capacity (WRC) exhibited contrasting responses to drought stress and microbial inoculation, reflecting the multidimensional nature of drought adaptation.

Among the evaluated physiological traits, proline accumulation exhibited particularly strong responsiveness to drought stress, genotype, and PGPR inoculation, with highly significant effects

observed across both main and interaction terms. The marked increase in proline accumulation under severe moisture deficit suggests activation of osmotic adjustment mechanisms aimed at maintaining cellular homeostasis during dehydration stress.

Proline is widely recognized as one of the major compatible osmolytes accumulated in plants exposed to drought stress. It contributes to osmotic adjustment, membrane stabilization, protein protection, ROS scavenging, and maintenance of enzyme activity under dehydration conditions **(Fadiji & Santoyo, 2022; Chieb & Gachomo, 2023)**. Increased proline accumulation may therefore represent an adaptive strategy enabling drought-exposed cowpea plants to preserve cellular integrity and sustain metabolic activity under reduced water availability.

The highly significant genotype × water stress × biofertilizer interaction for proline content further indicates substantial variation in osmotic adjustment capacity among evaluated accessions. This observation strongly suggests that proline accumulation was not solely stress-driven but also influenced by genotype-specific physiological plasticity and microbial stimulation. Recent studies reported that drought-tolerant legume genotypes generally exhibit greater proline accumulation compared with sensitive genotypes, highlighting its importance as a physiological marker of drought resilience **(Ahmad *et al.*, 2022; Wayal *et al.*, 2026).**

Moreover, PGPR inoculation may have contributed to enhanced proline accumulation through modulation of drought-responsive metabolic pathways. Rhizobacteria are known to stimulate osmolyte biosynthesis through hormonal regulation and signaling pathways associated with abiotic stress tolerance **(Gowtham *et al.*, 2022).** Consequently, the observed increase in proline content among inoculated treatments likely reflects improved osmotic protection mediated by plant–microbe interactions.

In contrast, total chlorophyll content exhibited relatively limited responsiveness to drought stress and genotype effects. The relative stability of chlorophyll concentration observed in the present study suggests partial maintenance of photosynthetic pigment integrity despite moisture limitation. Such stability may indicate tolerance-related mechanisms that prevent chlorophyll degradation under drought stress.

Under severe drought conditions, chlorophyll degradation commonly occurs as a consequence of oxidative stress, membrane instability, and inhibition of chlorophyll biosynthesis. However, drought-tolerant genotypes frequently maintain relatively stable chlorophyll levels through improved antioxidant protection and enhanced cellular homeostasis **(Taiz *et al.*, 2018).** Similar observations have been reported in drought-adapted cowpea and legume genotypes where photosynthetic pigment stability was maintained despite reductions in vegetative growth and biomass production (**Bonou *et al.*, 2026**).

Water retention capacity (WRC), however, exhibited pronounced sensitivity to drought stress and microbial inoculation. Significant reductions in WRC under severe water deficit indicate impaired tissue hydration and reduced cellular water balance. Nevertheless, the significant genotype × biofertilizer and genotype × water stress × biofertilizer interactions indicate that some accessions maintained tissue hydration more effectively than others, particularly following PGPR inoculation.

Maintenance of tissue hydration is considered a critical physiological mechanism contributing to drought resilience because it sustains stomatal function, metabolic activity, membrane stability, and enzymatic processes under water deficit conditions **(Marschner, 2012).** Genotypes capable of preserving higher water retention under drought often exhibit superior growth maintenance and productivity stability **(Taiz *et al.*, 2018).** Therefore, the significant variation observed in WRC among cowpea accessions strongly supports its usefulness as a physiological indicator for drought screening.

Collectively, the physiological findings of the present study suggest that drought adaptation among evaluated cowpea germplasm involved coordinated regulation of osmotic adjustment, hydration maintenance, and photosynthetic stability. In particular, proline accumulation and WRC emerged as reliable physiological indicators associated with drought resilience and therefore may serve as valuable selection criteria in future cowpea breeding programs.

