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Effect of seed coating and seed priming by bacterial strain *Microbacterium hydrocarbonoxydans* GHQ55 on germination and early seedling growth in *Oryza sativa*, *Brassica juncea*, and *Vigna mungo*

V. Laxman,[1] M. Shiva Kumar Reddy [2] Santoshini B[3] and Hameeda Bee*

Department of Microbiology, Osmania University, Hyderabad – 500007

Abstract

This study evaluated how early growth *Vigna mungo* (*Fabaceae*), *Brassica juncea* (*Brassicaceae*), and *Oryza sativa* (*Poaceae*) were treated by seed priming and seed coating with the bacterial strain *Microbacterium hydrocarbonoxydans* GHQ55 (available at P2BL) isolated from rice rhizosphere (GHQ55), and control. All crops benefited greatly from seed priming in terms of root and shoot length, germination, and seedling vigor, whereas biomass accumulation was boosted by seed coating with GHQ55. *Vigna mungo* exhibited the significant shoot and root length with GHQ55 priming and the best germination with coating (98%) as well. Seed priming yielded the vigor and longest roots (12.95 cm) in *Oryza sativa*, whereas seed coating with GHQ55 provided the most biomass. Seed priming with GHQ55 shown most effective germination rate and vigor index for *Brassica juncea.* These findings provide credence to seed priming as a low-cost, successful method of enhancing early seedling performance and crop establishment, particularly when used with microbial strain with GHQ55.

Key words: Plant growth-promoting bacteria, Seed coating, Seed priming, Seed germination, *Oryza sativa*, *Brassica juncea*, *Vigna mungo*, Vigor index.

1. Introduction

Early seedling vigor is a critical determinant of successful crop establishment and productivity. Seed enhancement techniques, such as seed priming and seed coating, have been widely studied for their role in improving germination, seedling growth, and stress tolerance. However, a number of environmental stressors, including dryness, inadequate soil nutrition, and infections, can hinder crop establishment and lower seed vigor throughout the early phases of plant growth. Microbial seed treatments particularly those utilizing plant growth-promoting rhizobacteria (PGPR) (Singh et al., 2021) (Choudhary et al., 2023) have become a viable way to address these issues. These helpful bacteria have been shown to increase nutrient availability, boost hormone production (such as auxins and gibberellins) (Khanal et al., 2024), and increase plant stress tolerance (Liu et al., 2023; Sharma et al., 2024).

Among the most popular methods of application are seed priming (Chatterjee et al., 2024) and seed coating. Using a carrier such carboxymethyl cellulose (CMC), seed coating applies a layer of microbial formulation directly to the seed, assisting microorganisms in remaining adherent and active on the seed surface (Kumawat et al., 2021). Before sowing, however, seeds are soaked in a bacterial slurry for seed priming, which enables early microbial contact and physiological activation (Singh et al., 2020).

The paper towel method, a standardized laboratory methodology, is frequently used by researchers to assess the efficacy of these approaches. In order to precisely measure the proportion of seeds that germinate, the lengths of the roots and shoots, and the seedling vigor index, it permits even moisture distribution and air circulation (ISTA, 2020).

This study compares the effects of bacterial strains GHQ55 on three important crops: *Oryza sativa* (rice), *Brassica juncea* (Indian mustard), and *Vigna mungo* (black gram) when they are applied via coating and priming. We evaluate biomass buildup, early seedling growth, and germination (Ali et al., 2022) success using the paper towel method to identify the best treatment combination for boosting seed vigor and early crop establishment (Radhakrishnan et al., 2021).

2. Materials and Methods

The study employed the bacterial strains GHQ55 (available at P2BL, Dept. of Microbiology), a known PGPR isolate from rice rhizosphere soil, and recognized for its characteristics that promote plant growth. The selection of *Oryza sativa* (rice), *Brassica juncea* (Indian mustard), and *Vigna mungo* (black gram) seeds was based on their differing physiologies and economic significance. *Oryza sativa* seeds, *Vigna mungo* and *Brassica juncea* seeds were purchased from the Hyderabad local market. After being surface sterilized for one minute with 0.1% (w/v) mercuric chloride ($HgCl_2$), followed by seeds were thoroughly rinsed three times with sterile distilled water.

