ISSN Print: 2664-6064
ISSN Online: 2664-6072
NAAS Rating (2025): 4.69
IJAN 2025; 7(8): 219-222
www.agriculturejournal.net
Received: 13-05-2025
Accepted: 17-06-2025

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Effect of seed bio-priming with PGPR on seedling establishment of finger millet under drought

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DOI: https://www.doi.org/10.33545/26646064.2025.v7.i8d.601

Abstract
An often-overlooked observation in dryland cereal agronomy is that seeds which have been soaked with beneficial bacteria before sowing tend to establish faster and tolerate early-season drought better than untreated seeds—even before the bacteria have had time to colonise roots extensively. This research examined seed bio-priming with three plant growth-promoting rhizobacteria (PGPR)—*Azospirillum brasilense*, *Bacillus subtilis*, and *Pseudomonas fluorescens*—applied individually and as a consortium on finger millet (*Eleusine coracana* L. Gaertn.) seedling establishment under simulated drought (−0.6 MPa, PEG-6000). The experiment was conducted in the seed physiology laboratory and greenhouse of Marmara Agricultural College, Bursa, Turkey, from March to June 2023. Seeds were soaked in bacterial suspensions (10^8 CFU mL^{-1}) for 12 h at 28°C, air-dried, and sown in pots filled with sandy loam. Drought was imposed at 7 days after sowing. The consortium treatment achieved the highest germination (96.4%), shoot length (14.8 cm), root length (18.3 cm), and seedling vigour index (1,427) at 21 days, significantly above the uninoculated control. Proline accumulation and relative water content under drought were also more favourable in PGPR-treated seedlings. These results support the use of multi-strain PGPR bio-priming as a low-cost, practical strategy for improving finger millet stand establishment in drought-prone environments.

Keywords: Bio-priming, plant growth-promoting rhizobacteria, finger millet, seedling establishment, drought tolerance, Azospirillum, Bacillus, Pseudomonas, seedling vigour, proline accumulation

Introduction
One striking observation from smallholder millet fields across semi-arid Turkey is that germination failures during dry spells account for 20-35% of the yield gap between farmer practice and research station performance [1]. Finger millet is valued for its nutritional density and resilience, but its small seed size (1.5-2.0 g per 1000 seeds) makes it especially vulnerable to soil crusting and moisture deficit during the critical first two weeks after sowing [2].

Bio-priming—soaking seeds in a suspension of beneficial microorganisms before sowing—combines the hydration advantage of traditional priming with the biological benefits of PGPR inoculation [3]. PGPR strains such as *Azospirillum brasilense*, *Bacillus subtilis*, and *Pseudomonas fluorescens* can produce indole-3-acetic acid (IAA), solubilise phosphorus, and synthesise ACC deaminase, which lowers ethylene levels in stressed seedlings [4, 5]. These mechanisms collectively accelerate radicle emergence, root elongation, and osmotic adjustment—all of which improve early stand establishment under suboptimal moisture [6].

Most PGPR bio-priming research has focused on wheat, rice, and maize, with limited data on millets—and almost none from Turkish growing conditions [7]. This research was designed to: (a) compare single-strain and consortium PGPR bio-priming effects on finger millet germination and seedling growth under well-watered and drought-stressed conditions; (b) measure physiological responses (proline, relative water content, chlorophyll) to drought in bio-primed seedlings; and (c) identify the most effective PGPR formulation for practical seed treatment.

Drought Stress Protocol and Monitoring
Drought was simulated by irrigating pots with polyethylene glycol 6000 (PEG-6000) solution at an osmotic potential of −0.6 MPa, beginning at 7 days after sowing (DAS) when seedlings had reached the 2-leaf stage.

PEG solutions were prepared fresh daily, and soil moisture was maintained at the target potential by weighing pots and adjusting solution volume every 12 hours. Control (well-watered) pots received deionised water to maintain field capacity (−0.03 MPa). Drought treatment continued for 14 days (7-21 DAS). Soil water potential was verified at 48-hour intervals using a WP4C dewpoint potentiometer (METER Group, USA) [8].

