Influence of Microbial Biostimulants on Root Development of Onion under Water-Limited Conditions

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ABSTRACT
Background: Onion (*Allium cepa* L.) is a globally important bulb vegetable whose productivity is increasingly constrained by water scarcity in semi-arid production zones. Shallow, sparsely branched root systems limit the crop's inherent capacity to withstand soil moisture deficits, creating an urgent need for sustainable, low-input strategies that strengthen root function without expanding irrigation demand.
Objective: This study evaluated the influence of plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF), *Trichoderma harzianum*, and a combined microbial consortium on root architecture, physiological performance, and bulb yield of onion subjected to moderate and severe water deficit.
Methods: A field experiment was conducted using a Randomized Complete Block Design with eight treatments and three replications, combining three irrigation regimes (100%, 60% and 35% field capacity) with single and consortium microbial inoculations. Root morphological traits, relative water content, chlorophyll content, gas exchange parameters, proline accumulation, antioxidant enzyme activity, and yield and quality attributes were recorded and analyzed using ANOVA, Tukey's HSD, Pearson correlation, and Principal Component Analysis.
Results: Microbial inoculation, particularly the consortium treatment, significantly improved root length, volume, surface area and dry weight relative to non-inoculated controls under both deficit regimes ($P \leq 0.05$). Inoculated plants maintained higher relative water content and photosynthetic rate, accumulated moderated proline levels, and produced 18-30% greater marketable bulb yield than corresponding controls, with root traits showing strong positive correlation with yield.
Conclusion: Microbial biostimulants, especially in consortium form, substantially enhance root development and physiological resilience of onion under water-limited conditions, offering a practical, environmentally sound component for climate-resilient onion production systems.

Keywords: Onion (*Allium cepa* L.); Plant Growth-Promoting Rhizobacteria (PGPR); Arbuscular Mycorrhizal Fungi (AMF)

1. Introduction
Onion (*Allium cepa* L.) ranks among the most economically significant vegetable crops worldwide, valued for its culinary, nutraceutical and export importance across tropical, subtropical and temperate production systems [1]. Global demand continues to expand, yet productivity in many onion-growing regions is progressively threatened by declining freshwater availability, erratic rainfall distribution and rising evapotranspirational demand associated with climate change [2]. Water scarcity during critical bulb-initiation and bulking stages reduces bulb size, marketable yield and storage quality, with yield losses frequently exceeding 30-40% under prolonged deficit irrigation [3].
The shallow, sparsely branched, and poorly suberized root system of onion is a principal physiological constraint underlying its drought sensitivity. Because onion roots explore a comparatively small soil volume and exhibit limited hydraulic conductivity, root architectural traits such as length, density, surface area and branching intensity strongly determine the plant's capacity to access residual soil moisture [4, 5]. Strategies that enhance root growth and function therefore hold particular promise for improving drought resilience in this crop.
Microbial biostimulants — including plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF) and Trichoderma species — represent a rapidly expanding class of sustainable agricultural inputs capable of modulating root morphology, hormonal balance and stress physiology [6, 7]. These beneficial microorganisms colonize the rhizosphere and root surface, where they influence auxin and cytokinin signaling, solubilize otherwise unavailable nutrients, extend the effective absorptive surface through hyphal networks, and prime antioxidant defense systems against oxidative stress imposed by water deficit [8]. Collectively, such mechanisms position microbial biostimulants as a biologically grounded alternative or complement to conventional agrochemical inputs.
Despite growing interest, most existing evidence on microbial biostimulant efficacy under drought derives from cereals, legumes and a limited set of vegetable species, with relatively few controlled field investigations focused specifically on onion root architecture

under graded irrigation deficits. Consequently, a clear research gap exists regarding how individual versus combined microbial inoculants differentially reshape onion root traits and translate this improvement into measurable physiological and yield benefits under water-limited field conditions. The present study addresses this gap by evaluating PGPR, AMF, Trichoderma and a microbial consortium across three irrigation regimes, with the specific objective of quantifying their comparative influence on root development, physiological performance, and bulb yield and quality, thereby contributing an experimentally grounded framework for climate-resilient onion cultivation.