For every variety of *Vigna mungo* (Blackgram), *Brassica* juncea (Indian Mustard) and *Oryza sativa* (Rice) there were three treatments established with the bacterial strain GHQ55 the overnight culture 1×10^8 cfu/mL (cfu-colony forming unit) was used as inoculum for seed coating, seed priming and control seeds were treated with sterile distilled water only, without any bacterial inoculum. The seeds were coated using a bacterial pellet that was combined with 1% carboxymethyl cellulose (CMC) as a binder and they were allowed to air dry before being paper towel germination. Seed priming technique, after soaking in the bacterial suspension for 12 hours, the seeds were allowed to air dry. The assay for germination the paper towel method was applied in accordance with ISTA regulations (ISTA, 2020). In a growth chamber set at 25°C, 30 seeds were spread out on brown paper towels for each treatment 30 seeds (figure.1-6).

2.1. Germination Percentage

After 10 days of germination for each variety *Vigna mungo*, *Brassica juncea* and *Oryza sativa* 30 seedling as taken for germination percentage (%).

2.2. Shoot Length

After 10 days of germination for each variety *Vigna mungo*, *Brassica juncea* and *Oryza sativa* 10 plants as taken shoot length in cm.

2.3. Root Length

After 10 days of germination for each variety *Vigna mungo*, *Brassica juncea* and *Oryza sativa* 10 plants as taken root length in cm.

2.4. Seedling Biomass

After 10 days of germination for each variety *Vigna mungo*, *Brassica juncea* and *Oryza sativa* 10 plants as taken seedling biomass as Total wet and dry weight.

2.5. Seedling Vigor Index

After 10 days of germination for each variety *Vigna mungo*, *Brassica juncea* and *Oryza sativa* 10 plants as taken seedling vigor index as for formula.

Seedling vigor index (SVI) = Germination (%) × (Root + Shoot length).

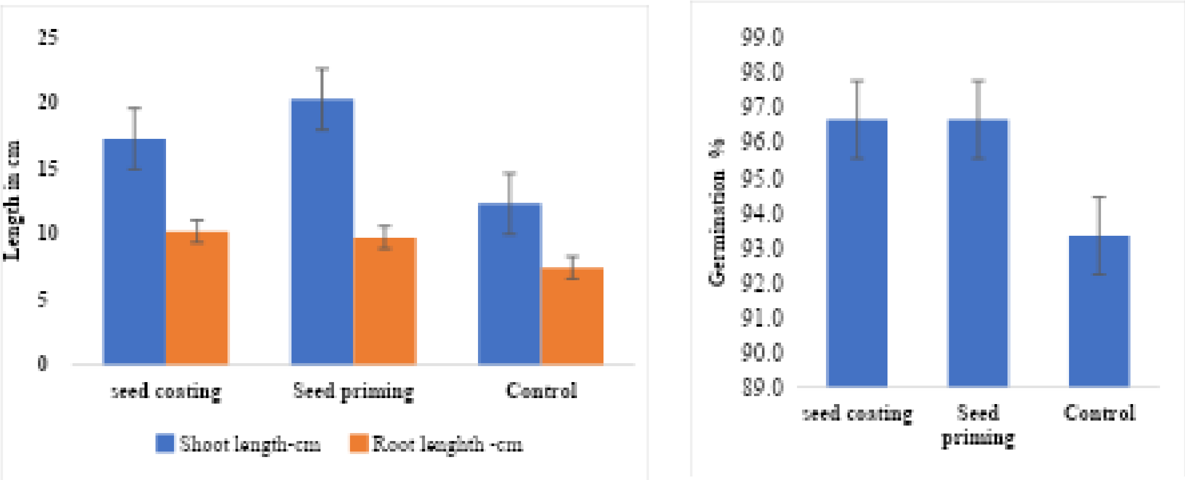
3. Results

Vigna mungo

The study evaluated the impact of three seed treatments seed coating, seed priming, and control (Figure.1 and Figure.2) on *Vigna mungo*.

