



THE EFFECT OF *BACILLUS* SPP. INOCULATION ON SEED GERMINATION AND SEEDLING GROWTH IN WINTER WHEAT (*TRITICUM AESTIVUM* L.)

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SUMMARY

Early seed vigor and uniform establishment have become the recognized key determinants of yield stability in winter wheat (*Triticum aestivum* L.), particularly under diverse environmental conditions. Determining early physiological performance and adaptation potential can be via seed imbibition efficiency, root elongation, coleoptile growth, and germination capacity. Therefore, the following study aimed to evaluate the effects of biological seed treatments on these traits in winter wheat cv. Asr under laboratory conditions. Wheat seeds received treatments with different dilution ratios (1:1–1:9) of *Bacillus megaterium*, *B. subtilis*, and *B. licheniformis*, assessing the variables such as hydration dynamics, seedling growth, and germination. The results showed concentration-dependent physiological responses in wheat seedlings. Moderate dilutions (1:7–1:9) improved water uptake and considerably stimulated root and coleoptile elongation compared with the control. Root and coleoptile length exhibited the robust coordinated response, revealing enhanced early vigor. The germination percentage varied moderately, suggesting that treatments mainly influenced the post-imbibition growth rather than basic viability. Correlation analysis confirmed a significant association between the root and coleoptile growth. Thus, the optimized biological seed treatments remarkably enhanced the early structural growth and establishment potential of winter wheat seedlings.

Keywords: Winter wheat (*T. aestivum* L.), *Bacillus* spp., biological seed treatments, seedling vigor, imbibition, early seedling development, root elongation, coleoptile growth

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Key findings: Biological seed treatment with *Bacillus* spp. significantly improved the early physiological performance of winter wheat (*T. aestivum* L.) cv. Asr seedlings. Moderate dilution ratios (1:7–1:9) enhanced water uptake, root length, and coleoptile elongation, while germination percentage showed moderate variations.

INTRODUCTION

Winter wheat (*Triticum aestivum* L.) is one of the most widely cultivated staple cereal crops that substantially contribute to global food security. However, increasing climate variability, soil salinity, nutrient depletion, and land degradation threaten wheat productivity (Buck and Palumbo-Compton, 2022). Sustainable intensification strategies are therefore essential to considerably improve crop establishment and yield stability while reducing the dependency on chemical inputs (Fukami *et al.*, 2018).

In wheat breeding programs, seed germination and early seedling vigor are crucial for successful field establishment, stress tolerance, and final grain yield in crops (Finch-Savage and Bassel, 2016). These important traits incur regulation by complex genetic and physiological mechanisms, including water imbibition, metabolic reactivation, and coordinated root-shoot development during early ontogenesis (Nonogaki *et al.*, 2010). Overall, the rapid and uniform germination enhances the competitive ability and resource-use efficiency, which are increasingly vital under regenerative and low-input agricultural systems (Khan and Bano, 2020).

Plant growth-promoting rhizobacteria (PGPR) have emerged as promising biological tools for improving seed performance and early plant growth and development (Nelson, 2004; Martínez-Viveros *et al.*, 2010). Among them, species of the genus *Bacillus* emerged to be particularly effective due to their ability to fix atmospheric nitrogen, solubilize phosphorus, produce phytohormones, synthesize siderophores, and mitigate abiotic stress conditions (Nelson, 2004; Martínez-Viveros *et al.*, 2010). Different *Bacillus* species, such as *B. megaterium*, *B. subtilis*, and *B. licheniformis*, have had wide reports of improving germination percentage, root elongation, and seedling vigor in cereal crops

through biochemical and hormonal modulation (Ahmad *et al.*, 2008; Adesemoye and Kloepper, 2009; Martínez-Viveros *et al.*, 2010).

Nitrogen-fixing bacteria, such as *Azotobacter chroococcum*, can considerably influence wheat seed germination and seedling biomass in a concentration-dependent manner (Erofeeva *et al.*, 2019). Similarly, the associative bacterial consortia have also shown positive effects on cereal seedling establishment under diverse soil environmental conditions (Sidorenko *et al.*, 2021). However, comparative evaluation of different *Bacillus* species and their dilution ratios on germination dynamics, imbibition processes, and early seedling growth traits of winter wheat remains insufficiently studied.

From a breeding perspective, understanding the relationship between seed mass, water uptake, root-coleoptile growth, and germination percentage under microbial inoculation is crucial for developing biologically enhanced seed treatment strategies compatible with sustainable wheat improvement through breeding programs. Therefore, the objective of this study was to comparatively evaluate the effects of different concentrations of *B. megaterium*, *B. subtilis*, and *B. licheniformis* strains on seed germination, imbibition dynamics, and early seedling growth and development in winter wheat (*T. aestivum* L.).

