# Seed Coating Formulations for Enhancing Seedling Vigor and Physiological Traits of Moong bean (Vigna radiata L.) and Rice (Oryza sativa L.)

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## Abstract

Seed coating with bioactive materials is a promising strategy to enhance seedling vigor, improve germination, and promote early growth. This study was conducted under laboratory conditions using a seed germinator to evaluate the effects of various seed coating formulations on seedling growth and physiological parameters of moongbean (*Vigna radiata*) and rice (*Oryza sativa*). Eight treatment combinations were tested, including biochar, NPK-solubilizing plant growth-promoting rhizobacteria (PGPR), and zinc oxide (ZnO) nanoparticles, either alone or in combination. The experiment assessed germination percentage, root and shoot length, fresh and dry weight, vigor indices, root/shoot ratio, water content, and fresh/dry weight ratio. Results revealed that the combined treatment (T8: Biochar + NPK Microbes + ZnO nanoparticles) significantly outperformed all other treatments, followed by T7 (NPK Microbes + ZnO nanoparticles), in both crops. PCA and correlation analysis underscored the positive interaction of these coating materials, revealing a strong association between vigor indices, germination percentage, and seedling biomass under T8: Biochar + NPK Microbes + ZnO nanoparticles treatment as compared to T1 Control. This study reveals the synergistic effect of nano and bio inputs integrated through seed coating technology for enhancing the early establishment of crops. The results provide insight on sustainable seed enhancement technologies with less chemical dependency that perform better in the crop during the initial stages.

## 1. Introduction

The problem of global food insecurity is urgent and worrying as it affects nearly 925 million individuals today, with the number expected to grow to 2 billion by mid-century [1]. There is a pressing need to mitigate the impacts of food systems on the environment and biodiversity while also preparing to meet the food demands of the future. Current intensive agricultural practices that rely on fertilizers, pesticides, and animal waste for higher yields are detrimental to the environment. Such practices lead to the pollution of air, soil and water as well as the loss of biodiversity [2, 3]. This calls for the immediate adoption of sustainable and innovative approaches to agriculture [4]. It is a knowledge-based framework for optimizing the total production of crops both ecologically and economically.

This paradigm integrates high technologies for crop management with accurate agrochemical applications by using quality seeds and improved crop varieties [5]. Seed enhancement technologies are regarded as the pre-sowing treatments that predominantly emerged as an effective tool in upgrading seed quality to overcome stresses related to germination and the early growth period of a plant [6, 7]. Among several methods available for seed enhancement, coating has assumed more importance since it energizes the seed and thereby improves field performance. Herein discussed is the method where active agents may be microbial inoculants, biochar, or nanomaterials applied to surfaces of seeds with binders and sometimes fillers. The coated seed can express rapid germination, good growing vigor, and strength under bad climatic conditions [8, 9].

Biochar is presently studied as a potential good seed coating material because it has a high content of carbon along with a porous structure, and high nutrient retention capacity [10]. This matter is created through the pyrolysis of biomass at a condition where oxygen has been limited, thus surface area, moisture retaining capacity, and organic carbon content are some of the attractive physical and chemical properties associated with biochar [8]. The other type of material that can result from burning biomass may include signalling chemicals in its content. Other smoke-derived compounds encourage seed germination as well as vigorous young plant growth by better soil texture and hence more water availability on soil [10].

The recent trend in nanotechnology and green inputs opens wider options of remedies to improve the performance of seeds and young plants. Metal-based nanoparticles (ZnO NPs) and carbon-based materials, such as biochar, have been proposed by several authors as promising seed pre-treatment agents. Many agricultural studies on nanoparticles compared with traditional priming: farmers usually use ZnO NPs for common treatments of seed focusing on the method called nano-priming [11]. The existing physiological and biochemical mechanisms that are revealed when applying nano-priming remain less discovered for different crop species under various environmental conditions [12]. Zinc (Zn) is a vital micronutrient, providing the composition of more than 300 plant enzymes and structural proteins [13]. It takes an active part in major physiological functions such as photosynthesis, protein synthesis, antioxidant activity, membrane stability, and gene expression function under stress conditions [14]. Zinc fertilizers supplied conventionally with zinc sulphate ($ZnSO_4 \cdot H_2O$ or $ZnSO_4 \cdot 7H_2O$) have low bioavailability because they rapidly become fixed in the soils and are solubilized poorly [15]. Among the high surface area and active forms of zinc compounds available for nanoparticles to compare among them is presented as a means that limitations can be more effectively addressed; hence uptake efficiency by the plant can possibly be improved [16]. Some of the crops on which ZnO NPs have shown considerable positive effects, apart from Vigna radiata, Cicer arietinum, tomato, cucumber, and alfalfa include seed germination vigor of seedlings and biomass accumulation [17–19]. They may or may not prove equally effective depending upon nanoparticle size and concentration as well as the plant species involved. A strategy for their integration into seed coating formulations, rather to be applied conventionally through priming methods, would be highly controlled and scalable to improve early seedling performance under field conditions. Moreover, alongside ZnO and biochar, the incorporation of beneficial microbial inoculants like NPK-solubilizing microbes enhances the functional capacity of the seed coatings. With the synergism of the plant and the microbes, the microbes help in better nutrient mobilization, root system improvement, and plant health. The combination of biochar for its carrier and soil-conditioning ability alongside plant growth promoting microbes, and micronutrient delivering ZnO NPs present a holistic and precision-based seed enhancement.

Microbes become one of the basic inputs for seed coating technologies with sustenance as well as the natural way of supporting increased seed germination and vigor of seedlings. Microbial inoculants, such as nitrogen-fixing Rhizobium bacteria, phosphate-solubilizing Bacillus bacteria, and potassium-solubilizing fungi of the genus Aspergillus applied to or with the seed prove very instrumental in enhancing nutrient bioavailability as well as uptake efficiency [20]. Such beneficial microorganisms establish either symbiotic or associative relations within the rhizosphere resulting in root enhancement together with hormone production (for example auxin and cytokinins) and also enhance stress resistance [21]. Microbial consortia coatings have already enhanced root morphology, vigor at the beginning, breaking of dormancy, and tolerance to both abiotic and biotic stress conditions [22]. One advanced technology in this regard includes bio-priming that can be defined as the combination of microbial inoculation with the hydration of seeds under controlled conditions whereby preconditioning of the seeds for rapid and uniform germination is achieved [23, 24]. These microbe-seed interactions establish a dynamic and responsive interface that supports plant growth from the very beginning with minimal use of chemical fertilizers and pesticides [22]. This study discusses the evaluation of a synergistic seed coating formulation containing biochar, zinc oxide nanoparticles (ZnO-NPs), and microbial inoculants for enhancing seed performance. Biochar has a porous structure accompanied by a high cation exchange capacity thus it can play the role of an efficient carrier matrix for microbes by aiding in water retention as well as improved nutrient exchange within the seed microenvironment [25]. ZnO-NPs, having a high surface area and reactivity, results in the slow release of bioavailable zinc which is an essential micronutrient considered very important in enzymatic activities, photosynthetic efficiency, and stress management [26]. The combination integrated seed coating with Plant growth promoting microbes is envisaged to further strengthen seed-soil-microbe interactions therefore improving seedling vigor for resource-efficient climate-resilient agriculture [21]. Therefore, by joining together these components the research work is aimed at developing a holistic method of seed treatment that optimizes the performance of the seeds and supports sustainable agricultural systems. This study moves forward eco-friendly seed technologies as well as offers a new approach to increased productivity of crops under varying agroecological conditions.