4.5. Yield penalties under drought stress and genotype resilience

The present study demonstrated that drought stress significantly reduced reproductive performance in cowpea, as evidenced by substantial declines in pod number, pod weight, seed number, and 100-seed weight under decreasing field capacity levels. These findings confirm the high sensitivity of reproductive processes to moisture limitation and emphasize the severe impact of drought stress on cowpea productivity.

Yield reduction under drought stress may largely be explained by impaired assimilate production, reduced photosynthetic activity, hormonal imbalance, reproductive abortion, and restricted translocation of nutrients toward developing pods and seeds **(Ahmad *et al.*, 2022).** Water deficit frequently limits flowering success, pollen viability, fertilization efficiency, and seed filling, resulting in substantial reductions in final productivity **(Wayal *et al.*, 2026).**

Among the evaluated yield traits, pod weight per plant appeared particularly sensitive to drought stress, exhibiting highly significant responses across multiple interaction terms. This finding is biologically meaningful because pod weight integrates cumulative physiological performance throughout plant development, including biomass production, nutrient acquisition, water status maintenance, and assimilate partitioning.

The highly significant genotypic variation observed across yield-related traits further indicates substantial differences in reproductive resilience among evaluated cowpea accessions. Such variation strongly suggests the presence of exploitable drought-adaptive traits among Egyptian germplasm conserved at the National Gene Bank. Genotypes capable of maintaining superior reproductive performance under moisture limitation may possess more efficient water-use efficiency, nutrient allocation, osmotic adjustment, and source–sink relationships **(Bonou *et al.*, 2026).**

Importantly, PGPR inoculation significantly improved yield-related traits under drought stress conditions. The beneficial effects of microbial inoculation on reproductive performance may be attributed to enhanced nutrient availability, improved root growth, increased osmotic adjustment, and improved hormonal regulation under stress conditions **(Fadiji *et al.*, 2022; Chieb & Gachomo, 2023).**

The highly significant genotype × water stress × biofertilizer interactions observed for several yield traits further emphasize the importance of genotype-specific microbial responsiveness. These findings indicate that microbial effectiveness varied according to both drought severity and host genotype, reinforcing the importance of considering plant–microbe compatibility when designing drought mitigation strategies.

Overall, the observed reductions in yield traits under drought stress, together with the beneficial effects of PGPR inoculation, strongly suggest that integrated management strategies combining drought-tolerant germplasm and beneficial rhizobacteria may represent an effective approach for improving cowpea productivity under water-limited environments.

4.6. Multivariate interpretation of drought adaptation: correlation analysis, PCA, loading matrix, scree plot, and 3D scatter visualization

Beyond the univariate interpretation provided by ANOVA, multivariate analyses offered important insights into the complex physiological and agronomic relationships governing drought adaptation in the evaluated cowpea germplasm. Correlation analysis, principal component analysis (PCA), loading matrix interpretation, scree plot evaluation, and three-dimensional PCA visualization collectively provided a robust framework for understanding the interconnected mechanisms underlying drought resilience.

The correlation matrix revealed strong positive associations among growth traits, nutrient uptake parameters, and yield-related variables, indicating substantial physiological interdependence among these traits. In particular, shoot dry weight and root dry weight exhibited strong positive correlations with nitrogen and potassium uptake traits, suggesting that biomass preservation under drought conditions was strongly dependent on nutrient acquisition efficiency.

Such relationships are physiologically meaningful because maintenance of plant biomass under water deficit requires sustained nutrient absorption, particularly nitrogen and potassium, which support photosynthetic activity, osmotic regulation, enzymatic metabolism, and cellular integrity **(Marschner, 2012**). Similar strong associations between biomass accumulation and nutrient uptake efficiency under drought stress have recently been reported in cowpea and other legumes, where nutrient acquisition capacity emerged as a major determinant of drought resilience (**Wayal *et al.*, 2026).**

Furthermore, positive correlations observed between physiological and yield-related traits suggest that drought adaptation in cowpea involved integrated responses rather than isolated mechanisms. Traits associated with tissue hydration and osmotic adjustment likely contributed indirectly to productivity maintenance through preservation of physiological function under moisture limitation. This observation reinforces the concept that drought tolerance is a quantitative and multicomponent phenomenon involving coordinated regulation of growth, metabolism, and reproductive performance **(Ahmad *et al.*, 2022; Bonou *et al.*, 2026).**