MATERIALS AND METHODS

Plant material and bacterial strains

The experiment commenced using seeds of winter wheat (*T. aestivum* L.) cv. Asr. The selection of uniform and healthy seed samples ensued before conducting the experiment according to the International Seed Testing Association standards (ISTA, 2020). The use of three plant growth-promoting rhizobacteria (PGPR) species comprised *Bacillus*

megaterium, *B. subtilis*, and *B. licheniformis*. However, the source and strain identification of the bacterial isolates underwent clear specifications. These species were the widely reported effective PGPR due to their ability to enhance nutrient availability, produce phytohormones, and stimulate early plant growth and development (Nelson, 2004; Martínez-Viveros *et al.*, 2010; Fukami *et al.*, 2018). Bacterial suspensions prepared involved diluting them with sterile distilled water to obtain five ratios: 1:1, 1:3, 1:5, 1:7, and 1:9 under laboratory conditions. Distilled water served as the control treatment.

Experimental design and germination assay

The experiment arrangement was in a completely randomized design following standard agricultural experimental procedures (Gomez and Gomez, 1984; Montgomery, 2013). Wheat seeds entailed placement in Petri dishes lined with filter paper before incubation at 20 °C in a thermostat. Each treatment comprised 30 seeds per replicate with three replications, totaling 1020 seeds used. Germination tests, as performed, were in accordance with ISTA (2020) rules. Germination percentage and early seedling growth parameters reached evaluation following standard seed vigor assessment approaches (Nonogaki *et al.*, 2010; Finch-Savage and Bassel, 2016).

Measurement of imbibition and growth traits

Seed mass recording transpired before incubation and after 24, 48, and 72 h using an analytical balance. Water uptake (imbibition) calculation was the difference between initial and final seed mass, expressed in mg (Nonogaki *et al.*, 2010). The calculation of the ratio of absorbed water to seed mass sought to evaluate imbibition efficiency. After 72 h, the data recorded on the following parameters included root length (cm), coleoptile length (cm), and germination percentage (%). The germination percentage calculation used the standard formula as follows (ISTA, 2020):

Germination (%) = the number of germinated seeds / Total seeds × 100

Statistical analysis

All recorded data based on various traits underwent the analysis of variance (ANOVA) to determine significant mean differences among the treatments following Gomez and Gomez (1984). Mean comparisons performed used the least significant difference (LSD_{0.05}) test. Correlation analysis proceeded to determine relationships between seed mass, water uptake, root length, coleoptile length, and germination percentage. Statistical analyses continued using SPSS software (Version 17.0) according to standard procedures for agricultural data analysis (Shishlyannikova, 2005).

RESULTS

The effect of different dilution ratios (1:1–1:9) of the *B. subtilis* strain suspension on imbibition and early growth traits of winter wheat (*T. aestivum* L.) cv. Asr seeds, expressed as a percentage relative to the control, appear in Figure 1. The results revealed the bacterial concentration significantly influenced the physiological activation during the early ontogenetic stages. Water uptake during imbibition displayed a considerable increase at 1:5 and 1:7 dilutions (approximately 101%–105%), suggesting improved permeability of the seed coat and enhanced metabolic activation. The 1:3 dilution resulted in reduced water absorption (≈83%), indicating that higher bacterial concentration may impose mild physiological stress. In water-to-seed mass ratio, the highest efficiency recording was at the 1:9 dilution (≈93%), while the 1:3 treatment showed the lowest value, further confirming the dose-dependent response of seed hydration processes to bacterial inoculation.

Root length exhibited considerable stimulation at 1:7 and 1:9 dilutions (≈122%), which may indicate an association with auxin-like compounds produced by the *B. subtilis*, thereby enhancing cell elongation and root

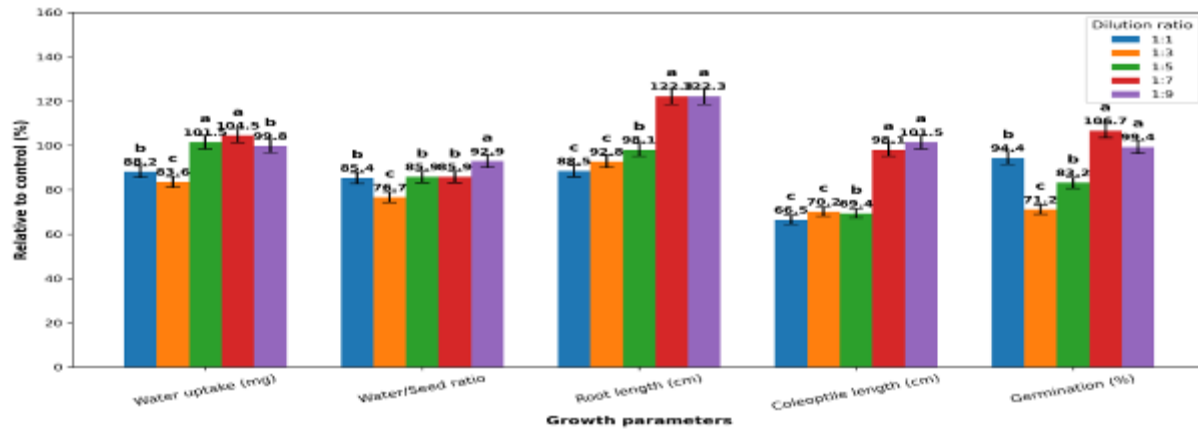


Figure 1. Effect of *B. subtilis* dilution ratios on seed imbibition and early seedling growth traits of winter wheat (*T. aestivum* L.).