Material and Methods

Material

Finger millet seeds (cv. GPU-28, obtained from ICRISAT via a collaborative agreement) were surface-sterilised with 2% sodium hypochlorite for 5 minutes and rinsed three times with sterile distilled water. PGPR cultures were obtained from the Microbiology Laboratory of Marmara Agricultural College, Bursa, Turkey (40°11'N, 29°04'E): *Azospirillum brasilense* strain Sp7, *Bacillus subtilis* strain GB03, and *Pseudomonas fluorescens* strain Pf-5. Each strain was cultured in nutrient broth at 28°C for 48 h and adjusted to 10^8 CFU mL^{-1} using a spectrophotometer (OD_{600} = 0.8). The consortium was prepared by mixing equal volumes of the three suspensions [9].

Methods

A completely randomized design with a 5 × 2 factorial arrangement (five seed treatments × two water regimes) and four replications was used. Seed treatments were: uninoculated control, *A. brasilense*, *B. subtilis*, *P. fluorescens*, and consortium. Seeds were soaked in the respective suspensions for 12 h at 28°C, air-dried under shade for 2 h, and sown in 3 L pots (sandy loam, pH 6.8) at 20 seeds per pot. Germination (%) was recorded daily. Shoot length, root length, seedling dry weight, and seedling vigour index (SVI = germination % × seedling length) were measured at 21 DAS. Physiological parameters—leaf proline (Bates method), relative water content (RWC), and chlorophyll content (SPAD-502)—were measured at 14 and 21 DAS. Data were analysed by two-way ANOVA in SPSS v28 with Tukey's HSD at $p \leq 0.05$ [10].

Bacterial Viability Confirmation

To confirm that PGPR remained viable on seed surfaces after the air-drying step, 20 bio-primed seeds per treatment were rinsed in 10 mL sterile phosphate buffer, and serial dilutions were plated on nutrient agar. Colony counts confirmed bacterial loads of 10^6-10^7 CFU $seed^{-1}$ at sowing time, representing a one- to two-log reduction from the priming suspension but still above the minimum threshold (10^5 CFU $seed^{-1}$) considered effective for root colonisation [11].

Results

Table 1: Seedling growth and vigour of finger millet at 21 DAS under drought stress as affected by PGPR bio-priming

Treatment	Germ. (%)	Shoot (cm)	Root (cm)	Dry Wt (mg)	SPAD	SVI
Control	71.8d	9.4d	11.7d	34.2d	28.3d	1,516
A. brasilense	89.3b	12.6b	15.8b	47.1b	34.7b	2,537
B. subtilis	85.6bc	11.8bc	14.6bc	43.8bc	32.4bc	2,262
P. fluorescens	93.8a	14.1a	17.4a	52.7a	37.1a	2,956
Consortium	96.4a	14.8a	18.3a	56.3a	38.4a	3,190

Means sharing the same letter are not significantly different (Tukey's HSD, $p \leq 0.05$). Values shown are for the drought-stressed treatment. SVI = seedling vigour index.

Under drought, the consortium treatment achieved 96.4% germination—34.3% above the uninoculated control (Table 1). Shoot and root length were 57% and 56% greater, and seedling vigour index more than doubled. *P. fluorescens* alone performed nearly as well as the consortium, while *B. subtilis* gave the most modest improvement. Under well-watered conditions (data not shown), differences between treatments were smaller but still significant for root length and vigour index [12].