Seed coating with GHQ55 **Seed priming with GHQ55** **Control**

Figure.1. Effect of seed coating and seed priming by bacterial strain *Microbacterium hydrocarbonoxydans* GHQ55 on *Vigna mungo* at 10 days seedling stage and control.



a

Figure.2. Effect of seed coating and seed priming by bacterial strain *Microbacterium hydrocarbonoxydans* GHQ55 on *Vigna mungo* at 10 days seedling stage and control. a) Root length, Shoot length, b). Gemination percentage (%)

3.1.1. Germination Percentage

The *Vigna mungo* seed coating with GHQ55 exhibited the highest germination rate showed 98%, followed by seed priming with GHQ55 had 96%, and control at 93% (Fig.2.b).

3.1.2. Shoot Length

The seed priming with GHQ55 resulted in the longest shoots (21 cm), with seed coating with GHQ55 had 18 cm, and control at 13 cm (Fig.2.a)

3.1.3. Root Length

The seed priming with GHQ55 also led to the longest roots (10 cm), compared to seed coating with GHQ55 (8 cm) and control (6 cm) (Fig.2.a).

3.1.4. Seedling Biomass

Total wet weight of *Vigna mungo* seed priming with GHQ55 recorded 2.25 g, seed coating 2.21 g, and control 1.78 g.

Total dry weight *Vigna mungo* of seed coating with GHQ55 had 0.199 g, seed priming 0.194 g, and control 0.159 g.

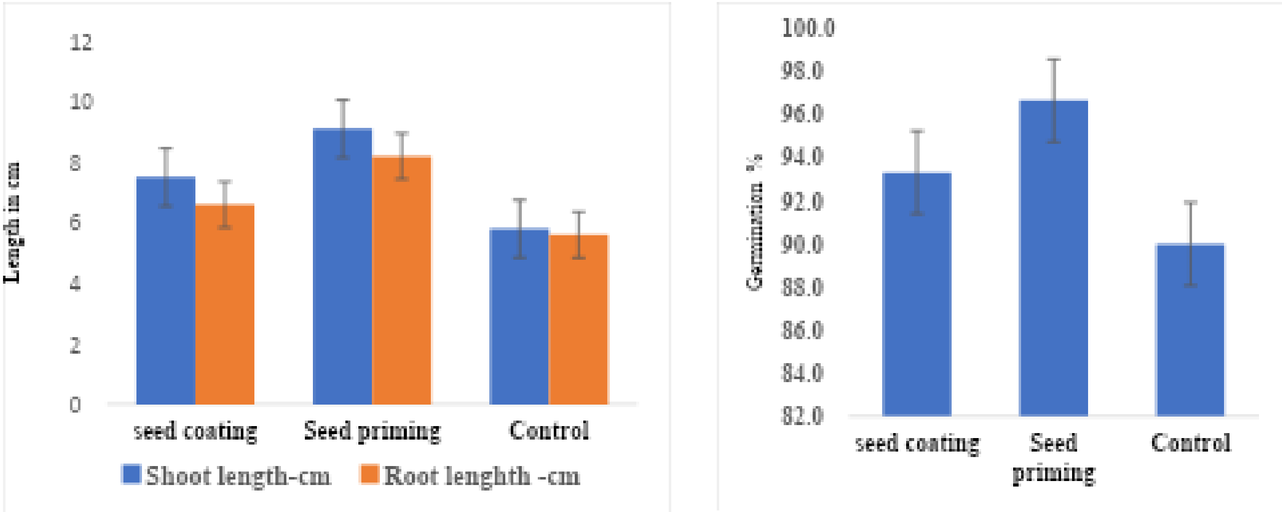
3.1.5. Seedling Vigor Index

The seed priming with GHQ55 achieved the highest index at 2890.33, followed by seed coating with GHQ55 had 2643.83, and control had 1832.96.

Brassica juncea

The study evaluated the impact of three seed treatments seed coating, seed priming, and control (Figure.3 and Figure.4) on *Brassica juncea*.