Develop weeding out strategies that are sustainable is of great importance for the improvement of early plant vigor, resource use efficiency, and yield stability in diverse agroecological settings. In this study we look at a new eco-friendly seed coating which is a mix of biochar, zinc oxide nanoparticles (ZnO-NPs) and plant growth promoting microbes to better out the issues of low germination, poor seedling development and stress. Biochar which has a very porous structure and high cation exchange capacity serves as a base for nutrients and microbes, also it improves water retention and seed soil interaction [25]. ZnO-NPs have better solubility and bioavailability over traditional Zn fertilizers which in turn supports enzyme action, membrane health and stress response [11, 26]. Use of microbial inoculants which may include nitrogen fixing and nutrient solubilizing species that in turn improve nutrient absorption, hormone production and tolerance to abiotic stress [21, 27]. This integrated approach shall endeavour to create a synergistic seed coating by merging nature-based plant bio-stimulants with green polymers, thus enhancing seed performance and supporting sustainable low-input agriculture. The combination thereof is expected to: (1) increase the level of seed germination and early seedling vigor; (2) improve physiological as well as biochemical characteristics related to nutrient absorption and stress resistance; (3) develop sustainable crop production with minimized usage of agrochemicals. Such an integrated formulation moves seed technology one step forward in bio-circular economy principles and climate-resilient agriculture. Thus, it enables further development of environmentally friendly innovations in the area of seed coatings, which provide high potential for increasing crop productivity in all soil-climate regions.

## 2. Material Methods

# 2.1 Experimental Setup

The study was conducted in the laboratory of Agronomy under controlled conditions at the Faculty of Agricultural Sciences, GLA University, Mathura. Coated with different seed coating materials, seeds of moong bean (*Vigna radiata* L.) and rice (*Oryza sativa* L.) were placed for incubation in a seed germinator at 23 ± 2°C temperature with relative humidity between 60–70% for moong bean and 80–90% for rice. Uniform conditions regarding light and moisture ensured by the germinator lead to optimum germination as well as seedling development. Biochemical observations about seedling vigor and growth parameters in addition to percent seed germination were recorded on the 7th and 12th day after sowing, respectively. Hence, it provided an exact evaluation of coating material effects under no interaction with any form of abiotic stress.

# 2.2 Preparation of Seed Coating Material

Certified seeds of moong bean (*Vigna radiata*, variety Gold STAR-444) of Shri Ram Seeds from Mathura and rice (*Oryza sativa*, variety Pusa Basmati-1509) from the Seed Distribution Kendra of Indian Agricultural Research Institute (IARI), New Delhi, India (28.08°N, 77.12°E) were used. These seeds were the same as those commonly obtained and cultivated by farmers, ensuring that the study addressed real field-level challenges and practical problems faced in crop production. STAR-444 is a short-duration (60–65 days), high-yielding moong bean variety with moderate resistance to Yellow Mosaic Virus and leaf crinkle diseases, while Pusa Basmati-1509 is an early-maturing (115–120 days) basmati rice variety with high yield potential compared to traditional basmati types. A synergistic combination of organic binders, bioactive materials, and microbial inoculants which assists in the germination, seedling vigor, and stress tolerance of moong bean (*Vigna radiata*) and rice (*Oryza sativa*) served as the seed coating material (Fig. 1). The adhesive base was prepared by dissolving 100g of jaggery in 500 mL of water and 50 mL of aloe vera gel. Through continuous stirring to maintain the solute mixture, 500g of agricultural waste biochar was added to improve adherence and, further, provide nutrients. Biochar not only increased the adhesive base’s efficacy as a microbial nutraceutical, but also served as a porous and highly adsorbent biochar carrier. The mixture was furthered enhanced by the addition of 0.5g of zinc oxide nanoparticles (ZnO-NPs) with a particle size of < 20 nm. ZnO-NPs supplemented with zinc, a vital micronutrient for the plants' metabolic processes, provided slow-release mechanisms crucial for enzymatic and metabolic processes. The covering substance was inoculated with microbial suspensions of *Bacillus subtillis* ($10^8$ CFU/mL), *Pseudomonas fluorescens* ($10^8$ CFU/mL), and *Aspergillus niger* ($10^7$ spores/mL) for all three crops, along with *Rhizobium* ($10^8$ CFU/mL) for moong bean, *Azotobacter* ($10^8$ CFU/mL) for rice. Moong bean and rice seeds were surface sterilized with 0.1% sodium hypochlorite for just one minute and then washed with distilled water, following by air drying. 1 kg of seeds was poured into a container and 100 mL prepared coating material was added to it for coating the seeds by manually rotating them so that uniform coating takes place over all the seeds. For small-scale mechanization, however, a hand-operated rotary coater has been used. The coated seeds are preferably dried under shade at ambient temperatures, which may vary between 25 to 30°C so as to facilitate microbial viability during a period of not less than 24 hours. The final product is stored in breathable cloth bags at about 20–25°C for further tests; this practice favours microbial life support and at the same time develops strong robustness towards an environmentally friendly seed coating that imparts beneficial effects from biochar, ZnO-NPs, and microbial inoculants together. Evaluation under controlled conditions included germination percentage plus seedling shoot and root length, fresh and dry weight together with vigor index to ascertain efficacy of the formulation.

## 2.3 Estimation of Seedling Growth Parameters

Parameters of seedling growth included percent germination, shoot and root lengths, dry weight, and vigor indices I and II determined in conformity with standardized procedures (Fig. 2). Percent germination was determined based on the number of normal seedlings that emerged after 7 days of incubation as prescribed by the International Seed Testing Association-ISTA (2015). Measurements for shoot and root lengths were taken using a digital ruler from the base of the hypocotyl up to the apex of the shoot and from the base up to the tip of the root, respectively [28]. The dry weight of seedlings was determined after oven drying at 70 ± 2°C for 72 h in an electronic balance as described by [29]. Vigor Index I is the product of the percentage of germination multiplied by the total length of seedling (shoot + root) and Vigor Index II is the product of percentage of germination multiplied by seedling dry weight as per [28]. These indices have been standardizing to measure seedling quality and vigor particularly under different seed coating treatments.

## 2.4 Principal Component Analysis and Correlation Estimation

To observe the basic patterns and relationships among seedling parameters, Principal Component Analysis (PCA) and Pearson correlation analysis were used. PCA was applied to reduce the dimensionality of multivariate data and to trace major components that contribute variation in seedling traits. The standardized dataset went into PCA using the scikit-learn package for Python [30]. Eigenvalues and their contributions to variance were checked with the help of a scree plot. A biplot was drawn, depicting trait associations as well as loadings on the principal components. Pearson's correlation coefficients, describing linear relations between every possible pair of variables were calculated with seaborn and panda's libraries [31]. The significance at p ($< 0.05$) was tested such that statistical correlations could be found. These multivariate techniques are much popular in the researches of physiology and treatment of seeds [32, 33], for bringing to bearing complex interactions of parameters related to germination, and identifying the most influential traits under variation of treatments.

## 2.5 Statistical Analysis

All experimental data were statistically analyzed to determine the level of significance of the treatment effects on seedling parameters using R software version 4.3.1. A one-way Analysis of Variance (ANOVA) was used in measuring differences between treatments, and means compared by Tukey's Honest Significant Difference (HSD) test at a 5% probability value ($p < 0.05$) as provided by the agricolae package for R developed by de Mendiburu in 2021. Normality and homogeneity of variances were tested before running ANOVA using the Shapiro-Wilk and Levene's tests, respectively, so that the model applied would be valid. Such a method has been very common in agronomic studies for evaluating treatment efficacy under controlled conditions referred to by Gomez and Gomez [34]. This will make it possible to interpret results correctly and compare accurately the effect that different seed coatings have on germination, vigor in seedlings, and associated attributes.