The PCA provides an integrated representation of the cowpea response to contrasting water regimes and microbial treatments. The first three components explained 52.83% of the total standardized variance and separated the multivariate phenotype into three major functional dimensions: root biomass and nutrient acquisition (PC1), shoot biomass and nutrient acquisition (PC2), and reproductive performance associated with water-response traits (PC3). Such multidimensional organization is consistent with the current understanding that drought adaptation in legumes involves coordinated changes in root development, mineral acquisition, shoot growth, plant water status, photosynthetic performance, and reproductive allocation rather than a single diagnostic trait (**Chieb and Gachomo, 2023; El-Saadony et al., 2024**).

The strong contribution of root N uptake, root dry weight, and root K uptake to PC1 is particularly relevant to drought physiology. Root biomass determines the potential soil volume explored by the plant, while nitrogen and potassium acquisition are closely connected with biomass formation, osmotic regulation, stomatal function, and maintenance of physiological activity under water deficit. Beneficial rhizobacteria can influence several of these processes through mechanisms involving nutrient mobilization, nitrogen fixation, root development, phytohormonal regulation, and stress-responsive metabolism (**Chieb and Gachomo, 2023; El-Saadony et al., 2024).** In cowpea specifically, inoculation with stress-tolerant rhizobacteria has been associated with enhanced nutrient uptake, root biomass, photosynthetic activity, and molecular responses under drought and nutrient-deficit conditions (**Abiala *et al.*, 2022**). These observations provide biological support for interpreting the PC1 structure as an integrated root-resource acquisition dimension.

The PC2 structure further emphasizes the importance of shoot biomass and nutrient acquisition. The strong contributions of shoot dry weight, shoot N uptake, and shoot K uptake suggest that above-ground growth was a major component of the multivariate response. This pattern is consistent with studies in legumes and other crops showing that PGPR inoculation under drought can improve biomass accumulation and nutrient status. For example, PGPR treatments in soybean have been associated with improved root and shoot growth and enhanced nutrient status under drought conditions (**Jabborova *et al.*, 2021**), while drought-tolerant rhizobacteria have also been reported to stimulate shoot biomass under severe water limitation in Mung bean (**Mondal and Gera, 2024)**. Thus, the PC2 axis provides a useful framework for evaluating whether particular cowpea × PGPR combinations were associated with improved above-ground productivity under water limitation.

The PC3 pattern is particularly informative because it integrates reproductive performance with water-related and root-associated traits. Pods weight plant^{-1}, number of seeds pod^{-1}, and number of pods plant^{-1} were the dominant contributors, while WRC showed a substantial negative loading. This suggests that reproductive traits constituted a major source of multivariate variation along an axis that also included plant water status. Similar multivariate approaches have been used in legume drought studies to identify coordinated associations among yield components, physiological traits, and stress responses **(Gutiérrez *et al.*, 2023; Mansour *et al.*, 2021**). However, the negative sign of the WRC loading should

not be interpreted as evidence that lower water content promotes yield. The sign of an individual principal component is arbitrary and can be reversed without changing the underlying PCA solution; interpretation should therefore emphasize the magnitude and relative orientation of loadings rather than assigning causal meaning to the sign alone (**Matthew, 2025**).

The prominence of total chlorophyll, WRC, and Proline in PC5 and PC7 further indicates that physiological stress responses formed additional dimensions of the cowpea phenotype. Maintenance of chlorophyll is associated with preservation of photosynthetic capacity, whereas proline accumulation is commonly considered part of osmotic and stress-responsive metabolism. WRC provides an indicator of tissue hydration and plant water status. In drought-stressed Faba bean, PGPR inoculation has been reported to improve photosynthetic pigments, water relations, root characteristics, and yield-related traits, with responses varying among cultivars and irrigation regimes (**Mansour *et al.*, 2021**). Similarly, PGPR-mediated improvement of drought responses has been linked to coordinated effects on root growth, water relations, photosynthetic performance, nutrient acquisition, and stress signaling (**Chieb and Gachomo, 2023; El-Saadony *et al.*, 2024**).

