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Optimization of hydropriming and biopriming treatments for enhancing seed quality in lentil (*Lens culinaris*)

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ABSTRACT

The present study was carried out during 2023–24 at ICAR-Indian Agricultural Research Institute, New Delhi aimed to standardize hydropriming and biopriming protocols to improve seed quality and seedling vigour in lentil (*Lens culinaris* Medik.). Hydropriming was evaluated using two seed-to-water ratios (1:1 and 1:2, w/v) across six soaking durations (4, 8, 12, 16, 20 and 24 h), while biopriming was optimized using four bacterial inoculum concentrations (0.1, 1, 10, and 100%) for 4, 8, 12 and 16 h. Seed quality parameters were assessed following standard germination test. Results indicated that hydropriming at 16 h with a 1:2 ratio significantly ($p \leq 0.05$) improved germination (93.0%), shoot and root length (8.58 and 16.9 cm), seedling fresh and dry weight (1.32 and 0.128 g/10 seedlings), and vigour indices (SVI-I: 2373; SVI-II: 11.92). Similarly, biopriming at 1% inoculum for 12 h recorded the highest performance, with germination (94.7%), shoot and root length (9.19 and 17.3 cm), fresh and dry weight (1.32 and 0.136 g/10 seedlings), and vigour indices (SVI-I: 2507; SVI-II: 12.90). This study demonstrates that hydropriming for 16 h (1:2 ratio) and biopriming for 12 h (1% inoculum) are optimal conditions for enhancing the physiological quality and initial seedling vigour of lentil under laboratory conditions, thereby providing a basis for future field validation.

Keywords: Biopriming, Germination, Hydropriming, Seed quality, Seedling vigour

Lentil (*Lens culinaris* Medik.) is an important cool season, protein rich legume that plays a vital role in food and nutritional security in South Asia. It enhances soil fertility through symbiotic nitrogen fixation, contributing to sustainable cereal-based systems (Sekhon *et al.* 2007). Globally, lentil is cultivated on about 5.6 Mha, producing 7.06 million tonnes with an average productivity of 1,245 kg/ha (FAO 2025). Although India ranks first in area (1.63 Mha) and third in production (1.56 Mt), its average productivity (952.3 kg/ha) remains lower than that of China. This productivity gap is often attributed to suboptimal crop establishment in specific high-yielding genotypes. For instance, L4717 (Pusa Ageti Masoor) is an early-maturing, high-yielding, and biofortified variety rich in zinc and iron, recommended for the Central Zone of India (Directorate of Pulses Development 2025). Despite its potential, irregular emergence and low early vigour under uneven soil moisture limit its performance, particularly in rainfed rice-fallow systems dependent on residual monsoon moisture (Mukherjee *et al.* 2023).

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To address these establishment challenges, seed priming techniques such as hydropriming and biopriming offer low-cost, eco-friendly solutions. These methods have been proven to enhance germination, field emergence, and seedling vigour without leaving chemical residues (Anbalagan *et al.* 2024, Hernández *et al.* 2024). Specifically, hydropriming ensures controlled seed hydration without radicle protrusion, whereas biopriming utilizes beneficial microorganisms to enhance nutrient uptake and early seedling growth. Recent research has further elucidated the physiological mechanisms underlying this primed state. Beyond physical hydration, priming initiates pre-germinative metabolism, which includes the activation of antioxidant enzymes (SOD, CAT and APX), repair of DNA and membranes, and mobilization of storage proteins (Jatana *et al.* 2024, Singh *et al.* 2026). In pulses, biopriming with Plant Growth-Promoting Rhizobacteria (PGPR) modulates hormonal signalling, specifically enhancing the GA/ABA ratio and indole-3-acetic acid (IAA) production, which promotes rapid radicle emergence and robust seedling establishment (Ahmad *et al.* 2025). These physiological memory effects allow primed seeds to better overcome environmental stressors such as moisture deficit in rice-fallow systems (Sarkar *et al.* 2021).