# 3. RESULT AND DISCUSSION

## 3.1 Effect of Seed Coating on Rice Seedling Parameters

The current research findings showed that seed coating treatment had a marked effect on rice seedling parameters on day 12, especially regarding the percentage of germination, seedling fresh and dry weight, as well as seedling vigor indices. Biochar, NPK Microbes, and Zinc Oxide Nanoparticles (T8), was the best of all the treatments and had the highest germination percentage of 89.40%, 5.16 cm shoot length, 8.99 cm root length, and 0.83 g dry weight which were all better than untreated control (T1). These results are consistent with those of Jain et al. (2021) as they reported improved germination and initial seedling development in rice as a result of microbial and nano-nutrient seed coating in direct-seeded systems. The proposed hypothesis of microbial inoculants and zinc oxide nanoparticles' steep impact on the volunteers' metabolism and seedling development early in the growth stages and subsequently on nutrient transport [6, 35]. T8 enhanced integrated seed coating seedling quality with seedling vigor indices I and II measuring 461.30 and 803.71 respectively. [36] Pant noted similar results with stress-resilient rice varieties treated with micronutrient priming. Although root-shoot ratio and water content did not differ significantly, treatments with ZnO nanoparticles (T4, T6, T7) exhibited higher water retention and fresh-to-dry weight ratios, suggesting improved physiological efficiency and cell hydration during early growth. Overall, the data confirms that seed coating with a combination of biochar, beneficial microbes, and zinc oxide nanoparticles significantly enhances the early seedling vigour and physiological performance of rice, supporting similar findings by Sivarathri Bala Subramanyam [37], who reported that priming and coating with micronutrients and growth regulators contributed to superior seedling establishment in dry direct-seeded rice.

## 1: Effect of Seed Coating on Rice Seedling Parameters

| Treatments | Germination (%) | Shooth Length (cm) | Root Length (cm) | Fresh weight (gm) | Dry weight (gm) | Vigour index 1 | Vigour index 1 | Water content % | Fresh weight / Dry weight ratio |
|---|---|---|---|---|---|---|---|---|---|
| **T1** | 72.4b | 2.27c | 4.48e | 1.65d | 0.28e | 164.35d | 324.35a | 1.44a | 5.51a |
| **T2** | 74.8ab | 2.23c | 4.46e | 2.09cd | 0.35e | 156.33d | 341.09a | 1.68a | 6.20a |
| **T3** | 76.2ab | 2.76bc | 5.82d | 2.64c | 0.53d | 216.41c | 430.53a | 1.46a | 5.20a |
| **T4** | 75.6ab | 2.44bc | 6.34d | 3.62b | 0.54cd | 184.46cd | 349.27a | 1.73a | 6.53a |
| **T5** | 78.5ab | 2.9bc | 6.73cd | 3.66b | 0.60bcd | 229.22c | 528.31a | 1.61a | 6.08a |
| **T6** | 80.3ab | 3.84abc | 7.80bc | 3.98ab | 0.69abc | 308.35b | 615.1a | 1.88a | 5.67a |
| **T7** | 88.6a | 4.19ab | 8.13ab | 4.38a | 0.75ab | 371.23ab | 720.32a | 1.89a | 5.78a |
| **T8** | 89.4a | 5.16a | 8.99a | 4.46a | 0.83a | 461.3a | 803.71a | 1.94a | 5.29a |
| **SEm ±** | 2.24 | 0.57 | 0.35 | 0.21 | 0.05 | 38.65 | 3.83 | 0.26 | 0.73 |
| **LSD** | 4.5*** | 1.75* | 1.07*** | 0.65*** | 0.15*** | 77.69** | NS | NS | NS |

Where, T1-Control, T2-Biochar, T3-NPK Microbes, T4-Zinc oxide nanoparticls, T5-Biochar + NPK Microbes, T6-Biochar + Zinc Oxide nanoparticles, T7-NPK Microbes + Zinc Oxide nanoparticles, T8-Biochar + NPK Microbes + Zinc oxide nanoparticles

# 3.2 Effect of Seed Coating on Moong bean Seedling Parameters

The results of the present investigation revealed that seed coating treatments had a significant influence on various seedling parameters of moongbean at 12 DAS, with the combined application of biochar, NPK microbes, and zinc oxide nanoparticles (T8) performing the best across most traits (Fig. 3). T8 recorded the highest germination percentage

(98.00%), shoot length (4.00 cm), root length (7.51 cm), fresh weight (3.00 g), and dry weight (0.64 g), indicating enhanced early seedling vigour and metabolic activity due to the synergistic interaction of organic, microbial, and nano-nutrient inputs. The improved vigour indices I (1099.2) and II (58.26) under T8, compared to the control (583.80 and 32.96, respectively), reflect better germination and seedling biomass accumulation [38, 39], who reported that seed coating with biofertilizers and micronutrients significantly improves germination and vigour in legumes. The enhanced root to shoot ratio in T8 (2.87) and high-water content (81.02%) further support the improved physiological efficiency and water uptake in coated seeds. Similar observations were made by Prasad [40], where zinc oxide nanoparticles and microbial inoculants enhanced root elongation and seedling water retention in pulses. The higher fresh weight to dry weight ratio in T8 (5.35) also indicates efficient biomass accumulation and nutrient utilization, aligning with the results of Yadav et al. [41], who reported that nano-seed treatments enhance metabolic performance and seedling growth. Collectively, these findings suggest that seed coating with biochar, beneficial microbes, and zinc oxide nanoparticles significantly enhances the germination, growth, and physiological traits of moongbean seedlings, promoting robust early-stage development and potentially improving crop establishment under field conditions.

Table 2
Effect of Seed Coating on Moong Bean Seedling Parameters

| Treatments | Germination (%) | Shooth Length (cm) | Root Length (cm) | Fresh weight (gm) | Dry weight (gm) | Vigour index 1 | Vigour index 1 | Water content % | Fresh weight / Dry weight ratio |
|---|---|---|---|---|---|---|---|---|---|
| **T1** | 79.33c | 2.26b | 4.23b | 1.78b | 0.38b | 583.80d | 32.96b | 1.44a | 3.16b |
| **T2** | 88.66abc | 2.86ab | 5.86ab | 1.74b | 0.42ab | 696.73cd | 37.61ab | 1.68a | 4.16ab |
| **T3** | 86.0bc | 3.10ab | 5.88ab | 2.00b | 0.57ab | 874.10abc | 44.52ab | 1.46a | 3.86ab |
| **T4** | 86.66bc | 2.50ab | 6.31ab | 2.05ab | 0.45ab | 683.36cd | 44.48ab | 1.73a | 4.61ab |
| **T5** | 86.00bc | 3.20ab | 6.33ab | 2.19ab | 0.48ab | 826.66bcd | 41.70ab | 1.61a | 3.83ab |
| **T6** | 91.33ab | 3.06ab | 5.44ab | 1.98b | 0.56ab | 815.64bcd | 44.76ab | 1.88a | 4.54ab |
| **T7** | 97.33a | 3.07ab | 7.22a | 2.31ab | 0.52ab | 967.93ab | 55.36a | 1.89a | 5.27ab |
| **T8** | 98.00a | 4.00a | 7.51a | 3.00a | 0.64a | 1099.2a | 58.26a | 1.94a | 5.35a |
| **SEm ±** | 3.15 | 0.49 | 0.79 | 0.32 | 0.07 | 88.17 | 7.37 | **0.26** | 0.17 |
| **LSD** | 9.58* | 1.50*** | 2.41*** | 0.97*** | 0.23 | 267.44** | 22.37*** | **NS** | 2.15*** |