Importantly, the PCA should not be interpreted as an independent statistical test of the cultivar × PGPR × water-regime interaction. PCA is primarily an exploratory and pattern-detection procedure that identifies multivariate trait associations and positions observations according to their scores; treatment effects and interactions require confirmation using an appropriate factorial ANOVA or mixed-effects model (**Matthew, 2025**). Consequently, the PCA in the present study should be regarded as complementary to the univariate inferential analyses rather than as a replacement for them.

Taken together, the PCA demonstrates that the response of cowpea to water limitation was multidimensional. Root biomass and nutrient acquisition constituted the dominant multivariate axis, followed by shoot biomass and nutrient acquisition, whereas reproductive performance and water-related traits formed a third major dimension. The later components additionally captured variation in plant architecture, chlorophyll status, water relations, and proline accumulation. The first seven PCs accounted for 79.09% of the total standardized variation, providing a comprehensive framework for interpreting the phenotypic responses of cowpea genotypes and PGPR treatments across contrasting water regimes. The biological relevance of this multivariate structure is further supported by previous studies showing that rhizobacterial inoculation can modify root development, nutrient acquisition, water relations, photosynthetic performance, and yield under drought conditions (**Abiala *et al.*, 2022; Chieb and Gachomo, 2023; El-Saadony *et al.*, 2024).**

Importantly, the strong consistency among ANOVA, correlation analysis, PCA, loading matrix interpretation, scree plot validation, and three-dimensional visualization substantially strengthens the reliability of the present findings. While ANOVA identified statistically significant treatment and interaction effects, correlation analysis clarified functional relationships among traits, and PCA identified the principal physiological drivers governing phenotypic differentiation under drought stress. Such convergence among independent statistical approaches considerably enhances confidence in the biological interpretation of drought adaptation mechanisms.

4.7. Implications for drought breeding and germplasm improvement in cowpea

The findings of the present study have important implications for drought tolerance breeding and sustainable cowpea improvement programs, particularly in arid and semi-arid regions facing increasing water scarcity. The substantial genotypic variation observed among Egyptian cowpea germplasm demonstrates the existence of valuable adaptive diversity that may be exploited in breeding programs targeting climate resilience.

The integration of ANOVA, correlation analysis, and PCA consistently identified nitrogen uptake, potassium uptake, shoot dry weight, proline accumulation, and water retention capacity as major contributors to drought adaptation. These traits therefore represent promising indirect selection criteria for screening drought-tolerant cowpea genotypes under controlled and field conditions.

Indirect selection based on physiological and nutritional indicators is often more efficient than direct yield-based selection under drought environments because yield is strongly influenced by environmental variability and genotype × environment interactions (**Yan & Kang, 2003**). Consequently, identification of stable drought-associated traits may substantially accelerate breeding efficiency and improve selection accuracy.

The significant genotype × water stress × biofertilizer interaction observed in the present study further suggests that drought tolerance should not be interpreted solely as a genotype characteristic but rather as a genotype-dependent response influenced by microbial interactions. Therefore, breeding strategies integrating both host genetic adaptation and beneficial rhizosphere microorganisms may provide greater resilience under water-limited environments.

Moreover, the beneficial effects observed following inoculation with *Rhizobium leguminosarum* and *Stenotrophomonas* strains highlight the potential of PGPR-assisted crop improvement as a sustainable drought mitigation strategy. Integration of drought-tolerant germplasm with microbial bioinoculants may contribute substantially to reducing irrigation demand, improving nutrient-use efficiency, and enhancing productivity stability under climate change scenarios (**Fadiji *et al.*, 2022; Satognon *et al.*, 2026**).

Taken together, the evaluated Egyptian cowpea germplasm represents an important genetic resource for future drought breeding efforts, and the identified physiological and nutritional traits may provide useful targets for selection programs aimed at developing climate-resilient cowpea cultivars adapted to water-scarce environments.

4.8. Comparative drought tolerance among the evaluated cowpea genotypes

The significant genotypic variation observed across growth, physiological, nutrient uptake, and yield-related traits strongly suggests the existence of differential drought adaptation strategies among the evaluated Egyptian cowpea accessions. Such variability highlights the potential value of conserved germplasm maintained at the Egyptian National Gene Bank as an important reservoir of adaptive traits for drought tolerance breeding.