Previous studies have demonstrated significant improvement in seed quality through these techniques. For instance, hydropriming for 16–24 h improved germination and seedling vigour in various lentil cultivars (Anbalagan *et al*. 2024), while biopriming with *Azotobacter* and *Pseudomonas fluorescens* increased seed viability and vigour (Balaji and Sathitya 2019). Similar benefits of priming have been reported in other crops, including mungbean (Shah *et al*. 2012), wheat (Forti *et al*. 2020) and maize (Basra *et al*. 2005). Despite these advancements, optimized priming protocols specifically tailored for the biofortified variety L4717 remain limited, particularly with respect to the use of novel microbial agents. Therefore, the present study was undertaken to optimize hydropriming and biopriming treatments using a newly isolated PGPR strain to evaluate their efficacy in enhancing seed quality and early seedling vigour of lentil.

MATERIALS AND METHODS

Source of PGPR strain and lentil seed material: The present study was carried out during 2023–24 at ICAR-Indian Agricultural Research Institute (28.08ºN and 77.12ºE; at an altitude of 228.61 m amsl), New Delhi. A novel PGPR strain, *Pseudomonas oleovorans* V3RM-33 (NCBI Accession No. OQ730031), previously isolated from the lentil rhizosphere and screened for superior growth promoting potential, was sourced from the Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi. The strain exhibited nitrogen fixation (2136.43 nmol ethylene/mg/protein/h), phosphorus solubilization (3.17 µg/mL), potassium solubilization (30.02 µg/mL), siderophore production (62.09 µg/mL), and phytohormone production including IAA (13.81 µg/mL), GA (6.06 µg/mL), and cytokinin (92.63% increase over the control). Freshly harvested seeds of the lentil variety L4717 (8.8% moisture content, 98% physical purity, 82% germination percentage) were obtained from the Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi.

Preparation of microbial culture: The bacterial inoculum was prepared by culturing the newly isolated PGPR strain in nutrient broth (*p*H 7.0) at 28 ± 1 °C for 48 h under agitation (150 rpm) in an incubator shaker (Kuhner ISF-1-W, Switzerland). The culture density was determined spectrophotometrically (SpectraMax M2, USA), where an OD_{600} of 1.0 was defined as the 100% undiluted inoculum (1×10^8 CFU/mL). This stock was subsequently serially diluted with sterile distilled water to obtain concentrations of 10% (1×10^7 CFU/mL), 1% (1×10^6 CFU/mL), and 0.1% (1×10^5 CFU/mL), with viable cell counts verified via the standard plate count method (Harringan and McCance 1976).

Surface sterilization of seeds: Seeds were surface sterilized with 0.1% $HgCl_2$ (3 min) followed by 70% ethanol (1 min) and rinsed five times with sterile distilled water. After sterilization, seeds were blotted dry under aseptic conditions to remove surface moisture. Owing to the short exposure duration, no significant water imbibition occurred during the sterilization process (Somasegaran and Hoben 2012).

Experimental design and treatments

Hydropriming: The hydropriming experiment was conducted in a two-factor factorial completely randomised design (F-CRD) with three replications. Treatments consisted of two seed-to-water ratios (1:1 and 1:2, w/v) across six soaking durations (4, 8, 12, 16, 20, and 24 h), resulting in 12 combinations; non-primed seeds served as the control. Seeds were hydroprimed at 20°C by complete immersion in distilled water in glass Petri plates to ensure uniform hydration. After priming, seeds were redried to their original moisture content and evaluated for physiological quality according to ISTA (2025) protocols.

Biopriming: The biopriming study was conducted in a two-factor factorial completely randomised design (F-CRD) with three replications. Treatments consisted of four inoculum concentrations (0.1, 1, 10, and 100%) across four soaking durations (4, 8, 12, and 16 h), resulting in 16 combinations. Biopriming was performed by aseptically immersing surface-sterilized seeds in the respective PGPR suspensions (1:2, w/v ratio) at 20°C under a laminar airflow chamber to maintain sterile conditions and prevent contamination. To isolate microbial effects from hydration effects, both hydroprimed seeds (soaked for corresponding durations) and non-primed seeds were maintained as comparative controls. Following treatment, all seeds were air-dried on sterile filter paper for 24 h to restore their initial moisture content (Basra *et al*. 2005) before physiological evaluation (Farooq *et al*. 2008, ISTA 2025).