Where, $T_1$-Control, $T_2$-Biochar, $T_3$-NPK Microbes, $T_4$-Zinc oxide nanoparticls, $T_5$-Biochar + NPK Microbes, $T_6$-Biochar + Zinc Oxide nanoparticles, $T_7$-NPK Microbes + Zinc Oxide nanoparticles, $T_8$-Biochar + NPK Microbes + Zinc oxide nanoparticles

## 3.3 PCA and Explained Variance of Seedling Traits

Principal Components Analysis (PCA) used to investigate the seedling trait data and the relationships among the seedling growth parameters under different seed coating treatments. This was done to reduce the dimensionality of the data. From the scree plot, the first three Principal Components (PCs) were sufficient, as these three cumulatively accounted for nearly 90% of the variation in the data set. Specifically, PC 1 accounted for 65.43% of the total variance, while PC 2 and 3 contributed 15.28% and 9.35% respectively. The eigenvalue indicative of the first principal component is much higher than the rest, the second will follow in a gradual manner. The “elbow point” that follows is a steep decline followed by flattening. This supports the reasoning of focusing three PCs for further interpretation. The data set is well structured, meaning that the vast majority of the variation observed could be explained with only a handful of factors, likely associated with the physiological processes influenced by the seed coating, which are derived from the processes and factors interacting in the seed and the coating. These findings are consistent with reports by Lone et al. [42], who demonstrated that PCA could efficiently cluster complex germination and early growth data into major trait axes in cereals. Likewise, Kumar et al. [43] noted a similar variance structure in their evaluation of bio-priming effects on wheat where PC1 relates to the explanation of the shoot and root vigor while PC2 pertains to moisture-related indices. Of note, the high proportion of variance captured by PC1 in our study suggests that the early growth traits, particularly shoot length, root length, and seedling vigor, strongly drive treatment-induced variability. These traits are frequently described as sensitive metrics of the effectiveness of seed treatment and the potential for early seedling establishment [44]. Moreover, the notable distinction between major and minor components underscores the point that a small number of PCs are all that is needed to accurately represent the underlying structure of the multidimensional dataset. The effectiveness with which PCA achieves this is a hallmark of its power in the simplification of data analysis but, in this case, is also consistent with studies on mungbean and rice where a small number of PCs captured the bulk of treatment-derived variation in seed performance traits [45, 46].

## 3.4 Biplot Analysis: Interrelationships Among Seedling Traits

The additional insights gained from the variable correlation plot (PCA biplot) are the impacts and interconnection of the traits of the seedling and how they change with the different seed coating formulations (Fig. 4). The biplot also shows strong clustering of shoot length, root length, and Vigor Index I along the first principal component (PC1). Therefore, these traits are the most important for early seedling growth and performance. This also indicates the seed coating materials greatly improved the elongation traits, which are important for the quick seedling emergence and establishment. In contrast, the traits of fresh weight, dry weight, and water content loading highly along PC2, showed varying moisture and biomass accumulation orthogonality. Their importance is likely linked to the second dimension of response which is moisture and biomass accumulation. Vigor Index II's placement in a different quadrant signifies a different set of mechanisms influencing it, likely a root:shoot ratio, energy reserves, or utilization during seedling emergence. Our PCA biplot's clustering patterns also match with Yadav et al. [47] where they showed bio-primed seeds with PCA segregated seedling traits into vigor and biomass. Accinneli et al. [48] showed a comparable grouping of traits in rice, where the application of zinc and microbial consortia seed coatings changed the PC loadings significantly towards root and shoot length. This goes in line with the theory that seed coating enhances germination performance and rewrites the expression of traits in the seedling phase, thus increasing vigor and physiological performance.

The acute angle between shoot-related traits on the biplot shows a strong positive correlation proving the coordinated nature of shoot elongation and root development under a good seed coating environment. Since water content is almost orthogonal to these elongation traits, this presents another independent axis of variability that may be attributed to hygroscopic or osmoprotectant elements in the coating. These observations further seed coating treatments as modifiers of seedling performance through several physiological pathways: enhanced water uptake; root-shoot balance; cellular energy use which come out as disparate trait clusters in multivariate space. It brings out visually such interrelations and hence makes PCA biplot a very powerful diagnostic tool that can be used in evaluating and optimizing different seed coating formulations for different crops and environmental conditions.

## 3.5 Correlation Analysis Among Seedling Parameters

The trellis diagram shows relationships of the different seedling growth traits under different seed coating treatments. Most of the traits showed considerable positive significant correlations, dominantly between the shoot length, root length, dry weight and the vigor indices (Fig. 5). This suggests that seed coating treatments led to enhanced seedling vigor parameter synergistic strengthening. Shoot length showed high positive correlations with root length and with vigor index I. This means that the seedling elongation could be one of the measures of the seedling vigor. Ali and Nawaz [49, 50] showed that growth-promoting coatings are able to enhance both shoot and root traits because of the increased water uptake and hormonal signaling. Furthermore, dry weight also showed significant correlation with vigor index II, affirming the theory that such index serves as an aggregate measure of the efficiency of biomass allocation. Interestingly, germination percentage was moderately but significantly correlated with vigor index I and shoot length, suggesting that treatments improving germination also tend to enhance early shoot growth, which is crucial for field establishment. These correlations reinforce the concept that seed coating not only supports higher germination but also enhances post-germination growth performance [51]. Negative or weak correlations only occurred infrequently and were mostly unimportant as demonstrated by a lack of strong brown or negative values. Crosses (X) in the plot represent unimportant associations which indicate a strong consistency among traits which further reinforces the reliability of the dataset. Correlation analysis illustrates that the seed coating treatments applied resulted in significant and meaningful changes, both statistically and biologically, in the performance of the seedlings [45]. Strong relationships among traits emphasize the integrated nature of seedling vigor traits which are enhanced collectively when optimized coating formulations are used. These results are in agreement with previous work in mungbean and rice where biochar and PGPR as well as micronutrient coatings were shown to enhance seedling traits [52].

# 4. Conclusion

The results of this study demonstrated the potential of composite seed coating using biochar, NPK-solubilizing microbes, and ZnO nanoparticles to significantly improve seed germination and seedling growth of moongbean and rice under laboratory conditions. Among the eight treatments, T8 (Biochar + NPK Microbes + ZnO nanoparticles) consistently showed the highest performance across all measured parameters, including shoot and root length, fresh and dry weight, germination percentage, and vigor indices. This was followed by T7 (NPK Microbes + ZnO nanoparticles), which also enhanced performance compared to the control. The PCA analysis revealed that the first two principal components accounted for the majority of variance in the dataset, with variables like vigor indices, germination percentage, and fresh/dry weight contributing most significantly to PC1. Laboratory studies have indicated that a composite seed coating using biochar, NPK-solubilizing microbes, and ZnO nanoparticles can significantly increase seed germination and growth of moong bean and rice seedlings. Among the eight treatments, T8 (Biochar + NPK Microbes + ZnO nanoparticles) showed the highest results of percentage germination, vigor indices, shoot and root length, and seedlings fresh and dry weight. The next best treatment was T7 (NPK Microbes + ZnO nanoparticles), which gave higher values over control and other treatments. The first two principal components absorbed the major variance in the data set, with the contributions of vigor indices, germination percentage, and fresh/dry weight making up the major share for PC1. The variable correlation plot indicated strong positive correlations among these traits, further affirming the efficacy of integrated seed coating treatments. These findings were supported by correlation analysis which showed significant inter-relationships among seedling traits. This research validates the hypothesis that a synergistic combination of nano and biological amendments in seed coatings can enhance the early seedling growth, which is critical for final crop yield and stress resilience. The study thus supports the adoption of advanced seed treatment technologies for enhanced crop productivity and offers a scientific basis for future validation.