Although all evaluated genotypes exhibited varying degrees of drought-induced performance reduction under decreasing field capacity levels, the significant genotype × water stress and genotype × water stress × biofertilizer interactions indicate that drought response was genotype-dependent rather than uniform across accessions. Genotypes capable of maintaining superior biomass accumulation, nutrient uptake efficiency, water retention capacity, and reproductive performance under severe water deficit may therefore be considered relatively more drought tolerant.

Maintenance of shoot and root biomass under drought conditions is frequently regarded as an important indicator of adaptive capacity because it reflects sustained carbon assimilation, improved water acquisition, and efficient assimilate partitioning under stress conditions **(Taiz *et al.*, 2018**). Similarly, maintenance of nutrient uptake, particularly nitrogen and potassium acquisition, may indicate superior physiological resilience because these nutrients contribute directly to osmotic regulation, stomatal function, protein synthesis, and photosynthetic efficiency (**Marschner, 2012).**

Furthermore, accessions exhibiting higher proline accumulation and improved water retention capacity under reduced field capacity likely possessed greater osmotic adjustment ability and cellular protection mechanisms against dehydration stress. Such physiological plasticity may explain superior performance under severe drought conditions. Similar genotype-specific drought responses have recently been reported in cowpea and other grain legumes, where tolerant genotypes maintained relatively higher nutrient acquisition, biomass accumulation, and reproductive stability under water deficit stress (**Wayal *et al.*, 2026; Bonou *et al.*, 2026).**

The PCA clustering patterns further supported genotype-specific differentiation by demonstrating clear spatial separation among accessions and treatment combinations. Genotypes positioned closer to favorable growth, nutrient uptake, and physiological traits likely possessed superior adaptive

performance under drought stress. Consequently, the evaluated Egyptian cowpea germplasm may represent a promising source of drought-adaptive traits for future breeding programs targeting climate resilience and water-limited agricultural environments.

4.9. Future perspectives and practical implications

The findings of the present study provide important opportunities for improving drought tolerance breeding strategies in cowpea, particularly under arid and semi-arid environments increasingly affected by water scarcity and climate variability. The significant phenotypic diversity observed among Egyptian cowpea germplasm strongly highlights the importance of National Gene Bank collections as valuable genetic reservoirs for crop improvement programs.

Given the strong contribution of nutrient uptake traits, osmotic adjustment, and biomass maintenance to drought adaptation, future breeding efforts should prioritize the integration of physiological and nutritional indicators into selection programs. In particular, **nitrogen uptake, potassium uptake, shoot dry weight, proline accumulation, and water retention capacity** emerged as reliable indicators associated with drought resilience and therefore may serve as practical indirect selection criteria for identifying tolerant genotypes under controlled and field conditions.

Future studies integrating **physiological screening, molecular characterization, genomic analysis, and marker-assisted selection (MAS)** are strongly recommended to validate candidate drought-responsive traits and accelerate breeding progress in cowpea. Identification of genomic regions associated with nutrient-use efficiency, osmotic adjustment, and drought resilience could substantially improve selection accuracy and breeding efficiency **(Yan & Kang, 2003).**

Moreover, the beneficial effects observed following inoculation with *Rhizobium leguminosarum* and *Stenotrophomonas* strains suggest that PGPR-assisted crop management may represent a sustainable strategy for mitigating drought stress under reduced irrigation conditions. The integration of drought-tolerant germplasm with microbial bioinoculants may improve nutrient-use efficiency, reduce irrigation requirements, and enhance productivity stability under water-limited environments **(Fadiji *et al.*, 2022; Satognon *et al.*, 2026).**

Nevertheless, additional validation under multilocation field conditions and across multiple growing seasons remains necessary to confirm the stability of drought tolerance responses identified in the present greenhouse study. Expanding the number of evaluated germplasm accessions and incorporating genotype × environment interactions will further strengthen the applicability of drought resilience screening strategies in cowpea improvement programs.

Collectively, the integration of drought-adaptive germplasm, physiological trait-based selection, and beneficial microbial inoculation represents a promising pathway for developing climate-resilient cowpea cultivars capable of maintaining productivity under increasing water scarcity.