Seed germination and seedling growth assay: Germination tests were conducted using the between-paper (BP) method in accordance with ISTA (2025) guidelines. For each replication, 100-seeds were placed on moistened germination paper, rolled, and incubated at 20 ± 2 °C with 90 ± 5% relative humidity. The final germination percentage was recorded on the 10^{th} day based on the count of normal seedlings. To evaluate growth parameters, 10 healthy seedlings were randomly sampled from each replication on the 10^{th} day. Following the Abdul-Baki and Anderson (1973), shoot length was measured from the base of the primary leaf to the hypocotyl base, and root length from the primary root tip to the hypocotyl base. Seedling fresh weight was recorded immediately, and dry matter was determined after oven-drying at 55°C for 48 h until a constant weight was achieved. Biomass was expressed as grams per ten seedlings (g/10 seedlings). Seedling Vigour Index I (SV-I) and Seedling Vigour Index II (SV-II) was calculated as:

$$\text{Seedling vigour index-I} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

$$\text{Seedling vigour index-II} = \text{Germination (\%)} \times \text{Seedling dry weight (g)}$$

Statistical analysis: The experiment was laid out in a two-FCRD with three biological replicates per treatment. Statistical analyses were carried out using the General Linear Model (GLM) procedure in IBM SPSS Statistics software (version 24.0; IBM Corp., Armonk, NY, USA). Treatment means were compared using Tukey's multiple range test at a significance level of $p \leq 0.05$. All data were

expressed as the mean ± standard error (SEM±) of three biological replicates.

RESULTS AND DISCUSSION

Hydropriming optimization for germination and seedling growth: The analysis of variance (ANOVA) indicated that soaking duration and seed-to-water ratio significantly influenced all seed quality parameters at $p \leq 0.05$ (Table 1). Germination percentage showed a progressive increase with soaking duration, reaching a peak at 16 h (91.8%), which was significantly superior to the unprimed control (80.0%). The 1:2 (w/v) seed-to-water ratio also recorded a significantly higher mean germination (88.3%) compared to the 1:1 ratio (86.0%).

Shoot and root lengths varied significantly among treatments, with the maximum mean shoot length (7.91 cm) and root length (16.5 cm) obtained at the 16 h soaking duration. These values were markedly higher than the control (5.79 cm and 13.4 cm, respectively). Biomass accumulation followed a similar trend, with seedling fresh weight reaching maximum at 1.32 g/10 seedlings (Fig. 1A) and seedling dry weight reaching 0.128 g/10 seedlings in the 16 h (1:2 w/v) treatment. This represents a significant increase over the control values of 0.81 g and 0.081 g, respectively. Furthermore, seedling vigour indices (SVI-I and SVI-II)