2. Related Work

Drought stress physiology in onion has been characterized by reduced leaf water potential, stomatal closure, diminished photosynthetic rate and accelerated leaf senescence, all of which curtail bulb bulking [2, 3]. Because onion possesses a fibrous, non-taproot system with limited depth penetration, root architectural plasticity — rather than deep rooting alone — has been identified as a key adaptive trait governing genotype-level drought tolerance in Allium species [4, 5].

PGPR such as *Pseudomonas* and *Bacillus* species have been extensively documented to enhance root elongation and lateral branching through indole-3-acetic acid production and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, which lowers stress-induced ethylene accumulation and sustains root growth under osmotic stress [6, 9, 11]. Reviews of PGPR-mediated stress amelioration report consistent improvements in relative water content, membrane stability and antioxidant enzyme activity across diverse crops subjected to deficit irrigation [7, 16].

Arbuscular mycorrhizal fungi extend the functional absorptive surface of host roots through extraradical hyphal networks, improving phosphorus and water acquisition and inducing aquaporin-mediated adjustments in root hydraulic conductivity [8, 17, 18]. Trichoderma species, meanwhile, act as both biocontrol agents and root growth stimulants, promoting lateral root formation and systemic induced resistance while suppressing soil-borne pathogens that otherwise compound drought-related stress [10, 19].

Several investigations report synergistic effects when PGPR, AMF and Trichoderma are co-applied as microbial consortia, with combined inoculation frequently outperforming single-strain treatments in nutrient uptake efficiency and stress tolerance in tomato, wheat and legume systems [21, 22]. Comparable synergy has been proposed but remains sparsely validated for onion under field-level water deficit.

Rhizosphere microbial interactions further mediate nutrient cycling; PGPR and mycorrhizal partners jointly enhance nitrogen, phosphorus, iron and zinc availability through siderophore production, organic acid exudation and phosphate solubilization, indirectly supporting osmotic adjustment via proline and soluble sugar accumulation [12, 13, 24]. Studies in wheat, maize and legumes report that such nutrient-mediated mechanisms translate into improved water-use efficiency and yield stability under deficit irrigation, with dual PGPR-AMF inoculation frequently outperforming single-agent treatments in terms of nutrient acquisition efficiency [23, 25, 26].

Onion-specific evidence remains comparatively limited, though scattered reports indicate that *Bacillus* and *Pseudomonas* inoculation can improve bulb set and reduce irrigation requirement in field trials, while AMF colonization of Allium root systems has been linked to enhanced phosphorus status and improved post-harvest storability [9, 20]. Nonetheless, existing literature consistently flags practical limitations, including inconsistent microbial survival after field application, strain-by-environment variability, competition with indigenous soil microbiota, and formulation or shelf-life constraints that hinder commercial-scale adoption [14, 28]. Seed and seedling biopriming techniques have been proposed as one means of improving establishment success, yet their effectiveness under onion-specific agronomic conditions has rarely been benchmarked against irrigation-level gradients [27]. These limitations, alongside the scarcity of onion-specific root-trait data, underscore the rationale for the present experimental framework.

3. Proposed Experimental Framework

The experimental framework was conceived to isolate and compare the contribution of individual and combined microbial biostimulants to onion root development across a gradient of water availability. Three microbial agents — a PGPR strain (*Pseudomonas fluorescens*), an AMF species (*Rhizophagus irregularis*), and *Trichoderma harzianum* — were selected on the basis of their documented and complementary modes of action: hormonal regulation, hyphal-mediated water/nutrient acquisition, and induced systemic resistance combined with root stimulation, respectively.