# Declarations

### Author Contributions

**Singh S.:** Manuscript Writing and performed the original research**; Kumar V.:** Supervised and Revised.

### Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

**Consent to Publish:** N/A

**Ethics Approval and Consent to Participate:** N/A

**Clinical Trial Registration:** N/A

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## References

1. Eldakak A, Milad SIM, Nawar AI, Rohila JS (2013) Proteomics: A promising approach for crop improvement. Frontiers in Plant Science 4:1–8
2. Jetiyanon K, Kloepper JW, Knecht K (2008) Seed treatment with biological agents for enhancement of seedling growth. Biological Control 44:198–206
3. Oliveira HC, Gomes EA, Costa MD, Ribeiro VP, Valicente FH (2016) Biofilm formation by Bacillus thuringiensis on maize seeds and root colonization. Brazilian Journal of Microbiology 47:632–642
4. Barnes AP, Soto I, Eory V, Beck B, Balafoutis A (2019) Exploring the adoption of precision agricultural technologies: A cross-country analysis. Land Use Policy 80:163–174
5. Duhan JS, Kumar R, Kumar N, Kaur P, Nehra K, Duhan S (2017) Nanotechnology: The new perspective in precision agriculture. Biotechnology Reports 15:11–23
6. Rocha I, Ma Y, Souza-Alonso P, Vosátka M, Freitas H, Oliveira RS (2019) Seed Coating: A Tool for Delivering Beneficial Microbes to Agricultural Crops. Frontiers in Plant Science 10: 1357
7. Rocha I, Ma Y, Souza-Alonso P, Vosátka M, Oliveira RS (2019) Synergistic effect of arbuscular mycorrhizal fungi and Bacillus spp. on seed germination and growth of maize. Applied Soil Ecology 142:120–127
8. Ehsanfar S, Modarres-Sanavy SAM (2005) A review on the technology of coating seeds. Asian Journal of Plant Sciences 4:463–469
9. O'Callaghan M (2016) Microbial inoculation of seed for improved crop performance: Issues and opportunities. Applied Microbiology and Biotechnology 100:5729–5746
10. Rouphael Y, Colla G, Bernardo L, Kane D, Trevisan S, Lucini L, Cardarelli M (2017) Biostimulant seed coating modulates root architecture and metabolic pathways involved in nitrogen assimilation in maize seedlings. Plant Biology 19:1–9
11. Chikkanna A, Ananth MS, Basavarajappa R (2018) Influence of nano-zinc on growth and yield of maize (Zea mays L. Journal of Pharmacognosy and Phytochemistry 7:2557–2560
12. Srivastava RK, Patel AK, Chauhan DR, Srivastava JP (2010) Seed nanopriming: A new tool to enhance germination and seedling growth. International Journal of Agricultural Research 5:59–64
13. Laity JH, Lee BM, Wright PE (2001) Zinc finger proteins: New insights into structural and functional diversity. Current Opinion in Structural Biology 11:39–46
14. Khanm MN, Mobin M, Abbas ZK (2018) Fertilizers and their contaminants in soils, surface and groundwater. In: Contaminants in Agriculture and Environment: Health Risks and Remediation. Springer, pp 57–78
15. Latef AAHA, Hashem A, Rasool S, Abd_Allah EF, Alqarawi AA, Egamberdieva D, Jan S (2016) Arbuscular mycorrhizal symbiosis and biochar improved the growth and mineral nutrition of maize under saline conditions. Archives of

Agronomy and Soil Science 62:533–545

16. Rameshraddy Y, Anantha MS, Sreeramulu K (2017) Response of zinc nano particles on growth, yield and quality of green gram (Vigna radiata L. Legume Research 40:564–567
17. Mahajan P, Dhoke SK, Khanna AS (2011) Effect of nano-ZnO particle suspension on growth of mungbean (Vigna radiata) seedlings using plant agar method. Journal of Nanotechnology 1–7
18. Rosa GJM, Faria MV, Oliveira JA (2013) Use of nanoparticles in seed priming: Perspective and challenges. Revista Brasileira de Sementes 35:15–29
19. Burman U, Saini M, Kumar P (2013) Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. Toxicological & Environmental Chemistry 95:605–612
20. Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255:571–586
21. Rashid MI, Mujtaba T, Mahmood T, Ahmad A (2016) Biological seed treatments with plant growth promoting rhizobacteria and their impact on seedling traits and nutrient uptake in maize. Applied Soil Ecology 107:380–389
22. Sarkar A, Rakshit A (2020) Microbial seed coating: The eco-friendly approach for sustainable agriculture. In: Seed Bio-priming for Stress Tolerance. Springer, pp 151–167
23. Ananthi T, Vijaya T, Arulmozhi R (2014) Bio-priming: A feasible strategy to improve seed germination and vigor. Journal of Applied and Natural Science 6:741–748
24. Devika B, Srimathi P, Sundaramoorthi A (2021) Bio-priming – A strategy for improving seed quality and vigor in pulse crops. International Journal of Current Microbiology and Applied Sciences 10:174–181
25. Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota – A review. Soil Biology and Biochemistry 43:1812–1836
26. Khot LR, Sankaran S, Maja JM, Ehsani R, Schuster EW (2012) Applications of nanomaterials in agricultural production and crop protection: A review. Crop Protection 35:64–70
27. Sarkar A, Rakshit A, Singh HB, Singh US (2017) Seed bio-priming with beneficial microbes: A review. Indian Journal of Agricultural Sciences 87:1–15
28. Abdul-Baki AA, Anderson JD (1973) Vigor determination in soybean seed by multiple criteria. Crop Science 13:630–633
29. Ruan YL, Jin Y, Yang YJ, Li GJ, Boyer JS (2002) Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. Molecular Plant 3:942–955
30. Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Duchesnay É (2011) Scikit-learn: Machine learning in Python. Journal of Machine Learning Research 12:2825–2830
31. Waskom M (2021) seaborn: Statistical data visualization (Version 0.11.2.
32. Adebisi MA, Kehinde TO, Ajala MO (2013) Multivariate analysis of seed quality traits in soybean [Glycine max (L.) Merr. International Journal of Plant Breeding and Genetics 7:180–190
33. Sharma D, Pramanik A, Agrawal PK (2016) Evaluation of bioactive secondary metabolites from endophytic fungus Pestalotiopsis neglecta BAB-5510 isolated from leaves of Cupressus torulosa D.Don. 3 Biotech 6:210
34. Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research, 2nd ed. John Wiley & Sons
35. Shahzad B, Tanveer M, Che Z, Rehman A, Cheema SA, Sharma A, Xu X (2017) Role of 24-epibrassinolide (EBL) in mediating abiotic stress tolerance in plants: Present status and future perspectives. Journal of Plant Growth Regulation 36:880–893
36. Pant B (2016) Effect of micronutrient seed priming on growth and yield of stress resilient rice varieties under aerobic condition (M.Sc. thesis. G.B. Pant University of Agriculture and Technology, Pantnagar, India
37. Subramanian A, Sivakumar S (2020) Nitrogen fixation enhancement in soybean by nanosilica. Soil and Tillage Research 199:104585