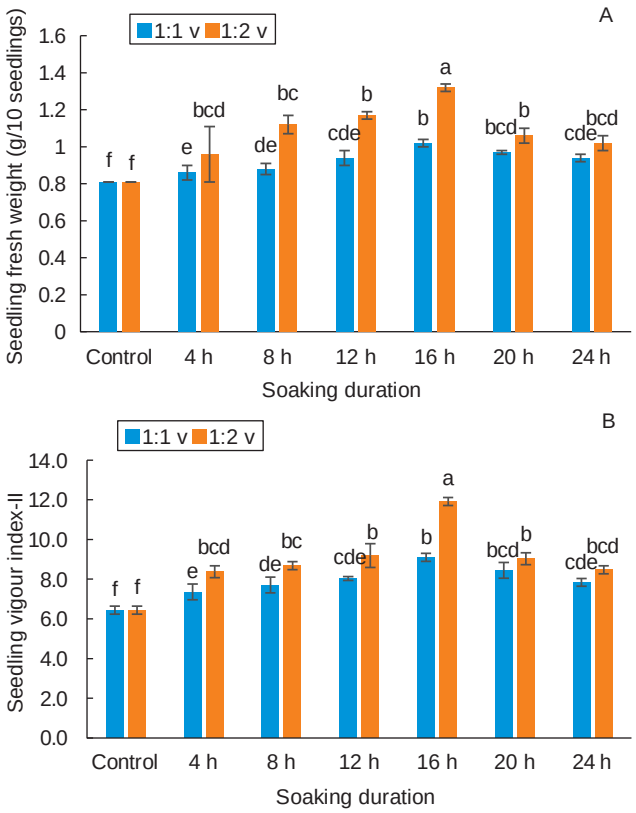


Fig. 1 Effect of hydropriming duration and seed-to-water ratio on (A) seedling fresh weight and (B) seedling vigour index-II of lentil variety L4717.
Vertical bars indicate the standard error of the mean (SEM±), and different lowercase letters denote significant differences between treatments at $p \leq 0.05$.

Table 1 Effect of hydropriming duration and volume on seed quality parameters of lentil

	Germination percentage (%)			Shoot length (cm)			Root length (cm)			Seedling dry weight (g/10 seedlings)			Seedling vigour index-I		
	1:1 (w/v)	1:2 (w/v)	Mean	1:1 (w/v)	1:2 (w/v)	Mean	1:1 (w/v)	1:2 (w/v)	Mean	1:1 (w/v)	1:2 (w/v)	Mean	1:1 (w/v)	1:2 (w/v)	Mean
Control	80.0	80.0	80.0 c	5.79	5.79	5.79 d	13.4	13.4	13.4 d	0.081	0.081	0.081 d	1535	1535	1535 e
4 h	85.3	88.7	87.0 b	6.31	6.98	6.65 c	14.8	15.8	15.3 c	0.086	0.094	0.090 c	1804	2018	1911 d
8 h	86.3	90.0	88.2 ab	6.62	7.48	7.05 bc	15.3	15.8	15.6 bc	0.089	0.097	0.093 bc	1897	2093	1995 bcd
12 h	87.0	90.7	88.8 ab	6.88	7.69	7.28 b	15.3	15.9	15.6 bc	0.092	0.101	0.097 b	1934	2143	2039 bc
16 h	90.7	93.0	91.8 a	7.24	8.58	7.91 a	16.0	16.9	16.5 a	0.100	0.128	0.114 a	2107	2373	2240 a
20 h	88.0	89.3	88.7 ab	6.93	7.89	7.41 ab	15.6	16.1	15.8 b	0.096	0.101	0.099 b	1979	2143	2061 b
24 h	84.3	86.7	85.5 b	6.52	7.31	6.92 bc	15.2	16.0	15.6 bc	0.093	0.098	0.095 bc	1834	2023	1929 cd
Mean	86.0 b	88.3 a	87.1	6.62 b	7.39 a	7.00	15.1 b	15.7 a	15.4	0.091 b	0.100 a	0.096	1870 b	2047 a	1958
A		4.225			0.529			0.513			0.006			117.476	
B		2.258			0.283			0.274			0.003			62.794	
A × B		NS			NS			NS			0.008			NS	

Different case letters in a column represents statistical differences of mean according to Tukey's multiple range test at a significance level of $p \leq 0.05$. NS-Non significant.

showed a similar trend, reaching their peak at 16 h with a 1:2 (w/v) ratio (Table 1), with maximum values (2373 and 11.92, respectively) being substantially higher than those of the non-primed control (1535 and 6.44, respectively).

The improved germination observed at 16 h with a 1:2 (w/v) seed-to-water ratio can be attributed to the optimization of pre-germinative metabolic processes. Controlled hydration initiates the repair of DNA and cellular membranes, activates antioxidant enzymes (SOD, CAT, and POX) and enhances the mobilization of stored reserves via increased α-amylase activity (Lutts *et al.* 2016). The superiority of the 1:2 (w/v) ratio suggests that adequate water availability is crucial for triggering these metabolic enzymes. Maintaining an ideal hydration level allows the seed to progress through Phase I and II of germination without reaching Phase III (radicle protrusion), ensuring a high-vigour primed state (Singh *et al.* 2026). Our findings align with reports by Anbalagan *et al.* (2024) and studies in chickpea (Ntshalintshali *et al.* 2025).