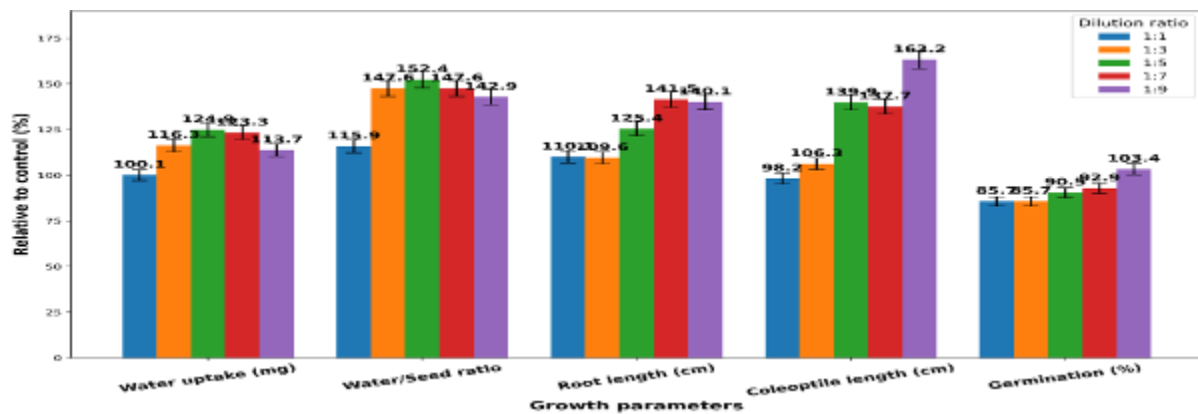


Figure 2. Effect of *B. licheniformis* dilution ratios on seed imbibition and early seedling growth traits of winter wheat (*T. aestivum* L.).

meristem activity. Coleoptile length also gained a significant improvement at 1:7 and 1:9 ($\approx 98\%$ – 101%), showing coordinated enhancement of below- and aboveground seedling growth. Germination percentage reached its maximum at the 1:7 dilution ($\approx 107\%$), showing significant ($P < 0.05$) superiority over other treatments, while the 1:3 dilution markedly reduced germination ($\approx 71\%$), suggesting that excessive bacterial concentration may exert inhibitory effects. Overall, the results revealed moderate dilutions (1:7) provided optimal physiological conditions for seed metabolic activation, leading to improved imbibition efficiency, enhanced root and coleoptile development, and higher germination performance. However, an excessive bacterial density negatively affects

seedling establishment, thereby highlighting the importance of determining optimal inoculum concentration for better seedling development in wheat.

The influence of different dilution ratios (1:1–1:9) of the *B. licheniformis* strain suspension on imbibition and early growth traits of winter wheat (*T. aestivum* L.) cv. Asr seeds, expressed as a percentage relative to the control, are notable (Figure 2). Bacterial treatments at moderate and lower concentrations revealed a well-defined stimulatory effect on physiological traits. During the imbibition phase, water uptake progressively increased from the 1:3 to the 1:5 dilution levels, reaching its maximum at the 1:5 dilution ($\approx 125\%$). This pattern indicates enhanced seed coat permeability and

accelerated metabolic activation. The slightly lower values, recorded at dilutions of 1:7 and 1:9, suggest that optimal hydration occurs within a specific concentration range. In water-to-seed mass ratio, the 1:5 dilution exhibited the highest value ($\approx 152\%$), significantly ($P < 0.05$) exceeding the control, thereby confirming that *B. licheniformis* markedly improved the imbibition efficiency and water retention capacity of wheat seeds.

Root length showed substantial enhancement at 1:7 and 1:9 dilutions ($\approx 141\%$ – 140%), indicating robust stimulation of root elongation processes, which may refer to bacterial production of phytohormones and auxin-like compounds and improve nutrient mobilization. Coleoptile length responded more prominently, reaching its highest value at the 1:9 dilution ($\approx 163\%$), suggesting that lower bacterial concentrations favor aboveground growth and promote coordinated seedling development. Germination percentage gradually increased with dilution, attaining its maximum at 1:9 ($\approx 103\%$), whereas lower dilutions (1:1 and 1:3) revealed comparatively reduced values, detailing that excessive bacterial density may partially constrain germination efficiency. Overall, the results revealed *B. licheniformis* exerts a considerable positive effect on imbibition-related traits and early seedling growth, observing optimal performance at intermediate to lower

concentrations (particularly 1:5–1:9). It highlights its potential as an effective biological agent for enhancing seed vigor and early establishment in winter wheat.