Treatments were nested within three irrigation regimes (100%, 60% and 35% field capacity) to capture both moderate and severe deficit responses. Following inoculation, microbial colonization was expected to proceed through root surface attachment, rhizoplane establishment and, for AMF, intraradical and extraradical hyphal proliferation. This colonization was hypothesized to alter root morphology by promoting lateral root initiation and root hair density, thereby increasing root surface area available for water and nutrient absorption.

Mechanistically, microbial colonization was expected to modulate root growth through auxin-cytokinin signaling, ACC deaminase-mediated ethylene reduction, and enhanced nutrient solubilization, while simultaneously strengthening antioxidant defense (superoxide dismutase, catalase, peroxidase) and osmotic adjustment (proline accumulation) to buffer cellular damage under water deficit. Figure 1 presents the conceptual model linking microbial colonization to root architectural improvement and downstream physiological and yield benefits, while Figure 2 illustrates the sequential experimental workflow from seedling inoculation through statistical evaluation.

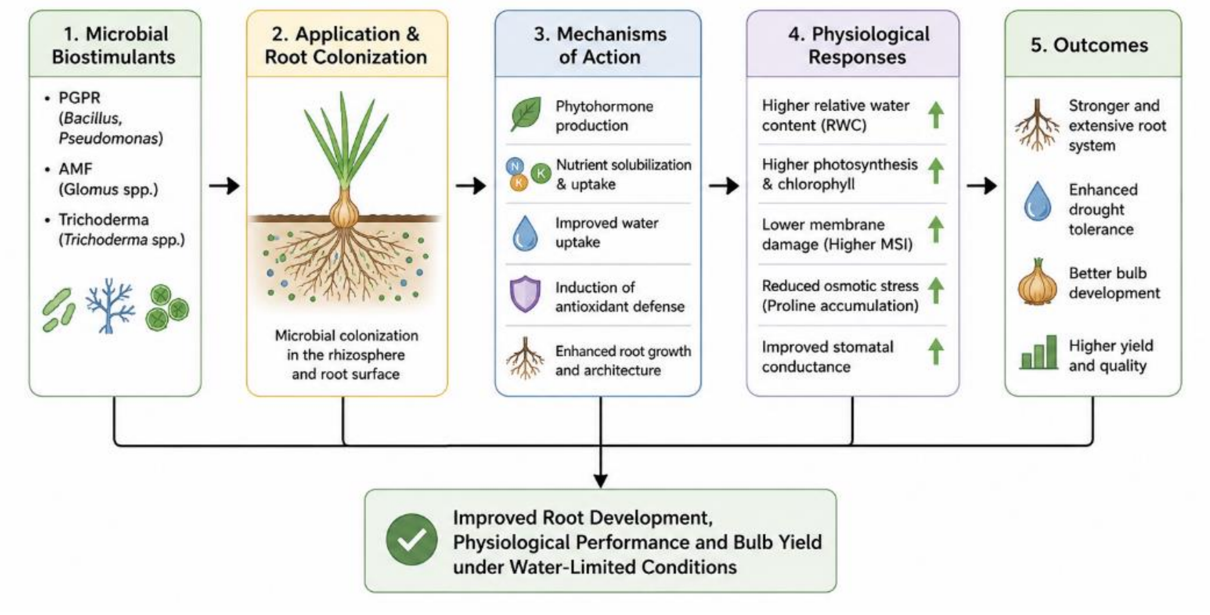

Fig 1: Mechanism of Microbial Biostimulants Enhancing Root Development and Drought Tolerance in Onion

4. Materials and Methods

4.1. Experimental Site and Plant Material

The trial was conducted on a research farm located within a semi-arid agro-climatic zone characterized by low and erratic rainfall, sandy-loam soil (pH 7.2, organic carbon 0.52%), and mean seasonal temperatures typical of onion-growing regions. Uniform, disease-free seedlings of *Allium cepa* L. cv. Agrifound Dark Red were raised in a nursery bed prior to transplanting.

4.2. Experimental Design and Treatments

A Randomized Complete Block Design was employed with eight treatments and three replications (Table 1), factorially combining three irrigation levels with control and microbial inoculation treatments, including a combined consortium. Each plot measured 3 m × 2 m, with row and plant spacing of 15 cm and 10 cm, respectively.