The significant increase in biomass and vigour indices is likely due to the efficient mobilization of reserves from the cotyledons to the developing embryonic axis. However, the decline in seed quality beyond 16 h likely resulted from over-priming and oxygen depletion. Prolonged submerged soaking induces hypoxic conditions, leading to anaerobic respiration, the buildup of toxic metabolites such as ethanol, and the leaching of essential solutes (Bewley *et al.* 2012, Anbalagan *et al.* 2024).

The enhanced growth parameters observed in this study (specifically, the 36% and 27.8% increases in SVI) are consistent with findings in other lentil varieties (Poomani *et al.* 2023, Rao *et al.* 2025) and pigeon pea (Sarkar *et al.* 2021). This confirms that an optimal hydration window is required to maximize physiological gains while avoiding irreversible cellular damage or loss of desiccation tolerance (Jatana *et al.* 2024).

Effect of biopriming on germination and seedling growth: The interaction between bacterial inoculum concentration and soaking duration significantly influenced germination percentage, shoot length, and root length at $p \leq 0.05$ (Table 2). The highest germination (94.7%), shoot length (9.19 cm), and root length (17.3 cm) were recorded with 1% biopriming (1×10^6 CFU/mL) for 12 h, which was significantly superior to the non-primed control (81.3%, 6.91 cm, and 13.5 cm) and the hydropriming control (Fig. 2).

The interaction also significantly influenced seedling fresh weight, dry weight, and vigour indices ($p \leq 0.05$) (Table 3). The maximum seedling fresh weight (1.32 g/10 seedlings) and dry weight (0.136 g/10 seedlings) were achieved with 1% biopriming for 12 h, which was significantly superior to both the non-primed control (0.81 g and 0.089 g) and the hydropriming control (1.10 g and 0.101 g). Seedling vigour indices (SVI-I and SVI-II) exhibited a similar pattern, attaining their maximum at 1% concentration with 12 h priming (2507 and 12.90, respectively), which were significantly higher than those of the non-primed control (1662 and 7.21) as well as all other

Table 2 Effect of bacterial inoculum concentration and biopriming duration on germination percentage, shoot length, root length of lentil

	Germination percentage (%)					Shoot length (cm)					Root length (cm)				
	4 h	8 h	12 h	16 h	Mean	4 h	8 h	12 h	16 h	Mean	4 h	8 h	12 h	16 h	Mean
Control	81.3	81.3	81.3	81.3	81.3 b	6.91	6.91	6.91	6.91	6.91 c	13.5	13.5	13.5	13.5	13.5 c
Hydropriming	87.3	88.7	89.3	92.3	89.4 a	7.19	7.33	8.54	8.79	7.97 b	15.6	16.1	16.2	16.7	16.2 a
Biopriming 0.1%	80.0	81.7	90.0	79.3	82.8 b	7.07	7.22	8.62	8.08	7.75 b	14.1	14.4	15.8	14.9	14.8 b
Biopriming 1%	82.7	90.3	94.7	84.0	87.9 a	7.98	8.75	9.19	8.80	8.68 a	14.8	16.0	17.3	15.9	16.0 a
Biopriming 10%	80.0	80.0	86.3	76.0	80.6 b	7.32	7.64	8.46	8.10	7.88 b	14.2	14.6	16.1	15.6	15.1 b
Biopriming 100%	58.3	22.0	14.3	8.3	25.8 c	6.59	5.94	5.51	4.24	5.57 d	12.6	12.0	11.7	10.8	11.8 d
Mean	78.3 a	74.0 c	76.0 b	70.2 d	74.6	7.18 c	7.30 bc	7.87 a	7.49 b	7.46	14.1 b	14.5 b	15.1 a	14.6 b	14.6
	C	T	C × T			C	T	C × T			C	T	C × T		
SEM±	0.861	0.7	1.7			0.13	0.11	0.27			0.2	0.2	0.4		
CD (p=0.05)	2.449	2.0	4.9			0.38	0.31	0.75			0.6	1.2	1.2		

