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# **Potential of Seed Priming to Ameliorate Plant Productivity and Augment Active Nodulation in** *Vigna mungo* **for Sustainable Agriculture**

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#### **Abstract**

Twenty-one genotypes of black gram were evaluated to study the impact of pre-sowing phytohormonal seed priming on root nodulation. From the present investigation, the genotype IC-261175 reported maximum root length under ascorbic acid treated seeds while the genotype IC-334268 had maximum shoot length under riboflavin treated seeds as riboflavin can lead to more vigorous seedling growth than ascorbic acid. Genotype IC-426495 reported the highest number of nodules per plant and the number of active nodules under ascorbic acid treated seeds. At both the genotypic and phenotypic levels, correlation coefficient analysis showed that seed yield per plant showed a substantial and positive link with number of major branches, number of pods per plant, pod length, seed index, and harvest index. The present study thus concludes that seed priming in *Vigna mungo* with ascorbic acid, riboflavin is capable of augmenting plant growth, nodulation and also ameliorates soil fertility as the active nodulation induced by seed priming aids in the conversion of atmospheric nitrogen into nitrogen compounds taken up by plants by the left behind nitrogenous compounds reclaim the soil fertility.

#### **Introduction**

After cereals, pulse crops are the most significant food crops; these are sometimes known as "grain legumes." Botanically called *Vigna mungo* (L.) Hepper, black gram is a grain legume that was

domesticated from *Vigna mungo* var. silvestris. It is also referred to as urdbean or mash. It has the ideal ratio of all the nutrients, including 40–47% starch, ash, lipids, carbs, and vital vitamins, and 20–25% protein—nearly three times that of cereals. In many

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nations, urad beans' susceptibility to unfavorable environmental circumstances has a detrimental impact on both their development and productivity. Drought stress is a result of extended water scarcity that significantly impacts black gram farming in India and other subtropical nations. One of the most significant abiotic factors that significantly lowers agricultural production of many crops is drought, particularly when it adversely affects vital physiological and biochemical processes in plants.<sup>1</sup> Pre-sowing seed priming, a technique involving the controlled hydration of seeds before planting can enhance germination rates, seedling vigor, and ultimately crop yield. By evaluating different black gram genotypes for their response to seed priming, farmers can identify cultivars that exhibit improved performance under varying environmental conditions, thereby optimizing seed treatment practices to maximize yield potential. It has been demonstrated that using primed seeds increases yield, early flowering, rapid growth, and germination rate and consistency.2 This method aims to modulate both the physiological and genetic mechanisms within plants, thereby enabling them to better withstand environmental stresses. It was discovered that the accumulation of proline in primed seeds treated with ascorbic acid, $^3$  for example, increased tolerance to salt by raising the accumulation of  $K^*$ and Ca<sup>2+</sup> and lowering the accumulation of Na<sup>2+</sup>. Ascorbic acid seed priming can positively impact

root nodulation by fostering improved root growth, increasing stress tolerance, and strengthening the symbiotic relationship between legumes and rhizobia. Therefore, the present investigation was done with objective to study the role of Ascorbic acid and Riboflavin on seed vigour and germination and to estimate the effect of phytohormonal seed priming on nodulation.

Assessments of correlation coefficients offer a chance to determine the strength and direction of the relationship between yield and its component traits as well as among various traits.4 An effective statistical method known as path coefficient analysis was created specifically to measure the relationships between various components and their direct and indirect impacts on grain yield. $5$  Therefore, the present research was also designed to evaluate the genetic variability parameters, correlation coefficients, and path analysis in the irrigated conditions of the Prayagraj region.

# **Methodology**

The investigation was done at the Field Experimentation Centre, Department of Genetics and Plant Breeding, SHUATS, Prayagraj, during the *Kharif* season of 2023. The experimental material for the present investigation comprised 03 treatments comprising control, ascorbic acid @ 0.2 g/L solution, and riboflavin acid @ 1.0 g/L solution-soaked black gram genotypes. The experimentation was laid out in Complete Randomised Block Design (CRBD). Steps involved in pre-soaking were;- in three beakers, 1 litre of water was taken in each. In the first beaker ascorbic acid @ 0.2 g/L, and in second beaker Riboflavin @ 1.0 g/L of water was added separately and in the third beaker only water was taken. Then seeds were soaked in each beaker overnight. The seeds soaked were shifted on blotting paper in a petri dish followed by transferring the seeds on germination paper and wrapped and placed in petri dish and covered with a cover slip. Random blotting and transferring were done to obtain 4 replications for each treatment. The petri dish was then transferred to the germinator for germination to take place. After 24 hours the germination rate was observed, and further observations were also recorded post seedling growth. A total of eight characters' worth of data were captured for each genotype and replication. (Table 1). Observations recorded were subjected to statistical analysis.<sup>6</sup>

