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Effect of different pre-sowing seed treatments on seed quality of subsequent lentil (*Lens culinaris* M.) crops grown under rainfed conditions **Mohammad Amir**^{*1}, C. B. Singh², Farooq Ahmad Khan³, Suhel Mehandi¹, Anam Khan⁴

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 ABSTRACT

Seed production of lentil under rainfed condition faces significant challenges that impact seed quality and yield. Water stress and *inconsistent rainfall lead to reduced germination rates and poor seedling establishment. Nutrient deficiencies in rainfed soils further compromise seed development, resulting in lower seed yield and vigor. Additionally, plants grown under these conditions are* susceptible to diseases and pests, exacerbating yield losses. To address these challenges, innovative techniques like seed priming play *a* crucial role. The present research examined how different pre-sowing seed treatments influence the seed germination potential of subsequent lentil crops grown under rainfed conditions. The experiment was conducted at Oilseed Farm, CSAUA&T, Kanpur, during *the Rabi seasons of 2019-20 and 2020-21 with two lentil varieties (K-75 -V₁ and KLB303 - V₂) and 14 pre-sowing seed treatments following* the Split Plot Design with three replications. Pre-sowing seed treatments were done by soaking seeds in different priming solutions for 8 hours at 25±2 ^oC. Treated seeds were sown in the field and harvested at appropriate maturity. The germination potential of freshly harvested seeds was tested in the laboratory. The result of the experiment revealed that KLB-303 exhibited improved seed germination attributes as compared to K-75. Seed coating with BioNPK and drought-alleviating bacteria on hydroprimed seeds excelled in germination behavior. This treatment resulted in peak seed viability (97.31%), significant first count (77%), and final germination (96%), along with faster germination speed (32.31), longer seedling length (20.69 cm), higher seedling dry *weight* (0.091 g), and robust vigor indices (1987.61 for index-I and 8.93 for index-II). Nutripriming using ZnSO₄ @ 0.3% + MnSO₄ @ *0.5% also showed encouraging outcomes.*

Keywords:hydropriming, biopriming, lentil, seed priming, seed germination, quality seed, seed vigor

Introduction

Lentil is a small legume seed belonging to the *Lens culinaris* species and the Leguminosae (Fabaceae or Papilionaceae) family. It is one of the most ancient food crops that has been grown in the world and originated from southwestern Asia dating back 7000-8000 years [1][2]. Lentil plays an important role in food and nutritional security. It is a sustainable source of protein, zinc, iron, prebiotic carbohydrates, and diverse healthpromoting nutrients. This crop is widely cultivated in semi-arid marginal areas and exposed to various environmental stressors. Beyond its agricultural significance, lentil plays roles in soil health maintenance and human/animal nutrition [3]. Globally, Canada is the major lentil producer (2.9 million tons), followed by India (1.2 million tons), Australia (0.5 million tons), and Turkey (0.3 million tons). The annual production of lentils in India ranges from 1.1 million tons to 1.5 million tons and Madhya Pradesh, Uttar Pradesh, Rajasthan, Punjab, and Bihar are the major lentil-producing states in India. Historically, lentils are rainfed in India. Generally, lentil is grown as rainfed crop during the season after rice, maize, pearl millet or kharif fallow.

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But in rainfed conditions adequate soil moisture condition is a major obstacle that leads to reduced germination percent, slow down the growth of the seedlings, and finally reduction in yield. Seed priming is an alternative that can improve the germination percentage and seedling establishment of lentils.

Biotic and abiotic stress conditions, such as drought, cold, insuficient rainfall, salt stress, and disease, are the most common restrictive conditions in lentil-growing areas and negatively affect lentil production. Germination and seedling emergence stages are critical for crop production; rapid and uniform field emergence is essential to achieve high yield and uniform plant stands, resulting in early maturity and reduced disease attack [4][5]. Finding ways to overcome environmental stresses, such as inadequate moisture during seed germination, is important for economic crop production. Seed priming as a pre-sowing seed treatment have been reported to have a positive effect on seed germination, seedling growth and uniform crop stand in many field crops [6][7]. Seed priming treatment activates some metabolic activity without actual germination [7]. For priming treatment, seeds are immersed in a solution of high osmotic potential for a certain period of time to initiate the germinative process and then re-dried to their original weight to suspend the further seed germination process [8]. Seed coating is a technique in which an active ingredient (e.g., microbial inoculant) is applied to the surface of the seed with the help of a binder or iller that can act as a carrier [9]. Seed coating with microbial inoculants produces two substantial benefits, *viz.*, less microbial inoculum per plant located at the seed–soil interface, and immediate contact between microbes and roots at the time of germination and early developmental stages [9][10]. Presently there are different types of seed priming techniques used to improve the seed germination and seedling stands. Seed priming, a recognized approach to ameliorate seed quality, involves transient activation of pregerminative metabolism involving antioxidant and DNA repair processes [11]. Seed pretreatment, including priming, orchestrates metabolic modifications preceding root emergence, thus bolstering germination and seedling emergence, pivotal for subsequent seedling development [5][7][12]. However, no information is available regarding the influence of pre-sowing seed treatment on the seed quality of subsequent crops. This research work focused on diverse presowing seed priming methods and their impact on lentil seed quality traits. The study aims to discern optimal seed priming techniques to enhance lentil seed quality for enhanced seed production.