Different case letters in a column represents statistical differences of mean according to Tukey's multiple range test at a significance level of $p \leq 0.05$. HP, Hydropriming; C, Concentration; T, Duration of soaking; CD, Critical difference.

biopriming treatments. Conversely, the highest concentration (100%, 1×10^8 CFU/mL) caused a drastic reduction in all parameters, with germination falling to 25.8% and vigour indices dropping to 127 (SVI-I) and 0.65 (SVI-II) at the 16 h.

The superiority of the 1% concentration for 12 h can be attributed to the establishment of an optimal microbial load on the seed surface. At this density (1×10^6 CFU/mL), the *Pseudomonas oleovorans* V3RM-33 strain effectively colonizes the spermosphere, triggering the synthesis of phytohormones such as indole-3-acetic acid (IAA) and gibberellins, which promote cell division and elongation in the radicle and plumule (Govindasamy *et al*. 2010). Furthermore, the 12 h duration provides sufficient time for the seeds to reach the lag phase of germination (Phase II), allowing for DNA repair and mitochondrial activation without inducing the physical stress of radicle protrusion. Similar growth-promoting effects of moderate PGPR inoculum (10^6–10^7 CFU/mL) have been reported in other legumes like soybean (Ajinde *et al*. 2023) and cowpea (Akpor *et al*. 2024), where balanced microbial populations enhanced nutrient mobilization and antioxidant defence.

Enhanced biomass accumulation at this optimal concentration may be further attributed to specific plant growth-promoting (PGP) traits of the *P. oleovorans* V3RM-33 strain, including phosphorus and potassium solubilization and siderophore release. As noted by Ahmad *et al*. (2025), such microbial activity enhances the efficiency of dry matter partitioning from the cotyledons to the growing embryonic axis. Similar synergistic gains in biomass have been reported in rice (Kokila and Bhaskaran 2016) and soybean (Ajinde *et al*. 2023), where optimized microbial loads improved seedling dry weight by over 25% compared to water-soaking alone.

In contrast, lower concentrations (0.1%) were likely insufficient to establish a functional population, while the decline in vigour at the 100% concentration (10^8 CFU/mL) indicates that excessive microbial density becomes a biological stressor. This inhibitory effect is likely associated with increased microbial competition for oxygen and nutrients at the seed-water interface. As observed by Raja and Anandham (2020), excessive inoculum leads to the rapid depletion of oxygen, inducing a hypoxic environment that promotes anaerobic respiration and the accumulation of toxic fermentation metabolites (ethanol), which irreversibly damage the seed embryo. Additionally, prolonged soaking (16 h) at high concentrations may lead to over-priming, where seeds lose desiccation tolerance or suffer from electrolyte leaching (Mekonnen and Kibret 2021). Therefore, a 1% inoculum concentration for 12 h represents the ideal equilibrium between microbial stimulation and physiological hydration for maximizing the seed quality of lentil variety L4717.

Multivariate visualization of biopriming eficiency: A combined heatmap was generated to comprehensively visualize the interaction between microbial concentration and soaking duration across all physiological parameters (Fig. 3). The normalized colour gradient, transitioning

Table 3 Effect of bacterial inoculum concentration and biopriming duration on seedling fresh weight, seedling dry weight, seedling vigour index-I and seedling vigour index-II of lentil