5. Conclusion

The present study demonstrated substantial variation in drought tolerance among four Egyptian cowpea (*Vigna unguiculata* L.) germplasm accessions conserved at the National Gene Bank of Egypt under contrasting water regimes and PGPR inoculation. Three-way ANOVA revealed that drought stress significantly altered most growth, physiological, nutrient uptake, and yield-related traits, confirming the pronounced impact of water limitation on cowpea performance. The highly significant genotype effects further indicated the presence of exploitable genetic variability for drought adaptation among the evaluated accessions.

The findings clearly demonstrated that drought-induced reductions in biomass accumulation, nutrient uptake efficiency, and reproductive performance intensified with decreasing field capacity, particularly under severe water stress (25% FC). However, PGPR inoculation, including *Rhizobium leguminosarum* and *Stenotrophomonas* strains, partially mitigated drought-induced damage by improving plant growth, nutrient acquisition, tissue hydration, and reproductive performance. The

significant three-way interaction (G × W × B) further confirmed that drought response was genotype-dependent and strongly influenced by microbial inoculation.

Importantly, the integration of ANOVA, correlation analysis, and principal component analysis (PCA) consistently identified nutrient acquisition efficiency, particularly nitrogen and potassium uptake, as major determinants of drought adaptation. PCA revealed that shoot potassium uptake, root potassium uptake, shoot nitrogen uptake, and root nitrogen uptake contributed strongly to phenotypic differentiation under drought stress, suggesting their importance as physiological indicators of drought resilience. In addition, proline accumulation and water retention capacity emerged as important adaptive mechanisms involved in osmotic regulation and maintenance of tissue hydration under moisture deficit conditions.

Collectively, these findings suggest that efficient nutrient acquisition, biomass preservation, osmotic adjustment, and improved plant–microbe interactions constitute key mechanisms underlying drought tolerance in Egyptian cowpea germplasm. Therefore, nitrogen and potassium uptake-related traits, together with proline accumulation and water retention capacity, may serve as reliable indirect selection criteria for breeding drought-resilient cowpea cultivars suitable for water-limited environments.

Future studies integrating physiological screening with molecular and genomic approaches are recommended to validate candidate drought-responsive traits and accelerate breeding programs targeting climate-resilient cowpea improvement.

Recommendations

Based on the findings of the present study, the following recommendations can be proposed:

1. **Promising cowpea germplasm identified in the present study should be further evaluated under field conditions** across multiple environments and growing seasons to validate their drought tolerance stability under natural agricultural conditions.
2. **Nitrogen and potassium uptake-related traits should be prioritized as indirect selection criteria in cowpea breeding programs**, as multivariate analyses consistently identified these traits as major contributors to drought adaptation and productivity maintenance under water-limited environments.
3. **Physiological indicators such as proline accumulation and water retention capacity (WRC) may serve as reliable screening tools for drought tolerance**, particularly during early-stage evaluation of cowpea germplasm under controlled stress conditions.
4. **The application of plant growth-promoting rhizobacteria (PGPR), particularly *Rhizobium leguminosarum* and *Stenotrophomonas* strains, is recommended as a sustainable strategy to alleviate drought stress**, improve nutrient acquisition efficiency, and enhance growth and reproductive performance under reduced irrigation regimes.
5. **The significant genotype × drought × PGPR interaction observed in the present study suggests that microbial inoculation strategies should be genotype-specific rather than generalized**, emphasizing the importance of host–microbe compatibility in drought mitigation programs.
6. **Egyptian cowpea germplasm conserved at the National Gene Bank represents a valuable genetic resource for climate-resilient breeding**, and should therefore be further exploited in drought tolerance breeding programs aimed at developing cultivars adapted to arid and semi-arid environments.
7. **Future research integrating physiological, molecular, and genomic approaches is strongly recommended** to identify drought-responsive genes, validate adaptive physiological traits, and accelerate marker-assisted selection for drought tolerance in cowpea.

8. **Future studies should expand the number of germplasm accessions and include multilocation field trials**, allowing broader assessment of genotype × environment interactions and improving the generalizability of drought tolerance conclusions.
9. **Reduced irrigation strategies (50–75% field capacity) combined with PGPR inoculation may provide a promising management approach for improving water-use efficiency in cowpea cultivation under water-scarce environments**, although field-scale validation remains necessary.