The effect of different dilution ratios (1:1–1:9) of *B. megaterium* strain suspension on imbibition and early growth traits of winter wheat (*T. aestivum* L.) cv. Asr seeds, expressed as a percentage relative to the control, are in Figure 3. The results revealed a distinctive response compared with other bacterial strains, with moderate influence on imbibition but pronounced stimulation of seedling growth traits. During the imbibition phase, water uptake remained relatively stable at 1:1–1:7 dilutions ($\approx 80\%$) and increased only at the 1:9 dilution ($\approx 100\%$), indicating those higher dilutions may alleviate possible osmotic and metabolic constraints associated with concentrated bacterial suspensions. For water-to-seed mass ratio, the values remained low ($\approx 64\%$) at 1:1–1:7 treatments; however, they increased at 1:9 ($\approx 80\%$), suggesting that optimal hydration efficiency occurred at reduced bacterial density. In contrast, root length exhibited robust stimulation, particularly at the 1:7 dilution ($\approx 159\%$), found significantly ($P < 0.05$) superior, confirming that *B. megaterium* significantly enhanced the root elongation by promoting cell division and root meristem. For coleoptile length, the highest values occurred at 1:5 ($\approx 155\%$),

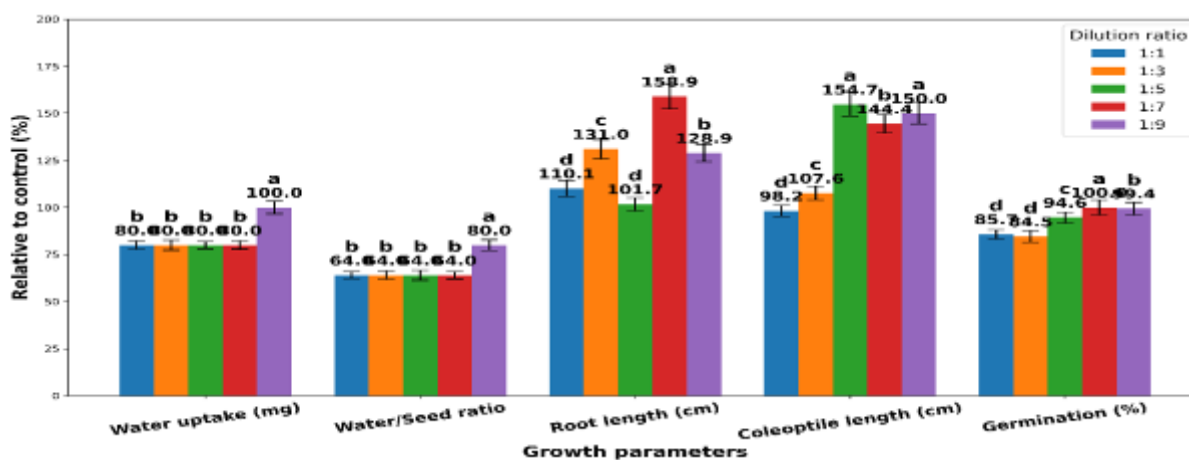


Figure 3. Effect of *B. megaterium* dilution ratios on seed imbibition and early seedling growth traits of winter wheat (*T. aestivum* L.).

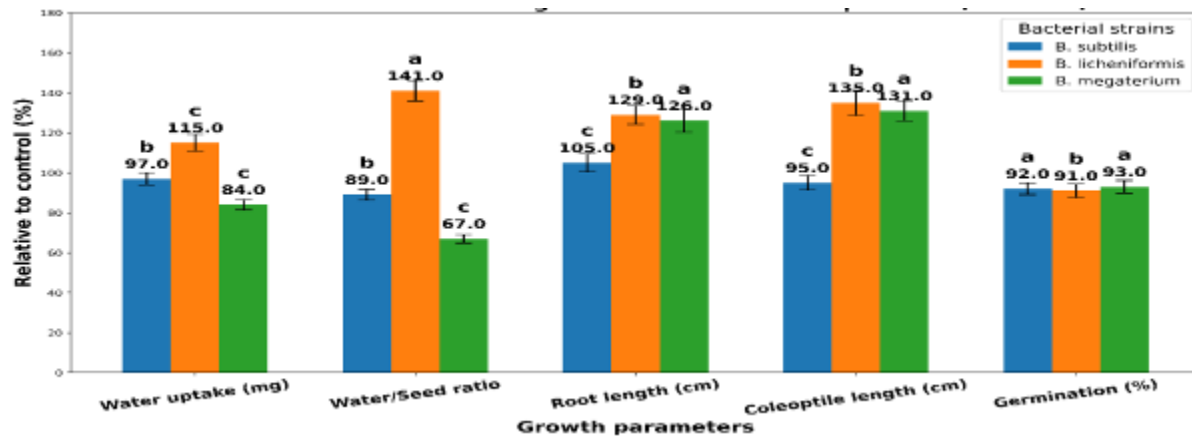


Figure 4. Comparative effects of *Bacillus* strains on seed imbibition and early seedling growth traits of winter wheat (*T. aestivum* L.).

sustaining enhancement at dilutions of 1:7 and 1:9, exhibiting coordinated stimulation of below- and aboveground seedling growth. The germination percentage was 100% at 1:7 and remained high at 1:9, confirming that moderate dilutions favor improved seed viability and emergence performance. Overall, the results revealed *B. megaterium* exerts a more robust impact on post-imbibition growth processes than on initial water uptake. This suggests that its primary mode of action may be in association with phytohormone production, improved nutrient mobilization, and enhanced metabolic processes.