	Seedling fresh weight (g/10 seedlings)				Seedling dry weight (g/10 seedlings)				Seedling vigour index-I				Seedling vigour index-II			
	4h	8h	12h	16h	Mean	4h	8h	12h	16h	Mean	4h	8h	12h	16h	Mean	
Control	0.81	0.81	0.81	0.81	0.81 c	0.089	0.089	0.089	0.089	0.089c	1662	1662	1662	1662	1662 c	7.21	7.21	7.21	7.21	7.21 d
Hydropriming	0.90	1.03	1.10	1.21	1.06 b	0.094	0.097	0.101	0.125	0.104b	1992	2079	2211	2352	2159 a	8.25	8.58	9.05	11.58	9.37 b
Biopriming 0.1%	0.96	1.03	1.14	1.09	1.06 b	0.098	0.093	0.115	0.101	0.102b	1695	1763	2192	1823	1868 b	7.84	7.59	10.40	8.02	8.46 c
Biopriming 1%	1.11	1.14	1.32	1.11	1.17 a	0.108	0.116	0.136	0.116	0.119a	1885	2236	2507	2073	2175 a	8.96	10.51	12.90	9.75	10.53 a
Biopriming 10%	1.03	1.06	1.21	1.11	1.10 b	0.102	0.103	0.116	0.103	0.106b	1722	1781	2120	1799	1856 b	8.15	8.24	10.04	7.79	8.56 c
Biopriming 100%	0.69	0.65	0.64	0.61	0.65 d	0.086	0.086	0.084	0.080	0.084c	1118	396	248	127	472 d	5.01	1.87	1.22	0.65	2.19 e
Mean	0.92 c	0.96 bc	1.04 a	0.99 b	0.97	0.096 b	0.097 b	0.107 a	0.102ab	0.101	1679 b	1653 b	1823 a	1639 b	1699	7.57 b	7.33 b	8.47 a	7.50 b	7.72
	C	T	C × T			C	T	C × T			C	T	C × T			C	T	C × T		
SEM±	0.02	0.02				0.003	0.002	0.005			24	19.811	48.528			0.24	0.198	0.484		
CD ($p\leq0.05$)	0.05	0.04				0.008	0.006	0.015			68.994	56.333	137.99			0.68	0.562	1.376		

Different case letters in a column represents statistical differences of mean according to Tukey's multiple range test at a significance level of $p \leq 0.05$. HP, Hydropriming; C, Concentration; T, Duration of soaking; CD, Critical difference.