Figure.3. Effect of seed coating and seed priming by bacterial strain *Microbacterium hydrocarbonoxydans* GHQ55 on *Brassica juncea* at 10 days seedling stage and control.



a b

Figure.4. Effect of seed coating and seed priming by bacterial strain *Microbacterium hydrocarbonoxydans* GHQ55 on *Brassica juncea* at 10 days seedling stage and control. a) Root length, Shoot length, b). Gemination percentage (%)

3.2.1. Germination Percentage

The *Brassica juncea* seed priming with GHQ55 showed the highest germination rate (96.67%), followed by seed coating with GHQ55 (93.33%) and control (90%) (Fig.4.b).

3.2.2. Shoot Length

The highest shoot length was observed in seed priming with GHQ55 (9.1 cm), followed by seed coating with GHQ55 (7.5 cm), and control (5.8 cm) (Fig.4.a).

3.2.3. Root Length

Seed priming with GHQ55 also resulted in the significant root length (8.2 cm), compared to seed coating with GHQ55 (6.6 cm) and control (5.6 cm) (Fig.4.a).

3.2.4. Seedling Biomass

Total wet weight: Seed priming with GHQ55 had the highest seedling wet weight (0.51 g), with seed coating (0.46 g) and control (0.45 g) showing lower values.

Total dry weight of Seed priming with GHQ55 showed the highest dry weight (0.17 g), followed by seed coating (0.153 g) and control (0.15 g).

3.2.5. Seedling Vigor Index

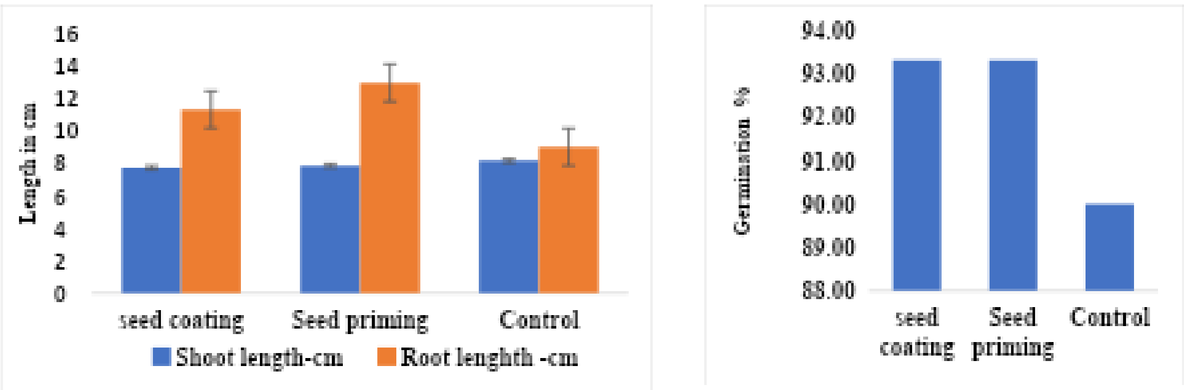
The seed priming with GHQ55 recorded the highest vigor index (1672.33), followed by seed coating (1316.00), and control (1026.00).

Oryza sativa

This study investigated the effects of **seed priming**, **seed coating**, and **control** treatments on early growth traits in *Oryza sativa* (Figure.5 and Figure.6).

Seed coating with GHQ55 **Seed priming with GHQ55** **Control**

Figure.5. Effect of seed coating and seed priming by bacterial strain *Microbacterium hydrocarbonoxydans* GHQ55 on *Oryza sativa* at 10 days seedling stage and control.



a b

Figure.6. Effect of seed coating and seed priming by bacterial strain *Microbacterium hydrocarbonoxydans* GHQ55 on *Oryza sativa* at 10 days seedling stage and control. a) Root length, Shoot length, b). Gemination percentage (%)

3.3.1. Germination Percentage

The *Oryza sativa* seed priming and seed coating both enhanced germination rates (93.33%) relative to the control (90%) (Fig.6.b).

3.3.2. Shoot Length

The *Oryza sativa* control showed the highest shoot length (8.15 cm), slightly ahead of seed priming (7.85 cm) and seed coating (7.73 cm) (Fig.6.a).

3.3.3. Root Length

Seed priming of *Oryza sativa* led to the longest root length (12.95 cm), outperforming seed coating (11.30 cm) and control (9.00 cm) (Fig.6.a).