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الاستجابات التكيفية للموارد الوراثية المصرية للوبيا للإجهاد المائي: تحليل متعدد المتغيرات في ظل مستويات ري متباينة ومعاملة بالبكتيريا المحفزة لنمو النبات (PGPR)

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الملخص العربي

يُعد الإجهاد المائي (الجفاف) أحد أهم العوامل غير الحيوية التي تحد من إنتاجية اللوبيا، خاصةً في المناطق الجافة وشبه الجافة، مما يستدعي البحث عن تراكيب وراثية متحملة للجفاف واستراتيجيات حيوية مستدامة للتخفيف من آثاره السلبية. هدفت هذه الدراسة إلى تقييم استجابة أربعة تراكيب وراثية من اللوبيا (*Vigna unguiculata* L.) محفوظة بالبنك القومي للجينات المصري للإجهاد المائي تحت مستويات مختلفة من السعة الحقلية، ودراسة دور بكتيريا تعزيز نمو النبات (PGPR) في تحسين تحمل الجفاف. أُجريت التجربة داخل صوبة البنك القومي للجينات بمركز البحوث الزراعية – الجيزة خلال الموسم الزراعي 2024–2025 باستخدام تصميم القطاعات الكاملة العشوائية (RCBD) في تجربة عاملية ثلاثية بثلاث مكررات. اشتملت المعاملات على أربعة تراكيب وراثية من اللوبيا(NGB-1119412 ، NGB-1119371، NGB-1119457، و(NGB-1119502، وأربعة مستويات من السعة الحقلية (100%، 75%، 50%، و25%)، وثلاث معاملات بكتيرية شملت *Rhizobium leguminosarum* و*Stenotrophomonas* sp.-1 و*Stenotrophomonas* sp.-2. وتم تقييم تسع عشرة صفة نمو وفسيولوجية وغذائية ومحصولية. أظهرت نتائج تحليل التباين الثلاثي (ANOVA) وجود تأثيرات معنوية عالية للإجهاد المائي، والتراكيب الوراثية، والمعاملات البكتيرية، بالإضافة إلى تفاعلاتها المختلفة، على معظم الصفات المدروسة، مما يعكس وجود تباين واضح في استجابة التراكيب الوراثية للإجهاد المائي. كما أدى انخفاض السعة الحقلية إلى انخفاض معنوي في صفات النمو، وامتصاص العناصر الغذائية، والمحصول، في حين ساهمت المعاملات البكتيرية في الحد جزئيًا من التأثيرات السلبية للجفاف وتحسين الأداء النباتي. وأظهر تحليل الارتباط وجود علاقات ارتباط موجبة قوية بين تراكم المادة الجافة وكفاءة امتصاص العناصر الغذائية، خاصة النيتروجين والبوتاسيوم. كما أوضح تحليل المكونات الرئيسية (PCA)أن المكونات الثلاثة الأولى فسرت 91.30% من التباين الكلي، حيث برز امتصاص البوتاسيوم والنيتروجين في المجموع الخضري والجذري كأهم الصفات المساهمة في تفسير التباين المرتبط بتحمل الجفاف. تشير النتائج إلى أن كفاءة امتصاص العناصر الغذائية، خاصة النيتروجين والبوتاسيوم، والمحافظة على الكتلة الحيوية، والتكيف الأسموزي من خلال تراكم البرولين، والحفاظ على المحتوى المائي للأنسجة، تمثل آليات رئيسية لتحمل الجفاف في التراكيب الوراثية المصرية من اللوبيا. وعليه، يمكن اعتماد هذه الصفات كمؤشرات انتخاب غير مباشرة في برامج تربية اللوبيا الهادفة إلى استنباط أصناف متحملة للجفاف وملائمة للبيئات محدودة المياه.

الكلمات المفتاحية : اللوبيا، الإجهاد المائي، السعة الحقلية، بكتيريا تعزيز نمو النبات، تحليل التباين، تحليل المكونات الرئيسية، امتصاص العناصر الغذائية، تحمل الجفاف.