Under field conditions, morphological characteristics were examined and their genetic variability parameters were investigated. The formulas from Johnson7 were used to determine PCV and GCV. Burton and Devane's<sup>8</sup> formula excluded heritability (in the broadest sense). Al-Jibouri recommended methods were used to calculate correlation<sup>9</sup> while path coefficient analysis was worked out by the method suggested by Dewey and Lu.<sup>10</sup> Three replications of the experiment were conducted using a Randomized Block Design. The spacing between plants and rows was 10 and 30 cm, respectively. From randomly selected five plants for each genotype for each replication, thirteen characteristics were recorded: days to 50% flowering, days to 50% pod setting, days to maturity, plant height (cm), number of primary branches, number of clusters per plant, number of pods per plant, pod length (cm), number of seeds per pod, seed index (g), biological yield (g), harvest index, and seed yield per plant (g).



IC-325256 0.34 0.35 0.20 0.09 0.15 0.11 14.01 14.15 15.46 7.60 8.51 8.33 IC-261175 0.13 0.16 0.25 0.04 0.10 0.11 17.16 19.95 18.58 9.40 10.63 11.91 IC-328904 0.13 0.33 0.17 0.04 0.16 0.10 18.93 23.56 23.87 11.58 13.47 13.65

**Table 1: Average mean performance of 21 genotypes for 7 characters in black gram**



### **Results and Discussion**

**Characters Studied under Laboratory Conditions** The statistical analysis revealed that the variations in treated seeds of black gram genotypes for days to germination were significant. With a grand mean of 8.76, 8.28, 8.15 days for control, Ascorbic acid treated, and Riboflavin treated seeds respectively, the average performance of days to germination varied from 6.33 to 10.67 days for untreated control seeds; from 5.19 to 10.67 days for ascorbic acid treated seeds and from 2.87 to 11.40 days for riboflavin treated seeds. (Table 1). While with a grand mean of 9.72, 7.66 and 8.62 cm for control, Ascorbic acid treated, and Riboflavin treated seeds respectively, the average performance of root length varied from 3.72 to 14.81 cm for untreated control seeds; from 2.65 to 13.34 cm for ascorbic acid treated seeds and from 2.99 to 18.18 cm for riboflavin treated seeds. (Table 1).

The average performance of shoot length varied from 2.35 to 7.63 cm for untreated control seeds; from 3.40 to 13.49 cm for ascorbic acid treated seeds and from 3.59 to 16.52 cm for riboflavin treated seeds. (Table 1) with a grand mean of 4.51, 7.22, and 8.17 cm for control, Ascorbic acid treated, and riboflavin treated seeds respectively. With a grand mean of 0.24, 0.27, and 0.22 grams for control, Ascorbic acid treated, and Riboflavin treated seeds

respectively, the average performance of nodule fresh weight varied from 0.13 to 0.40 grams for untreated control seeds; from 0.16 to 0.39 grams for ascorbic acid treated seeds and from 0.14 to 0.29 grams for riboflavin treated seeds. (Table 1). The average performance of nodule dry weight varied from 0.04 to 0.11 grams for untreated control seeds; from 0.05 to 0.19 grams for ascorbic acid treated seeds and from 0.05 to 0.14 grams for riboflavin treated seeds. (Table 1) with a grand mean of 0.07, 0.12, 0.09 grams for control, Ascorbic acid treated, and riboflavin treated seeds respectively,