Expressing the comparative effects of three *Bacillus* strains (*B. subtilis*, *B. licheniformis*, and *B. megaterium*) on seed imbibition and early growth traits of winter wheat (*T. aestivum* L.) seeds was in percentages relative to the control (Figure 4). The results clearly detailed strain-specific variations in physiological stimulation patterns. Among the tested strains, *B. licheniformis* exhibited the robust effect on water uptake ($\approx 115\%$), significantly ($P < 0.05$) exceeding *B. subtilis* ($\approx 97\%$) and *B. megaterium* ($\approx 84\%$), revealing considerable improvement in seed hydration processes. For water-to-seed mass ratio, the *B. licheniformis* also produced the highest increase ($\approx 141\%$), while *B. subtilis* showed moderate ($\approx 89\%$) and *B. megaterium* resulted in lower stimulation ($\approx 67\%$). The root length reached marked stimulation from both

B. licheniformis ($\approx 129\%$) and *B. megaterium* ($\approx 126\%$), whereas *B. subtilis* revealed a moderate effect ($\approx 105\%$). Coleoptile length followed a similar pattern, with *B. licheniformis* ($\approx 135\%$) and *B. megaterium* ($\approx 131\%$) remarkably outperforming *B. subtilis* ($\approx 95\%$), highlighting the robust role of these two strains in promoting aboveground seedling growth and development. For germination percentage, the strains showed less variation; the *B. subtilis* ($\approx 92\%$) and *B. megaterium* ($\approx 93\%$) exhibited comparable values, while *B. licheniformis* displayed a slightly lower response ($\approx 91\%$). Overall, the comparative analysis revealed *B. licheniformis* appeared with the most consistent and pronounced stimulatory effect on both imbibition-related and growth parameters. Meanwhile, *B. megaterium* primarily enhances post-imbibition growth traits, and *B. subtilis* exerts moderate and stable influence across all measured traits. The results emphasized the potential application of *B. licheniformis* as the most effective bioinoculant for improving early vigor and seedling establishment in winter wheat.

The overall effect of bacterial dilution ratios (1:1–1:9) on wheat seed imbibition and early seedling traits had percentage expressions relative to the control (Figure 5). The results revealed an obvious dose-dependent response pattern across the physiological traits. Water uptake progressively increased with dilution, rising from 89% (1:1)

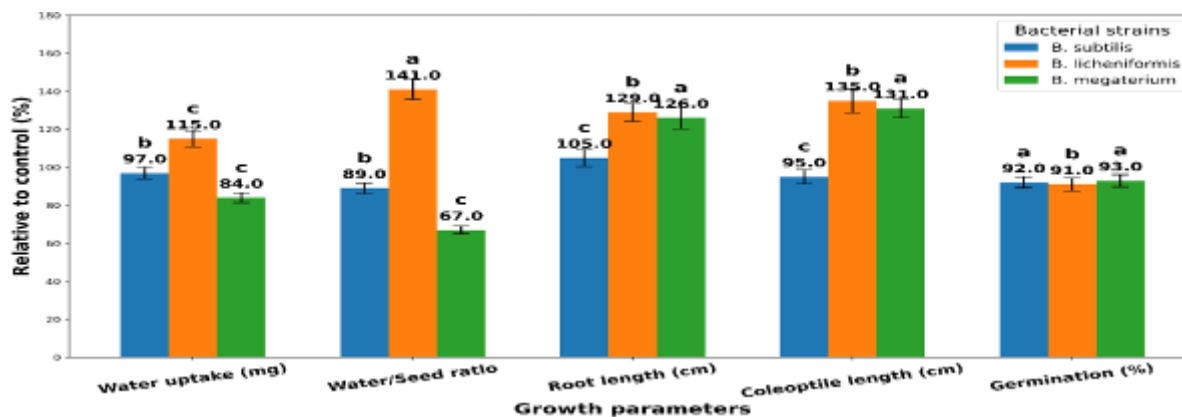


Figure 5. Effect of dilution ratios on seed imbibition and early seedling growth traits of winter wheat (*T. aestivum* L.).