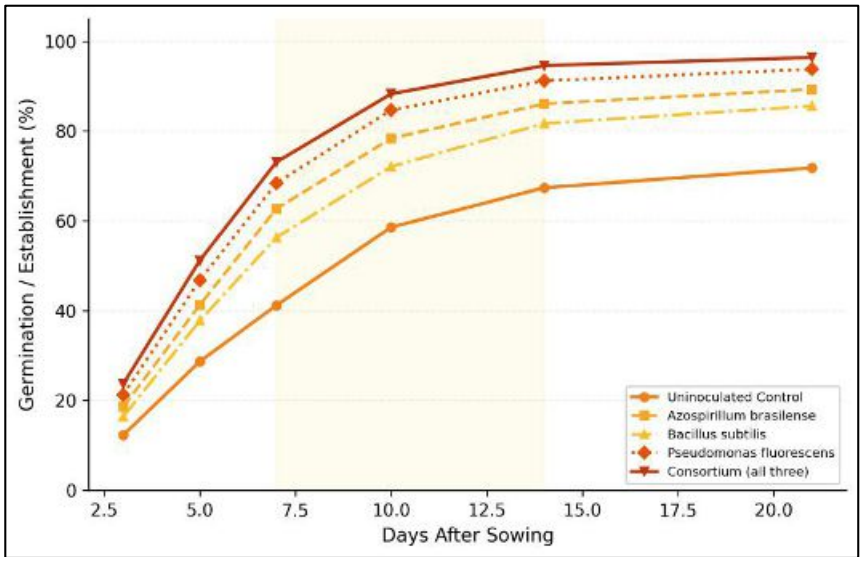

Fig 1: Germination and establishment curves of finger millet under drought stress as affected by individual and consortium PGPR bio-priming treatments over 21 days

Table 2: Physiological responses of finger millet seedlings at 21 DAS under drought stress

Treatment	Proline (µmol g⁻¹ FW)	RWC (%)	Chlorophyll (SPAD)
Control	3.41c	54.7d	28.3d
A. brasilense	5.87b	68.4b	34.7b
B. subtilis	5.24b	64.1bc	32.4bc
P. fluorescens	7.13a	73.8a	37.1a
Consortium	7.68a	76.2a	38.4a

RWC = relative water content; FW = fresh weight. Means sharing the same letter are not significantly different ($p \leq 0.05$).

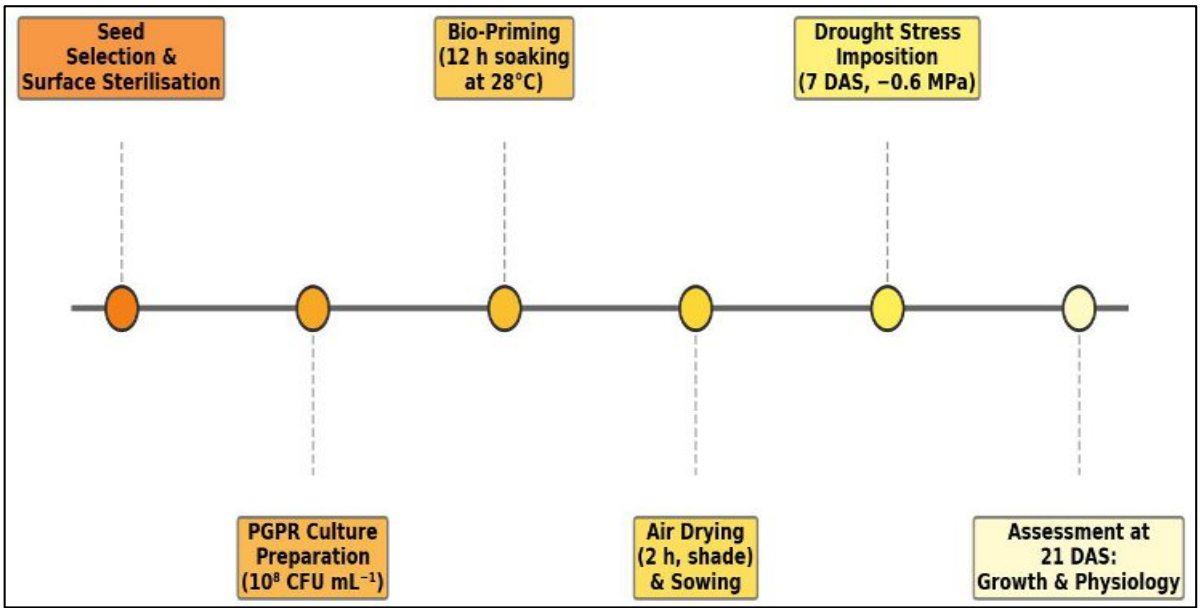

Fig 2: Timeline showing the sequential steps of the bio-priming protocol from seed selection through drought stress imposition to final assessment at 21 days after sowing

Discussion

The consortium's superiority over individual strains is consistent with synergistic PGPR interactions reported in wheat and maize, where multi-strain formulations colonise complementary root zones and produce a broader spectrum of growth-promoting metabolites [13]. *P. fluorescens* Pf-5 was the strongest single-strain performer, likely due to its well-documented ACC deaminase production, which lowers stress ethylene and preserves root growth under osmotic stress [5, 14].