38. Rai S, Singh PK, Mankotia S, Swain J, Satbhai SB (2021) Iron homeostasis in plants and its crosstalk with copper, zinc, and manganese. Plant Stress 1:100008
39. Kumar V, Raghuvanshi N, Pandey AK, Kumar A, Thoday-Kennedy E, Kant S (2023) Role of Halotolerant Plant Growth-Promoting Rhizobacteria in Mitigating Salinity Stress: Recent Advances and Possibilities. Agriculture 13:168
40. Prasad T, Prakash R (2018) Enhancing the efficiency of nitrogen fixation using nanoparticle inoculants. Microbial Biotechnology 11:1274–1285
41. Yadav PV, Kumari M, Ahmed Z (2011) Seed Priming Mediated Germination Improvement and Tolerance to Subsequent Exposure to Cold and Salt Stress in Capsicum. Research Journal of Seed Science 4:125–136
42. Lone RA, Bhat MA, Khan MH (2021) Use of multivariate techniques for classification and characterization of germination traits in cereals under stress. Indian Journal of Plant Physiology 26:278–285
43. Kumar R, Verma P, Singh D (2022) Multivariate analysis of seed germination and seedling growth parameters under bio-priming in wheat. Journal of Cereal Research 14:45–52
44. Sankaran M, Vijayalakshmi C, Chinnusamy C (2015) Multivariate analysis in seed priming studies for enhancing seedling vigour in rice. Madras Agricultural Journal 102:83–86
45. Patel KD, Parmar KB, Chauhan JB (2020) Effect of biochar and microbial seed coating on seed quality and early seedling traits in mungbean [Vigna radiata (L.) Wilczek. Legume Research 43:779–784
46. Patel RR, Jat BL, Patel MR (2019) Principal component and cluster analysis in mungbean [Vigna radiata (L.) Wilczek] genotypes under different seed treatment practices. International Journal of Current Microbiology and Applied Sciences 8:1723–1733
47. Yadav SK, Dave A, Sarkar A, Singh HB, Sarma BK (2013) Co-inoculated Biopriming with Trichoderma, Pseudomonas and Rhizobium Improves Crop Growth in Cicer arietinum and Phaseolus vulgaris. 6:255–259
48. Accinelli C, Abbas HK, Zablotowicz RM, Wilkinson JR (2018) Eco-friendly seed treatments: Biodegradable coatings for delivery of beneficial microbes. Agronomy for Sustainable Development 38:1–11
49. Nawaz A, Shahbaz M, Asadullah, Imran A, Marghoob MU, Imtiaz M, Mubeen F (2020) Potential of Salt Tolerant PGPR in Growth and Yield Augmentation of Wheat (Triticum aestivum L.) Under Saline Conditions. Frontiers in Microbiology 11: 2019.
50. Ali B, Wang X, Saleem MH, et al (2022) PGPR-Mediated Salt Tolerance in Maize by Modulating Plant Physiology, Antioxidant Defense, Compatible Solutes Accumulation and Bio-Surfactant Producing Genes. Plants. 11 (3), 345. https://doi.org/10.3390/plants11030345
51. Singh UB, Malviya D, Singh S, et al (2021) Salt-Tolerant Compatible Microbial Inoculants Modulate Physio-Biochemical Responses Enhance Plant Growth, Zn Biofortification and Yield of Wheat Grown in Saline-Sodic Soil. Int J Environ Res Public Health 18:9936
52. Verma S, Yadav A, Singh RK (2022) Micronutrient and PGPR seed priming improves seedling vigor and nutrient uptake in rice. International Journal of Chemical Studies 10:158–162