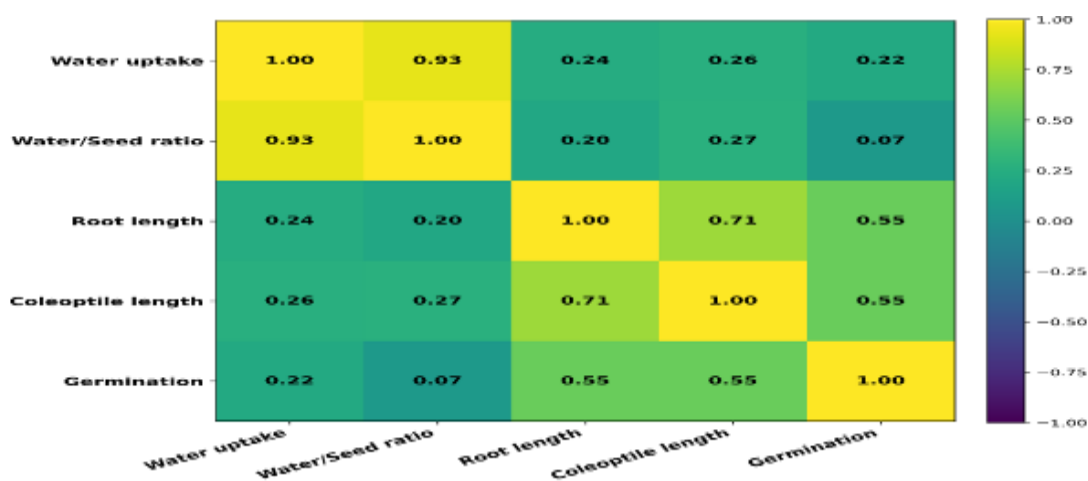


Figure 6. Correlation among seed imbibition and early seedling growth traits of winter wheat (*T. aestivum* L.).

to 105% (1:9), showing that lower bacterial concentration favors improved hydration efficiency and balanced osmotic regulation during the imbibition phase. Root length exhibited a pronounced stimulatory trend, increasing steadily from 103% (1:1) to 138% (1:9), suggesting that moderate-to-high dilutions significantly promote root elongation and meristematic growth. Coleoptile length followed the similar trend, reaching its highest value at 1:9 ($\approx 130\%$), revealing coordinated stimulation of aboveground seedling development and improved early vigor under optimal bacterial dilution. The germination percentage showed moderate variations;

although, it decreased at 1:3 ($\approx 81\%$) and subsequently increased with dilution 1:9 (101%), signifying that appropriate dilution may enhance seed viability. Generally, the results confirmed the dilution ratio was a crucial factor of biological treatment efficiency, and the bacterial dilutions 1:7 and 1:9 provided the most favorable conditions for enhanced imbibition dynamics, root and coleoptile growth, and stable germination performance, thus highlighting the importance of optimizing inoculum concentration for maximizing biofertilizer efficacy in winter wheat.

According to the correlation coefficient among imbibition-related and early seedling growth traits of winter wheat, the matrix revealed distinct patterns of physiological interdependence between hydration dynamics and developmental responses (Figure 6). A significant positive correlation ($r = 0.93$) was evident between water uptake and the water-to-seed mass ratio, indicating that these two traits represent closely related aspects of the imbibition process and jointly reflect seed hydration efficiency. In contrast, water uptake showed weak positive correlations with root length ($r = 0.24$), coleoptile length ($r = 0.26$), and germination percentage ($r = 0.22$), suggesting that hydration is a prerequisite for germination, with the early seedling growth influenced by additional metabolic and hormonal factors beyond simple water absorption. Similarly, the water-to-seed mass ratio exhibited weak associations with root length ($r = 0.20$) and coleoptile length ($r = 0.27$), and a very low correlation with germination ($r = 0.07$), further confirming that imbibition intensity alone does not fully determine the final germination and seedling vigor. Inversely, a significant positive correlation emerged between the root length and coleoptile length ($r = 0.71$), indicating a coordinated development of below- and aboveground seedling structures. Moreover, root length showed a moderate-to-strong positive correlation with germination percentage ($r = 0.55$), demonstrating that enhanced early growth had a close association with improved seed viability and emergence potential. These relationships revealed post-imbibition growth traits have more interconnection than hydration traits and may serve as more reliable indicators of seed vigor. Overall, the correlation analysis suggested that although imbibition-related traits were in a strong correlation with each other, their influence on germination and seedling growth was indirect. Contrastingly, root and coleoptile elongation play a central integrative role in early growth performance, thereby highlighting the importance of physiological parameters when assessing the effectiveness of biological seed treatments in winter wheat.

DISCUSSION

The timely study revealed that seed inoculation with *Bacillus* spp. exerted an apparent concentration-dependent effect on imbibition dynamics, early seedling growth, and germination performance of winter wheat (*T. aestivum* L.) cv. Asr. This confirms the practical relevance of PGPR-based seed treatments for improving early vigor under sustainable production systems. These results were greatly analogous to the concept that germination and early establishment had the control of coordinated physiological processes, including rapid water uptake, metabolic reactivation, and synchronized root-shoot development. These microbial inoculants can modulate these processes through multiple biochemical routes (Kloepper et al., 2004; Nonogaki et al., 2010; Finch-Savage and Bassel, 2016).