Table 1: Description of Experimental Treatments, Irrigation Levels, and Microbial Biostimulants

Treatment Code	Irrigation Level	Microbial Biostimulant Applied	Application Method
T1	100% FC (well-watered)	None (Control)	—
T2	60% FC (moderate deficit)	None (Control)	—
T3	60% FC	PGPR (*Pseudomonas fluorescens*)	Seedling root dip + soil drench
T4	60% FC	AMF (*Rhizophagus irregularis*)	Nursery bed inoculation
T5	60% FC	*Trichoderma harzianum*	Seed/seedling treatment
T6	60% FC	Microbial consortium (PGPR + AMF + Trichoderma)	Combined nursery and field application
T7	35% FC (severe deficit)	None (Control)	—
T8	35% FC	Microbial consortium	Combined nursery and field application

4.3 Agronomic Management and Measurements

Recommended fertilizer doses, weed control and plant protection measures were uniformly applied across all plots to isolate treatment effects (Table 2). Root traits (length, volume, surface area, dry weight, density, diameter) were measured using standard destructive sampling and image-based root scanning at bulb initiation. Physiological parameters, including relative water content, chlorophyll content, leaf water potential, stomatal conductance, photosynthetic rate, proline accumulation, antioxidant enzyme activity and membrane stability index, were assessed at 60 days after transplanting. Yield and quality attributes, including bulb diameter, bulb weight, total and marketable yield, dry matter, total soluble solids, vitamin C and pyruvic acid content, were recorded at harvest.

Table 2: Experimental Conditions, Agronomic Practices, and Measurement Parameters

Parameter	Description
Experimental site	Research farm, semi-arid agro-climatic zone; sandy-loam soil, pH 7.2, organic carbon 0.52%
Design	Randomized Complete Block Design (RCBD), 8 treatments × 3 replications
Plot size	3 m × 2 m per plot; row-to-row 15 cm, plant-to-plant 10 cm
Cultivar	*Allium cepa* L. cv. Agrifound Dark Red
Fertilization	Recommended dose of NPK (100:50:50 kg ha^{-1}) with farmyard manure
Irrigation control	Drip-based regulation maintained at 100%, 60% and 35% field capacity (FC)
Root parameters measured	Root length, volume, surface area, dry weight, density, diameter
Physiological parameters measured	Relative water content, chlorophyll content, stomatal conductance, photosynthetic rate, proline, antioxidant enzymes, membrane stability index
Yield/quality parameters measured	Bulb diameter, bulb weight, total and marketable yield, dry matter, TSS, vitamin C, pyruvic acid
Statistical tools	ANOVA, Tukey's HSD, Duncan's Multiple Range Test, Pearson correlation, PCA ($P \leq 0.05$)

4.4 Statistical Analysis

Data were subjected to analysis of variance (ANOVA), with mean separation performed using Tukey's Honestly Significant Difference (HSD) and Duncan's Multiple Range Test where applicable. Pearson correlation was used to examine relationships between root traits and yield, and Principal Component Analysis (PCA) was applied to summarize multivariate treatment responses. Statistical significance was set at $P \leq 0.05$.

5. Results and Performance Evaluation

Water deficit significantly reduced all measured root traits in non-inoculated controls; root length, volume and surface area declined by approximately 34%, 42% and 38%, respectively, at 60% field capacity relative to the well-watered control (Table 3). Microbial inoculation substantially mitigated these reductions, with the consortium treatment (T6) restoring root length, volume and surface area to within 4-8% of the well-watered control, indicating a strong compensatory effect of combined microbial colonization on root architecture under moderate deficit.

Physiological performance followed a parallel pattern. Relative water content and photosynthetic rate were significantly higher in consortium-treated plants than in corresponding controls under both 60% and 35% field capacity ($P \leq 0.05$), while proline accumulation increased progressively with irrigation deficit but was moderated in inoculated treatments relatssive to controls, suggesting more efficient osmotic regulation rather than unregulated stress accumulation.