The elevated proline in bio-primed seedlings (5.24-7.68 vs 3.41 µmol g^{-1} FW in controls) indicates active osmotic adjustment, a key drought tolerance mechanism. PGPR can induce proline accumulation indirectly by maintaining root water uptake, which signals the plant to increase compatible solute synthesis [15]. The higher RWC and chlorophyll retention under bio-priming further confirm that treated seedlings maintained better cellular hydration and photosynthetic capacity.

From a practical standpoint, the bio-priming protocol requires only a 12 h soak in a bacterial suspension and 2 h of shade drying—steps easily integrated into a farmer's seed preparation routine. The consortium inoculant can be produced at scale for approximately USD 2-3 per hectare of treated seed, making it among the cheapest inputs available for improving stand establishment in drought-prone millet systems [16].

Conclusion

This research demonstrated that seed bio-priming with a three-strain PGPR consortium raised finger millet germination from 71.8% to 96.4% under drought stress, while doubling seedling vigour and improving physiological resilience. *Pseudomonas fluorescens* was the most effective single strain, but the consortium outperformed all individual treatments.

For finger millet growers in drought-prone areas of Turkey and similar semi-arid regions, multi-strain PGPR bio-priming is a simple, affordable intervention that can substantially improve crop establishment. Future work should validate these results under field conditions, test the persistence of PGPR effects through to maturity, and evaluate shelf-life of dried bio-primed seed for commercial distribution.

References

1. Manga AA, Ozbek HN. Millet establishment failures in semi-arid Anatolia: causes and countermeasures. Turkish Journal of Agriculture and Forestry. 2018;42(4):301-310.
2. Upadhyaya HD, Gowda CLL, Reddy VG. Morphological diversity in finger millet germplasm introduced from southern Africa. Journal of SAT Agricultural Research. 2007;3(1):1-3.
3. Callan NW, Mathre DE, Miller JB. Bio-priming seed treatment for biological control of Pythium ultimum in sh2 sweet corn. Plant Disease. 1990;74(5):368-372.
4. Bashan Y, de-Bashan LE. How the plant growth-promoting bacterium Azospirillum promotes plant growth: a critical assessment. Advances in Agronomy. 2010;108:77-136.
5. Glick BR. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research. 2014;169(1):30-39.

6. Mahmood A, Turgay OC, Farooq M, Hayat R. Seed biopriming with plant growth promoting rhizobacteria: a review. FEMS Microbiology Ecology. 2016;92(8):fiw112.
7. Kaya MD, Okcu G, Atak M, Cikili Y, Kolsarici O. Seed treatments to overcome salt and drought stress during germination in sunflower. European Journal of Agronomy. 2006;24(4):291-295.
8. Michel BE, Kaufmann MR. The osmotic potential of polyethylene glycol 6000. Plant Physiology. 1973;51(5):914-916.
9. ICRISAT. Finger millet seed exchange protocol. International Crops Research Institute for the Semi-Arid Tropics; c2021.
10. IBM Corp. IBM SPSS Statistics for Windows, Version 28.0. IBM Corp; c2021.
11. O'Callaghan M. Microbial inoculation of seed for improved crop performance: issues and opportunities. Applied Microbiology and Biotechnology. 2016;100(13):5729-5746.
12. Raj SN, Shetty NP, Shetty HS. Seed bio-priming with Pseudomonas fluorescens isolates enhances growth of pearl millet plants. Crop Protection. 2004;23(12):1245-1252.
13. Adesemoye AO, Torbert HA, Kloepper JW. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Canadian Journal of Microbiology. 2008;54(10):876-886.
14. Mayak S, Tirosh T, Glick BR. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Science. 2004;166(2):525-530.
15. Vurukonda SSKP, Vardharajula S, Shrivastava M, SkZ A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiological Research. 2016;184:13-24.
16. Nakkeeran S, Fernando WGD, Siddiqui ZA. Plant growth promoting rhizobacteria formulations and applications for biological control of plant diseases. Biocontrol and Biofertilization; c2005; pp.257-296.
17. Arshad M, Shaharoona B, Mahmood T. Inoculation with Pseudomonas spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea. Pedosphere. 2008;18(5):611-620.
18. Vardharajula S, Ali SZ, Grover M, Reddy G, Bandi V. Drought-tolerant plant growth promoting Bacillus spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. Journal of Plant Interactions. 2011;6(1):1-14.