The average performance for the number of nodules per plant varied from 13.36 to 24.48 nodules for untreated control seeds; from 14.06-30.25 nodules for ascorbic acid treated seeds and from 13.10 to 26.71 nodules for riboflavin treated seeds. (Table 1) with a grand mean of 16.79, 20.25 and 19.17 nodules for control, Ascorbic acid treated, and riboflavin treated seeds respectively while With a grand mean of 10.40, 12.62 and 12.48 nodules for control, ascorbic acid treated, and riboflavin treated seeds respectively, the average performance for a number of active nodules per plant varied from 7.02 to 15.42 nodules for untreated control seeds; from 7.41 to 20.94 nodules for ascorbic acid treated seeds and from 7.51 to 16.92 nodules for riboflavin treated seeds. (Table 1). The superior performance of black gram genotypes treated with ascorbic acid and riboflavin compared to the control treatment can be attributed to the multifaceted roles of these compounds in plant growth and nodulation processes. Ascorbic acid, a potent antioxidant, and riboflavin, a crucial component of plant metabolism, play pivotal roles in enhancing root and shoot development, stimulating nitrogen fixation, and promoting nodule formation in leguminous plants like black gram. Ascorbic acid and riboflavin likely facilitate the uptake and assimilation of nutrients, leading to improved root and shoot elongation. Moreover, these compounds may enhance the symbiotic relationship between black gram and nitrogen-fixing bacteria by creating a conducive environment for nodule initiation and development. Overall, the supplementation of black gram with ascorbic acid and riboflavin augments plant growth,

nitrogen assimilation, and nodulation efficiency, culminating in superior performance compared to untreated plants. Similar findings were reported in black gram working with GA<sub>2</sub> to break seed dormancy and impact seedling growth.<sup>11</sup>

# **Characters Studied under Laboratory Conditions Genetic Variability**

The GCV showed a broad range of variances, from 2.66% (days to maturity) to 25.86% (number of pods per plant), suggesting a significant level of genetic variability (Table 2). PCV varied from quantity of pods per plant (30.47%) to days to maturity (4.06%). A comparison of the coefficients of variance showed that for each character, the phenotypic coefficient of variance (PCV) was greater than the genotypic coefficient of variance (GCV).<sup>12,13</sup>

under field conditions							
SI. No.	<b>Characters</b>	Range	<b>GCV</b> $(\%)$	<b>PCV</b> $(\% )$	$h2$ (Broad <b>Sense)</b> (%)	<b>Genetic</b> <b>Advance</b>	Gen. Adv. as percent of Mean
$\mathbf{1}$	Days to 50% flowering	37.00-44.67	3.44	5.46	39.68	1.83	4.47
2	Days to 50% pod setting	44.27-55.46	4.94	5.87	70.77	4.40	8.57
3	Days to Maturity	65.00-74.33	2.66	4.06	42.93	2.56	3.59
4	Plant height (cm)	38.73-66.89	11.42	17.49	42.59	7.68	15.35
5	Number of primary branches	9.12-13.96	9.37	13.39	48.93	1.57	13.50
6	Number of clusters per plant	9.20-17.58	14.95	20.93	51.03	2.77	22.00
$\overline{7}$	Number of pods per plant	15.22-45.07	25.86	30.47	72.00	12.21	45.20
8	Number of seeds per pod	5.31-7.20	7.48	9.31	64.57	0.79	12.38
9	Pod length (cm)	3.55-4.70	6.15	7.87	61.09	0.40	9.91
10	Biological yield (g)	11.87-29.13	23.01	26.47	75.60	7.77	41.22
11	Seed Index	3.40-4.90	8.40	9.11	84.94	0.65	15.95
$12 \overline{ }$	Harvest Index	33.04-44.41	8.05	8.29	94.39	6.17	16.11
13	Seed yield per plant	6.37-12.20	10.62	11.86	80.27	2.58	19.61

**Table 2 : Genetic Parameters for 13 characters of 21 Black gram genotypes studied** 

For black gram, high PCV and GCV were noted for the number of pods per plant, which was previously reported for biological yield.14 The outcomes for PCV and GCV indicated that there is a good chance that these characters can be selected appropriately and hybridized to achieve even greater improvement. The current analysis found that the black gram germplasm had high heritability (broad sense) for the days to 50% pod setting, number of pods per plant, number of seeds per pod, pod length, biological

yield, seed index, harvest index, and seed yield per plant. An earlier report<sup>15</sup> found that the quantity of pods per plant had a significant heritability. Earlier research on the number of seeds per pod, pod length, and biological yield revealed high heritability.<sup>16,17,18</sup> Similar findings were drawn from their studies on the harvest index and seed output per plant, which likewise showed high heritability.<sup>19,20,21</sup> The current study found high GCV, high estimate of heritability, and strong genetic advancement as a percentage

of the mean for both biological yield and the number of pods per plant. In addition, plant height, number of clusters per plant, and seed yield per plant were found to have high to moderate genetic advance as a percentage of the mean, along with moderate