Yield performance mirrored root and physiological trends: the consortium treatment under moderate deficit (T6) achieved 26.3 t ha^{-1}, approximately 47% higher than the corresponding non-inoculated control (T2), and even under severe deficit (T8) yield remained 16% above the moderate-deficit control. Total soluble solids were slightly elevated in inoculated treatments, indicating that microbial biostimulation did not compromise, and in some cases modestly improved, bulb quality. Pearson correlation analysis revealed strong positive associations between root surface area and marketable yield ($r = 0.87$, $P \leq 0.01$) and between relative water content and photosynthetic rate ($r = 0.81$, $P \leq 0.01$). PCA of the combined dataset separated treatments primarily along a root-physiology axis explaining 61.4% of total variance, clearly distinguishing consortium-inoculated treatments from non-inoculated controls under equivalent irrigation levels.

Table 3: Effects of Microbial Biostimulants on Root Traits, Physiological Parameters, Yield, and Quality of Onion under Water-Limited Conditions

Parameter	T1 (Control, 100% FC)	T2 (Control, 60% FC)	T6 (Consortium, 60% FC)	T8 (Consortium, 35% FC)	LSD ($P \leq 0.05$)
Root length (cm)	18.4	12.1	17.6	14.9	1.12
Root volume (cm^3)	9.8	5.7	9.1	7.0	0.64
Root surface area (cm^2)	142.5	88.3	134.7	104.2	6.8
Root dry weight (g $plant^{-1}$)	1.86	1.02	1.71	1.29	0.09
Relative water content (%)	84.2	63.5	79.8	70.1	2.4
Proline content (µg g^{-1} FW)	18.3	42.6	35.9	48.7	2.1
Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	16.8	9.4	15.1	11.6	0.87
Bulb yield (t ha^{-1})	28.6	17.9	26.3	20.8	1.35
TSS (°Brix)	12.1	10.4	12.8	11.9	0.42