A key outcome of the presented research was that the three *Bacillus* species differed in their performance across traits, revealing strain-specific modes of action rather than a uniform PGPR effect. In the *B. subtilis* treatments, moderate dilutions, particularly 1:7, maximized germination and promoted root and coleoptile elongation, whereas the 1:3 dilution showed inhibitory tendencies. This pattern was consistent with a dose-response phenomenon widely reported for rhizobacterial inoculants, where an optimal inoculum density enhanced the physiological activation; however, the excessive microbial density may impair seed hydration balance and early metabolic coordination (Nelson, 2004; Martínez-Viveros et al., 2010; Erofeeva et al., 2019). *Bacillus* strains can stimulate early seedling development via phytohormone-like activities, improved nutrient mobilization, and synthesis of bioactive metabolites (Nelson, 2004; Martínez-Viveros et al., 2010; Glick, 2012; Backer et al., 2018).

In contrast, *B. licheniformis* produced the most pronounced stimulation of imbibition-related traits, especially water uptake and water-seed ratio, and enhanced the root and coleoptile growth at intermediate-to-low concentrations. It suggests that this strain may

be particularly effective in improving seed hydration efficiency and early growth vigor. The seed imbibition was not merely a physical process; it initiates cascades of enzymatic activation, reserve mobilization, and embryo growth, and improvement in hydration efficiency can accelerate uniformity of emergence under suboptimal conditions (Nonogaki *et al.*, 2010; Finch-Savage and Bassel, 2016). The considerable stimulation of coleoptile length at higher dilutions, together with robust root elongation, revealed that *B. licheniformis* could better support the coordinated seedling architecture, which is critical for early competitive ability and stand establishment (Lucy *et al.*, 2004; Finch-Savage and Bassel, 2016).

The *B. megaterium* response was distinctive, and the imbibition metrics had less improvement at higher bacterial density, while post-imbibition growth traits—particularly root length and coleoptile length—gained remarkable enhancement at selected dilutions. The results suggested that *B. megaterium* may contribute more to growth regulatory processes after hydration has begun, potentially through nutrient mobilization and growth-promoting metabolites that stimulate meristem activity and cell elongation, rather than directly increasing water uptake itself (Nelson, 2004; Martínez-Viveros *et al.*, 2010). This interpretation aligns with previous findings that *Bacillus*-associated consortia can enhance early seedling performance in cereals by facilitating nutrient availability and stimulating growth (Sidorenko *et al.*, 2021).

The comparative synthesis further clarified the selection priorities: *B. licheniformis* emerged as the most consistent strain for improving both hydration metrics and early growth, while *B. megaterium* excelled primarily in enhancing seedling elongation traits, and *B. subtilis* showed moderate and stable improvement with an optimum at specific dilutions. These strain-specific differences are not surprising because PGPR effects depend on multiple interacting factors, including the microbial metabolic repertoire, exudate composition, inoculum density, and plant genotype responsiveness (Ahmad *et al.*, 2008; Martínez-Viveros *et al.*, 2010). Importantly,

the germination percentage itself varied less across the strains than seedling growth traits, which supports the concept that PGPR treatments may improve seedling vigor and establishment potential through multiple biochemical pathways and beneficial plant-microbe interactions (Adesemoye and Kloepper, 2009; Glick, 2012; Backer *et al.*, 2018).

The dilution-ratio analysis provides a robust practical message that increasing dilution generally improved water uptake and enhanced root and coleoptile elongation, with the most favorable responses at 1:7 and 1:9. The results revealed overly concentrated bacterial suspensions can induce inhibitory effects—potentially through altered osmotic balance, and excessive microbial metabolites—whereas moderate dilutions preserve growth stimulation and minimize negative effects. The same pattern had a previous report for nitrogen-fixing bacteria influencing cereal germination in a concentration-dependent manner (Erofeeva *et al.*, 2019). The outcomes disclosed that selecting an appropriate dilution is as important as selecting the strain itself, and the more inoculum does not necessarily translate into better performance, especially at the critical imbibition-to-germination transition stage.

The correlation analysis expressed that imbibition indicators had strong interrelations, whereas their relationship with seedling growth and germination was weaker, while root and coleoptile lengths displayed robust interdependence and moderate positive association with germination. This pattern was biologically meaningful because imbibition represents an early enabling step; however, the subsequent seedling development depends on the coordinated hormonal regulation, reserve mobilization, and cell expansion processes (Nonogaki *et al.*, 2010). Therefore, root and coleoptile elongation could serve as more integrative proxies of functional vigor under PGPR treatment than water uptake alone. It has a crucial role in selection criteria and seed quality screening programs, where rapid and uniform establishment is a priority (Finch-Savage and Bassel, 2016).

From the breeding and seed technology perspectives, the results suggested *Bacillus*-based biological seed treatments can be integral to enhance the early vigor traits that contribute to stand establishment, particularly under low-input and stress-prone environments. Meanwhile, the strain by dilution interaction must be at optimum to ensure consistent performance. Although, the generation of these present results happened under controlled laboratory conditions using standardized germination testing procedures (ISTA, 2020). It provides a foundation for subsequent greenhouse and field validation, where soil microbiome interactions, temperature fluctuations, and moisture variability can further influence the bioinoculant performance and stability. Future work should therefore be focusing on the evaluation of the best-performing combinations (*B. licheniformis* at 1:5–1:9 and *B. subtilis* around 1:7) under abiotic stress conditions relevant to wheat production. Likewise, it should evaluate whether these early vigor improvements translate into yield stability and improved stress tolerance at later developmental stages, as expected for sustainable intensification strategies based on biological inputs.