**Table 3a : Correlation coefficient between yield & its attributing traits in 21 black gram genotypes at phenotypic level (below** 

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heritability with a moderate GCV. Direct selection may be employed to improve these traits since additive gene effects define them. Similar findings were obtained for the harvest index by Saran.<sup>16</sup>











# **Character Association**

Generally, there was more genotypic association than phenotypic correlation. This suggested a natural correlation between different characteristics. The results of the current investigation's phenotypic and genotypic correlation coefficient analysis showed that while seed yield per plant showed a non-significant correlation with the remaining characters, it exhibited a highly significant and positive correlation with the number of primary branches, number of pods per plant, pod length, seed index, and harvest index (Table 3a). The traits listed above had a strong

positive correlation, indicating that they were largely influencing the yield of black gram seeds. Selection procedures for the improvement of one character will therefore invariably result in the improvement of the other character, even in the case where direct selection for improvement has not been done for the yield character. The findings of the present study were in line with earlier studies, demonstrating a significant positive association between seed production and other characteristics.

The number of major branches for the phenotypic correlation coefficient showed a strong and positive link with seed yield per plant.<sup>22</sup> The number of pods per plant, pod length, and seed index were found to be significantly and positively linked with seed yield per plant.18,23,24 Harvest index showed a positive and significant connection with seed yield per plant.<sup>25</sup> In the present investigation, at the phenotypic level, the highest positive direct effects on seed yield at the phenotypic level were depicted by harvest index (0.2627), days to 50% pod setting (0.2655), plant height (0.1145), number of primary branches per plant (0.2409), pod length (0.2713), number of pods per plant (0.0234), seed index (0.5907). The residual component of phenotypic path analysis indicated that 81.10% of the variability of seed yield was accounted for by these thirteen characters. At the genotypic level, the highest positive direct effects on seed yield at genotypic level were depicted by harvest index (0.2380), days to 50% pod setting (0.2739), plant height (0.0733), number of primary branches per plant (0.2442), pod length (0.3248), number of pods per plant (0.0181), seed index (0.5669) and biological yield (0.0294). The residual component of genotypic path analysis indicated that 76.10% of variability of the seed yield was accounted for by these thirteen characters (Table 3 b,c). Similar results have been reported by from earlier researchers. Seed yield per plant was found to be most positively impacted by the harvest index. There was a favorable direct effect on seed yield from the seed index, number of pods per plant, and pod length,22,23 similar conclusion was also drawn.26 Hence selection based on these characteristics would bring an improvement in seed yield in black gram.

#### **Conclusion**

Because of their functions in lowering oxidative stress and increasing cellular metabolism, riboflavin and ascorbic acid-primed seeds have demonstrated improved root development. At the genotypic and phenotypic levels, correlation coefficient analysis showed that seed yield per plant showed a substantial and positive link with number of major branches, number of pods per plant, pod length, seed index, and harvest index. Characters such as harvest index, days to 50% pod setting, plant height, number of primary branches per plant, pod length, number of pods per plant, seed index, and biological yield were found to exhibit a direct positive effect at both the genotypic and phenotypic levels, according to path coefficient analysis information. This showed that the following contributing characteristics' direct and indirect effects were mostly responsible for the seed yield, and these characteristics should be prioritized while selecting Blackgram for improvement. The present research affirms the potential of seed priming agents (ascorbic acid and riboflavin) in enhancing crop productivity and reclaiming soil fertility through augmenting active nodulation in *Vigna mungo*.

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#### **Conflict of Interest**

The authors do not have any conflict of interest.

#### **Data Availability Statement**

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#### **Ethics Statement**

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

#### **Informed Consent Statement**

This study did not involve human participants, and therefore, informed consent was not required.

#### **Author Contributions**

- **• Prajapati Divyansukumar:** Performed field experiment, data collection
- **• Sam A. Masih:** Draft editing, analysis.
- **• Ann Maxton:** Draft writing, conceptualization

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