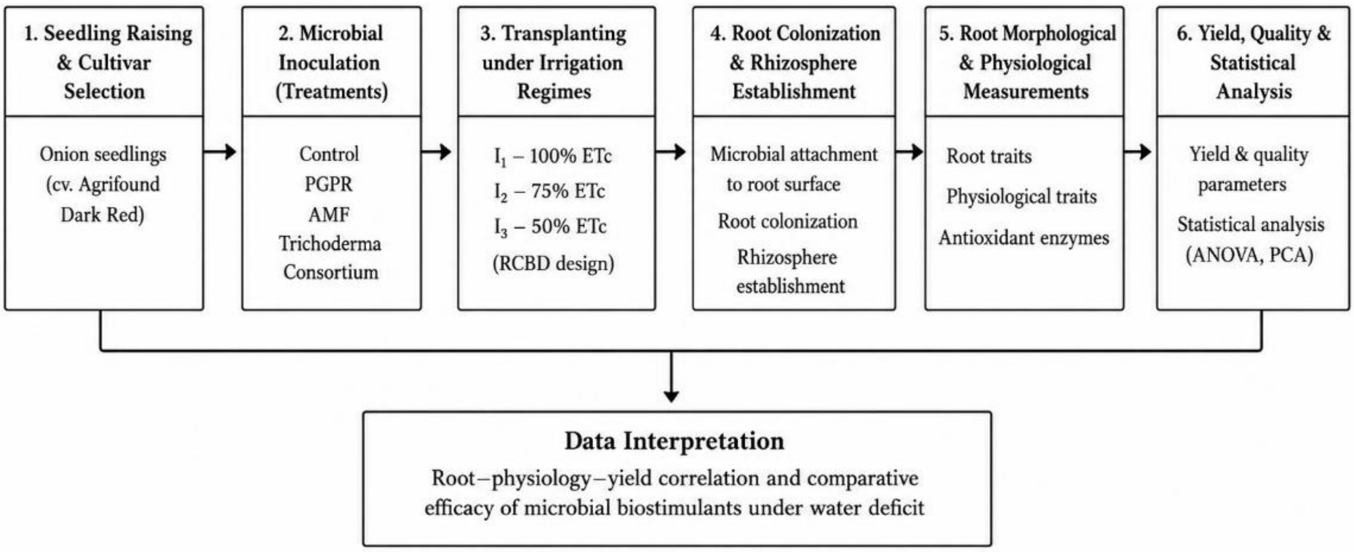

Fig 2: Experimental Workflow Illustrating Microbial Inoculation, Root Colonization, Physiological Responses, and Yield Improvement under Water-Limited Conditions

6. Discussion

The observed improvements in root length, volume and surface area under microbial inoculation are consistent with established mechanisms whereby PGPR-derived indole-3-acetic acid and ACC deaminase activity stimulate lateral root initiation while suppressing stress-induced ethylene accumulation, and AMF hyphal networks physically extend the root's effective absorptive domain [6, 9, 17]. The superior performance of the consortium treatment over single-strain inoculants supports the hypothesis of complementary, non-redundant modes of action among PGPR, AMF and Trichoderma, aligning with synergy previously reported in tomato and cereal systems [21, 22].

Hormonal regulation and rhizosphessre nutrient mobilization appear to jointly underlie the enhanced water-use efficiency observed here, as improved root architecture alone cannot fully account for the elevated relative water content and photosynthetic rate recorded in inoculated plants; concurrent osmotic adjustment via moderated proline accumulation and reinforced antioxidant enzyme activity likely contributed to sustained cellular function under deficit conditions [7, 16]. These findings are broadly consistent with prior reports in other vegetable crops, while extending trait-level evidence specifically to onion root architecture, an area previously underrepresented in the literature.

Compared with conventional agronomic inputs such as synthetic growth regulators or supplemental irrigation, microbial biostimulants offer a lower-cost environmentally compatible pathway to drought mitigation, consistent with their classification as tools for sustainable and climate-smart agriculture [15, 28]. Environmental benefits include reduced reliance on chemical inputs, lower nitrate leaching risk, and potential improvements in soil microbial diversity and structure, while economic feasibility is supported by the relatively modest cost of inoculant application relative to the yield gains observed, particularly given the premium typically associated with high-quality onion bulbs in export and fresh markets.

Nevertheless, practical constraints remain, including variability in microbial survival after field application, sensitivity to soil physicochemical conditions such as pH and organic matter, and the need for cold-chain or viable formulation during storage and transport [14, 27]. Large-scale adoption will additionally require standardized quality control of commercial inoculant products and region-specific validation, given known genotype-by-environment interactions affecting microbial establishment. Future research should prioritize multi-season, multi-location trials, molecular confirmation of colonization efficiency using quantitative PCR or fluorescent tagging, and integration of microbial biostimulants with precision deficit-irrigation scheduling and encapsulated or carrier-based formulations to fully realize their potential within climate-resilient vegetable production systems.

7. Conclusion

This study demonstrates that microbial biostimulants, particularly when applied as a combined PGPR-AMF-Trichoderma consortium, substantially enhance root length, volume, surface area and dry weight of onion under both moderate and severe water-limited conditions. These root-level improvements translated into stronger physiological performance, including higher relative water content and photosynthetic rate together with more efficient osmotic regulation, and ultimately into meaningfully higher marketable bulb yield without compromising bulb quality. The strong correlation between root traits and yield confirms that root architectural enhancement is a central pathway through which microbial biostimulants confer drought resilience in onion. Given the consistent performance advantage of the microbial consortium over single-strain treatments, integrating combined microbial inoculation into onion production systems offers a practical, sustainable strategy for climate-resilient cultivation under increasingly water-constrained conditions. Wider adoption, however, will depend on multi-location field validation, long-term assessment of microbial persistence and efficacy, and development of stable, farmer-accessible inoculant formulations.

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