# Figures

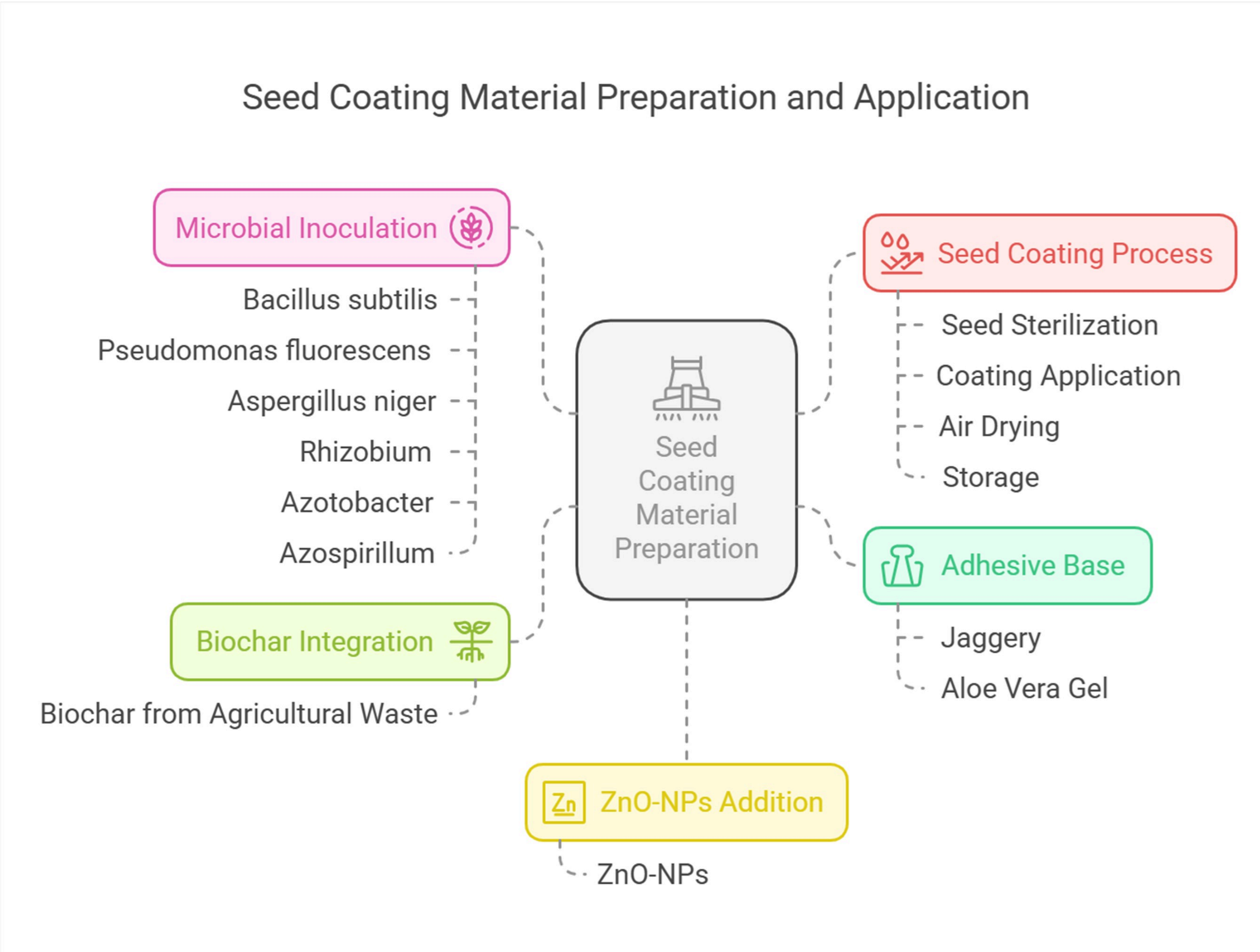



**Figure 1**

**Seed Coating material preparation**

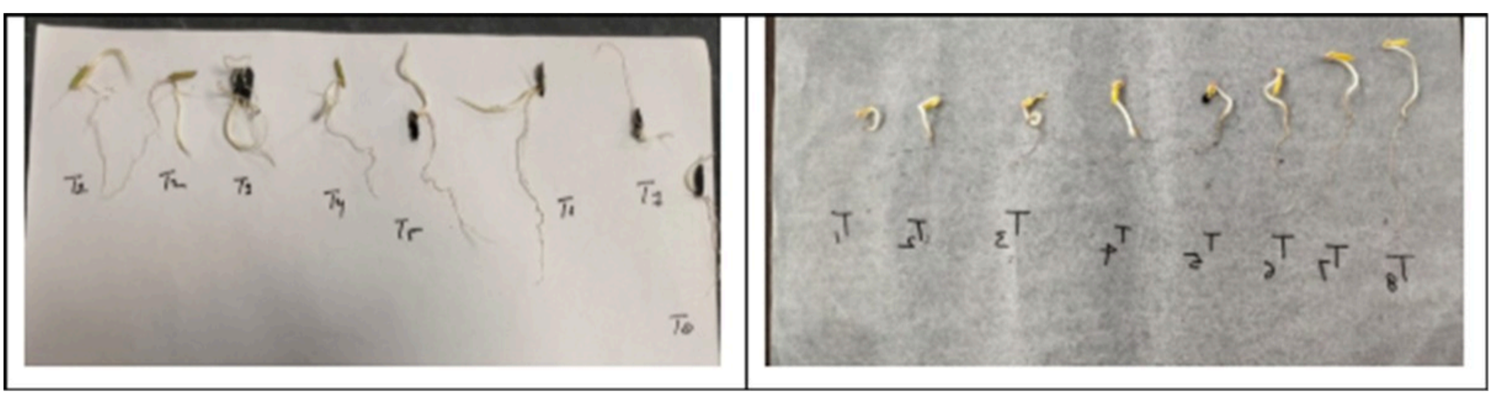


**Figure 2**

**Seedling growth at 5 DAS under control condition**

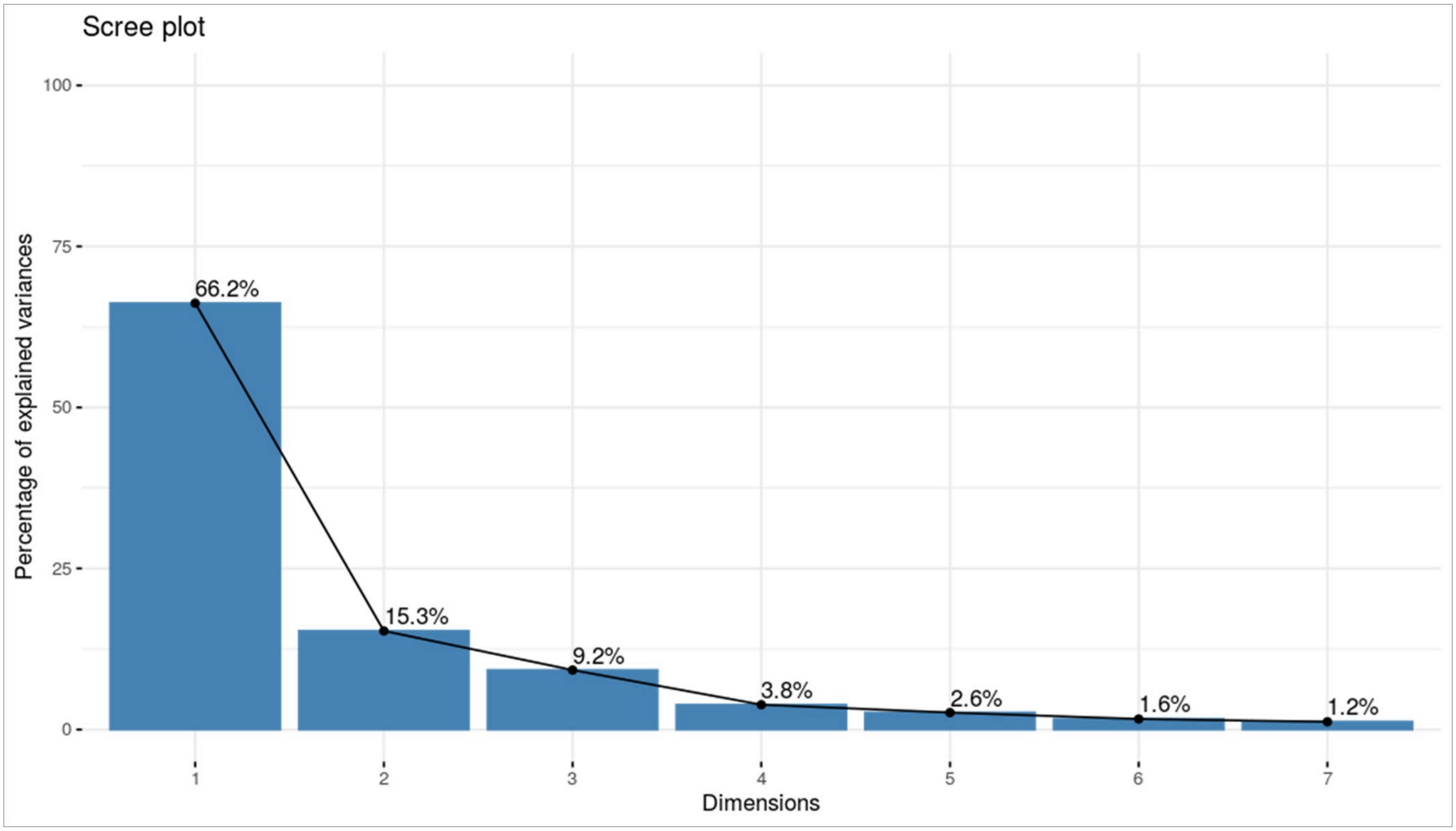



**Figure 3**

**A scree plot of PCA for seedling growth attributes under different treatments of seed coating will be presented here. The scree plot shows the variance explained by each component, where in most cases the first two components (PC1 and PC2) explain a maximum part of total variation suitable for dimensional reduction as well as interpretation of traits.**