3.3.4. Seedling Biomass

Total wet weight of *Oryza sativa* the seed coating had the highest wet weight (0.522 g), followed by seed priming (0.51 g) and control (0.49 g).

Total dry weight of *Oryza sativa* seed coating also showed the highest dry matter accumulation (0.188 g), compared to seed priming (0.184 g) and control (0.172 g).

3.3.5. Seedling Vigor Index

Seed priming with GHQ55 recorded the highest vigor index (1941.33), followed by seed coating (1776.13) and control (1543.50).

Discussion

The present study shows that, in comparison to untreated controls, seed priming and seed coating, especially when paired with the bacterial strain *Microbacterium hydrocarbonoxydans* GHQ55, significantly improve early seedling growth features in Brassica, rice (Oryza sativa), and blackgram (Vigna mungo L.). The germination rate, seedling biomass, and vigor index were all increased by seed augmentation methods for all three crops, with seed priming frequently producing the best results.

While seed priming gave the highest vigor index (2890.33) and larger shoot (21 cm) and root lengths (10 cm), seed coating in blackgram yielded the highest germination rate (98%). These gains are in line with those of (Sutradhar et al., 2023), who showed that blackgram bio-primed with Rhizobium leguminosarum had improved vigor and germination. Similarly, after using gibberellic acid and hydropriming, Khanal and Shrestha (2024) noted improved seedling performance in blackgram. Positive contributions from seed coating were also noted by (Sivakumar et al., 2024), who demonstrated increased rhizosphere microbial activity through prebiotic coatings, and (Vijayalakshmi et al., 2024), who found that biopolymer-based coatings enhanced emergence and field performance.

The most effective method of treatment in Brassica was seed priming, which produced the maximum vigor index (1672.33), germination rate (96.67%), and shoot (9.1 cm) and root lengths (8.2 cm). Following seed priming, (Patil et al., 2023) observed increased water absorption efficiency and enzyme activity in Brassica juncea under stress. These results corroborate their findings. Furthermore, it was shown by (Bakhsh et al., 2022) that biostimulant-based priming greatly enhanced Brassica napus root development, allowing for optimal establishment. Seed coating beat the control even though it was marginally less effective than priming. This is consistent with (Singh et al., 2023) findings that biopolymer coatings enhanced the vigor and emergence of seedlings in Brassica campestris.

Both coating and seed priming increased germination in rice (93.33%) as compared to the control (90.00%). Priming produced the largest root length (12.95 cm) and vigor index (1941.33), indicating improved root development at an early stage. This is consistent with (Verma et al., 2022) findings that seed priming is linked to the activation of metabolic and enzymatic pathways that promote early development. Regarding root development and vigor, seed coating did not outperform priming, although producing somewhat better wet (0.522 g) and dry weight (0.188 g). An elongation mechanism brought on by stress, which is frequently linked to weaker root systems, could be the cause of the control's surprisingly longer shoots (8.15 cm). According to (Kumar et al., 2023), seed priming offers more balanced and strong early development, which is essential for successful field establishment. This is demonstrated by the superior vigor index in primed seedlings.

Our results highlight how well seed improvement technologies specifically, seed priming with bacterial strain *Microbacterium hydrocarbonoxydans* GHQ55 improve germination, seedling vigor, and early biomass gain. To build a solid plant stand and eventually increase agricultural output, such enhancements are essential. Seed priming methods are highly advised for use in sustainable agriculture practices due to their affordability, simplicity of use, and uniformity across a variety of crops.

Conclusion

Early growth characteristics in *Vigna mungo* (*Fabaceae*), *Brassica juncea* (*Brassicaceae*), and *Oryza sativa* (*Poaceae*), including germination %, root and shoot length, biomass, and seedling vigor, were markedly improved by seed priming, particularly with bacterial strain *Microbacterium hydrocarbonoxydans* GHQ55. While seed priming consistently produced better outcomes than untreated seeds, seed coating also increased performance. According to these results, seed priming is an easy and affordable way to encourage robust crop establishment and early growth, which supports its use in sustainable farming methods.

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