CONCLUSIONS

The results revealed that biological seed treatment with *Bacillus* spp. significantly influenced imbibition dynamics, early seedling growth, and germination of winter wheat (*Triticum aestivum* L.) cv. Asr. Moderate dilution ratios (1:7 and 1:9) provided the most favorable conditions for improving water uptake, root elongation, and coleoptile growth compared with the control. The biological treatments primarily stimulated structural seedling development rather than substantially altering germination percentage. A significant positive correlation between root length and coleoptile length indicated coordinated regulation of early seedling growth processes. Among the evaluated strains, *B. licheniformis* showed the most consistent stimulatory effect on both hydration and growth traits, while *B.*

megaterium significantly promoted post-imbibition seedling elongation. Overall, the findings demonstrate that optimized *Bacillus*-based biological seed treatments can enhance early seedling vigor and improve the establishment potential of winter wheat under sustainable cropping systems.

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CONCLUSIONS

The existing study explained the ability of the utilized rRNA sequences to detect the investigated *Veronica* species and discriminate them from each other. These ribosomal amplicons can efficiently help identify the biological diversity of a wider spectrum of *Veronica* DNA sequences. These promising amplicons can also serve to determine the further details within these identified *Veronica* sequences in various biological and geographical sources.

REFERENCES

- Adesemoye AO, Kloepper JW (2009). Plant-microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol.* 85: 1–12. <https://doi.org/10.1007/s00253-009-2196-0>
- Ahmad F, Ahmad I, Khan MS (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth-promoting activities. *Microbiol. Res.* 163(2): 173–181. <https://doi.org/10.1016/j.micres.2006.04.001>
- Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S, Smith DL (2018). Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization.

- Front. Plant Sci.* 9: 1473. <https://doi.org/10.3389/fpls.2018.01473>
- Buck HJ, Palumbo-Compton A (2022). Soil carbon sequestration as a climate strategy: What do farmers think? *Biogeochemistry*. 161: 59–70. <https://doi.org/10.1007/s10533-022-00949-0>
- Erofeeva EA, Rechkin AI, Savinov AB (2019). Germination and seedling development of wheat under *Azotobacter chroococcum* cell suspension exposure. *Principles Ecol.* (2): 4–11. <https://doi.org/10.15393/j1.art.2019.8405>
- Finch-Savage WE, Bassel GW (2016). Seed vigour and crop establishment: Extending performance beyond adaptation. *J. Exp. Bot.* 67(3): 567–591. <https://doi.org/10.1093/jxb/erv490>
- Fukami J, Cerezini P, Hungria M (2018). *Azospirillum*: Benefits that go far beyond biological nitrogen fixation. *AMB Express*. 8(1): 73. <https://doi.org/10.1186/s13568-018-0608-1>
- Glick BR (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica*. 2012: 963401. <https://doi.org/10.6064/2012/963401>
- Gomez KA, Gomez AA (1984). Statistical Procedures for Agricultural Research. 2nd Ed. John Wiley & Sons, New York.
- ISTA (2020). International rules for seed testing. Int. Seed Test Assoc., Bassersdorf.
- Khan N, Bano A (2020). Role of plant growth promoting rhizobacteria in improving growth and yield of wheat under stress conditions. *SABRAO J. Breed. Genet.* 52(1): 1–14.
- Kloepper JW, Ryu CM, Zhang S (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology*. 94(11): 1259–1266. <https://doi.org/10.1094/PHYTO.2004.94.11.1259>
- Lucy M, Reed E, Glick BR (2004). Applications of free-living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek* 86(1): 1–25. <https://doi.org/10.1023/B:ANTO.0000024903.10757.6e>
- Martínez-Viveros O, Jorquera MA, Crowley DE, Gajardo G, Mora ML (2010). Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *J. Soil Sci. Plant Nutr.* 10(3): 293–319. <https://doi.org/10.4067/S0718-95162010000100006>
- Montgomery DC (2013). Design and Analysis of Experiments. 8th Ed. Wiley, New York.
- Nelson LM (2004). Plant growth promoting rhizobacteria (PGPR): Prospects for new inoculants. *Crop Manag.* 3(1): 301–305. <https://doi.org/10.1094/CM-2004-0301-05-RV>
- Nonogaki H, Bassel GW, Bewley JD (2010). Germination—Still a mystery. *Plant Sci.* 179(6): 574–581. <https://doi.org/10.1016/j.plantsci.2010.02.010>
- Shishlyannikova LM (2005). Mathematical support of scientific research using SPSS for Windows 11.5. Moscow.
- Sidorenko ML, Sleptsova NA, Bykovskaya AN, Berezhnaya VV, Klykov AG (2021). Germination of cereal seeds under influence of nitrogen-fixing and phosphate-mobilizing bacteria. *AgricBiol* 5a6(1): 146–157. <https://doi.org/10.15389/agrobiol.2021.1.146rus>