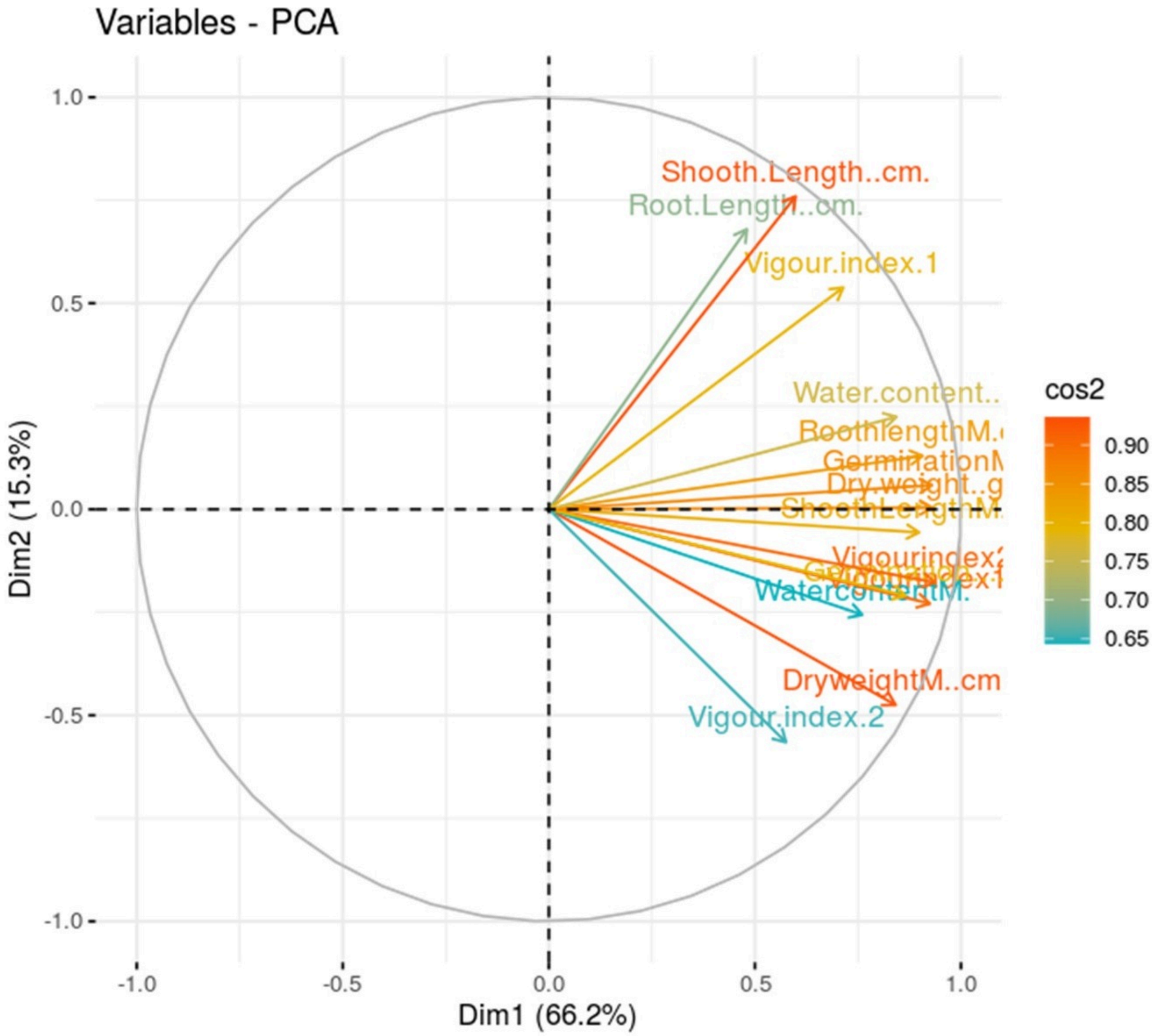
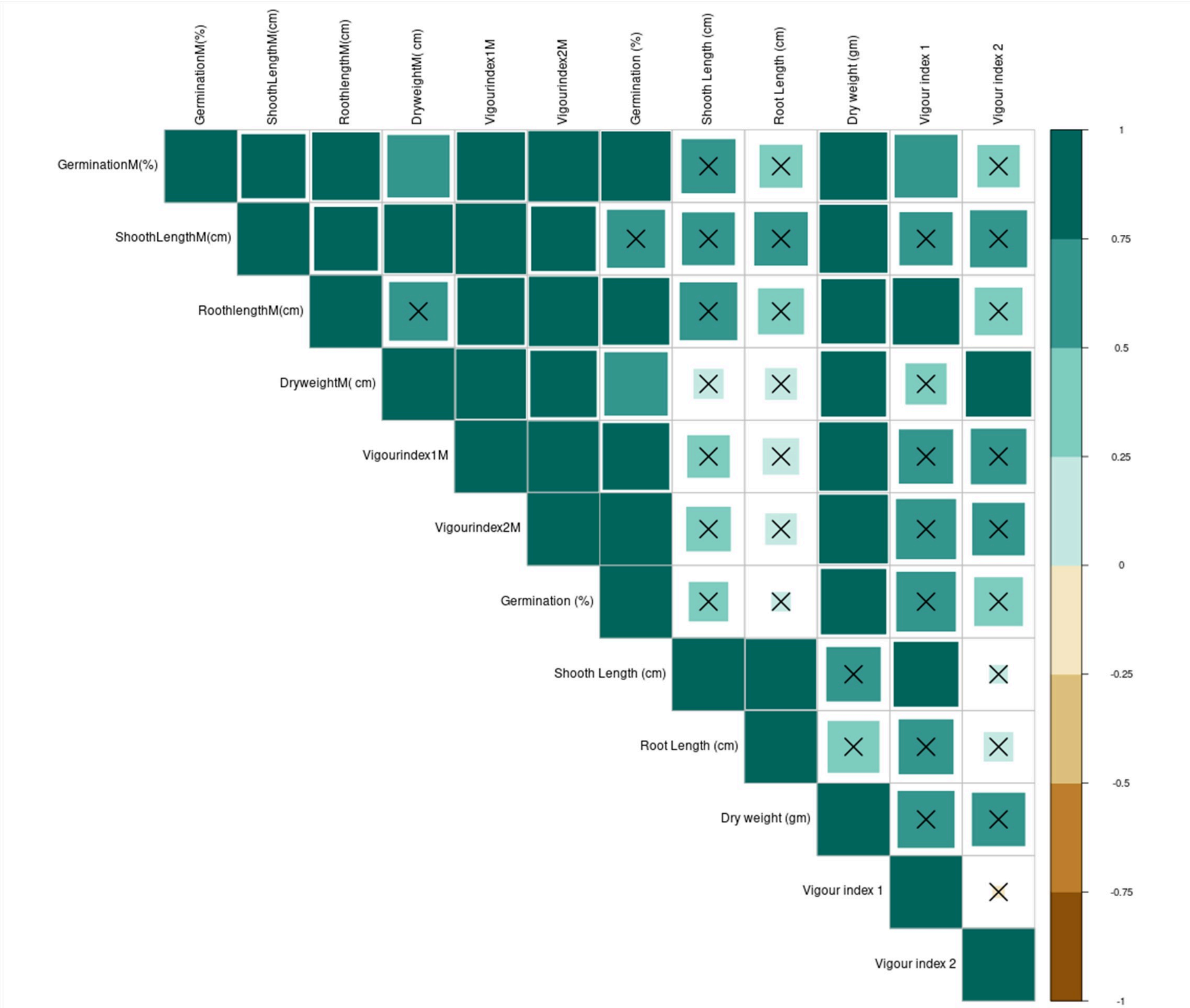



Figure 4

PCA biplot, which shows the relation of seedling traits under different seed coating treatments. This chart displays how these parameters relate to each other and explain their contributions to principal components. A good positive relationship and clustering were observed for shoot length, root length, dry weight, and vigor indices I; hence treatment optimization gives mutual reinforcement.




## Figure 5

**Correlation matrix of seedling parameters with different seed coating treatments. This heatmap illustrates the Pearson correlation coefficients among germination, shoot/root length, dry weight, and vigor indices. Strong, positive correlations are shaded darker, while crosses (X) denote marks of no significance (p > 0.05). This depicts consistent and significant relationships among vital seedling parameters.**