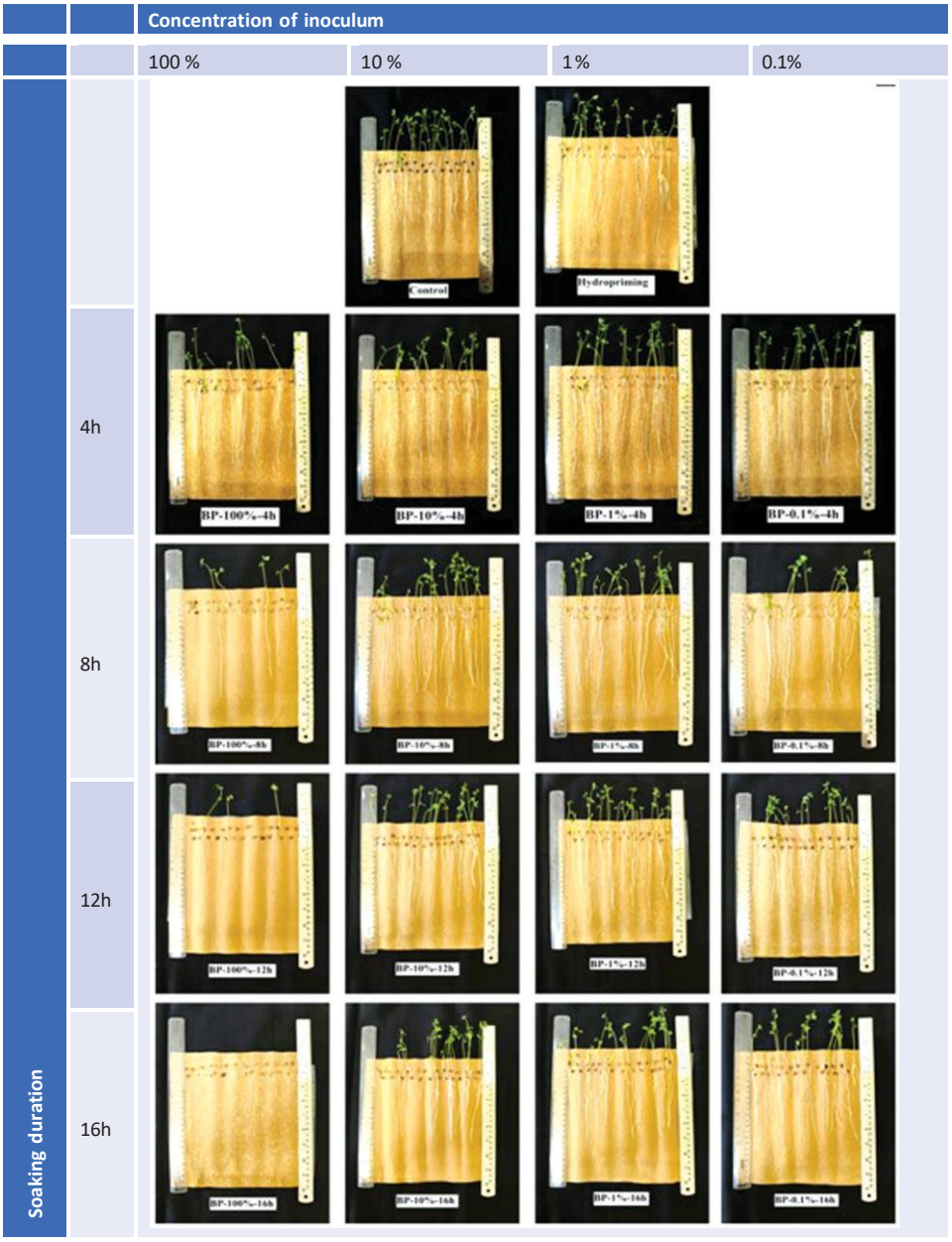


Fig. 2 Morphological comparison of lentil seedlings (variety L4717) recorded on the 10th day of the standard germination test under varying biopriming concentrations and soaking durations.

from purple (low) to yellow (high), clearly delineates the physiological shifts across the treatment matrix.

The heatmap reveals a prominent yellow zone concentrated at the 1% inoculum level for 12 h, signifying the optimal equilibrium for germination, seedling elongation, and vigour indices. This visual clustering confirms that a moderate microbial load (1×10^6 CFU/mL) provides the most consistent stimulus for lentil development. In sharp contrast, the 100% inoculum level (1×10^8 CFU/mL) appears as a distinct purple band across all durations, indicating a strong inhibition of growth. Similar multivariate trends have been reported in pulse crops to delineate optimal priming zones, providing a robust approach for standardizing complex biopriming protocols (Jatana *et al*. 2024).

The present study established that precise optimization of hydration duration and microbial concentration is fundamental to enhancing the physiological quality of the lentil variety L4717. Hydropriming for 16 h at a 1:2 (w/v) seed-to-water ratio significantly enhanced seed germination (91.8%) and vigour compared to the non-primed control. For biopriming, the application of the newly isolated PGPR strain (*Pseudomonas oleovorans* V3RM-33) at a 1% concentration (1×10^6 CFU/mL) for 12 h was found to be the most effective treatment, yielding the highest germination (94.7%) and seedling vigour index (2507). Conversely, an excessive microbial load (100% inoculum) and prolonged soaking (>16 h) acted as biological stressors, severely inhibiting seedling development. These findings demonstrate that precise standardization of hydration duration and microbial concentration is essential for maximizing the physiological quality of lentil seeds. While these laboratory results provide a robust baseline for enhancing early seedling establishment, future field-scale evaluations are required to validate these optimized protocols across diverse agro-climatic conditions.

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Fig. 3 Heat map indicates the variation in the germination and seedling growth parameters across different durations and concentration of biopriming in lentil L4717.
Control, Non-treated seeds; HP, Hydropriming; h, Hours of priming; Germ, Germination%; Shoot, Shoot length (cm); Root, Root length (cm); FW, Seedling fresh weight (g/10 seedlings); DW, Seedling dry weight (g/10 seedlings); SVI-I, Seedling vigour Index-I; SVI-II, Seedling vigour index-II. The colour gradient from blue-green-yellow represents low to high values.

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