Methods and Materials

The field trial was conducted at Oilseed Farm and produced seeds were analyzed in the seed testing laboratory, CSAUA&T, Kanpur, Uttar Pradesh, India, during the *Rabi* seasons of 2019- 20 and 2020-21. Seed materials of Lentil varieties K-75 (V_1) and KLB303 $(V₂)$ were obtained from CSAUAT's seed processing plant in Kanpur and utilized for the experiment. Bioinoculants were sourced from the ICAR-IISS, Mau, Uttar Pradesh India. Liquid formulations of BioNPK, BioGrow, BioPhos, and Drought Alleviating Bacteria, each containing 1 x 109 (cfu), were diluted in a ratio of 1:10 ml with distilled water. The pre-sowing seed treatments *viz*., seed priming and coatings were done by soaking of seeds in different priming solutions along with pure water with a seed-to-solution ratio of 1:4 (volumetrically) for a period of 8 hours at a temperature of 25±2°Cand subsequently shade dried to their original weight while as coating of hydro-primed as well as non-primed seeds was done using bio-inoculants (Table 1).The treated seeds were sown in the experimental plots with three replications. Seeds were harvested and sampled from each plot and seed quality traits were assessed in the seed testing laboratory adopting a two-factor randomized complete block design i.e., variety × seed priming with three replicates.

Seed viability test was performed using Tetrazolium salt (2,3,5 triphenyl tetrazolium chloride) as described [13]. One-half of the imbibed seeds of lentil were kept into the 1.0% tetrazolium solution at 35 $\pm 2^{\circ}$ C to for complete coloration. Thereafter, they were removed from the solution and rinsed 2-3 times with distilled water. Seeds that stained were considered as viable. Seed germination test was performed as per the standard protocols established by ISTA [14] with slight modifications. A hundred seeds of lentil were placed at equidistance between two layers of paper towel and kept them in a Seed Germinator at 25 \pm 1 $^{\circ}$ C. The germination count was recorded on daily basis till the final germination was over. Seeds were considered germinated when the radicle appeared for at least 2 mm. The germination lasted for 16 days with data being collected on a daily basis. The final germination percentage (FGP) was calculated by the following formulae:

No. of seed germinated on final day Final germination percent (FGP) = $\frac{N_{0.0}$ of seed germinated on final day
Total no. of seeds sown for the germionation test

For the first count $(\%)$, a number of seeds germinated on the 5th day was recorded and calculated in percent. Speed of germination which is an expression of vigor was calculated by dividing the highest cumulative germinate on percentage, Σ nt

with number of days taken to reach that germination percentage, Σ n [15]:

Germination Speed (%/day) = $\frac{\sum nt}{\sum n}$

From the seed germination tests, ten random seedlings were chosen from each replication to assess average seedling length (cm). Subsequently, the seedlings were subjected to ovendrying at 80°C for 48 hours to ascertain their dry weight.

The seedling vigour index I and II are the product of percent seed germination and total seedling length (cm), and per cent seed germination and seedling dry weight (g), respectively [16]. Seedling Vigor index I & II were calculated as under:

Seedling Vigour Index $-I = Germanation (\%)x$ Seedling Length (cm) Seedling Vigour Index - II = Germination $(\%)x$ Seedling dry weight (g)

Data obtained from the two years experiments were pooled and subjected to statistical analysis [17]. The ANOVA with a twofactor statistical analysis was employed to compare the absolute control treatment against the other treatments.

Table 1: Details of pre-sowing seed treatments

Treatment	Description
T_1	Control - No seed priming
T ₂	Rhizobium inoculation @ 10ml/kg of seeds
T_3	Hydropriming: in water
T ₄	Nutripriming I: Potassium nitrate (KNO ₃) 0.3% solution
T ₅	NutriprimingII: Monopotassium phosphate(KH ₂ PO ₄) 0.5% solution
T ₆	NutriprimingIII: ZnSO ₄ 0.3% + MnSO4 0.5% solutions
T ₇	Seed coating of non-primed seeds with Trichoderma harzianum
T ₈	Coating hydro-primed seeds with Trichoderma harzianum
T ₉	Coating of hydroprimed seeds with BioNPK
T_{10}	Coating of hydroprimed seeds with BioGrow
T_{11}	Coating of hydroprimed seeds with BioPhos
T_{12}	Coating of hydroprimed seedswith Drought Alleviating Bacteria + BioNPK
T_{13}	Coating of hydroprimed seeds with Drought Alleviating Bacteria +
	Biogrow
T_{14}	Coating of hydroprimed seed with Drought Alleviating Bacteria + Biophos
BioNPK – comprising NFB, PSB and KSB; BioGrow-contorium of diverse bacterial strains	
known for their ability in phosphorus solubilization, IAA production, and siderophore	

synthesis., BioPhos - liquid formulations of PSB containing Kluyvera sp.

Experimental Findings

Perusal of the data (Table 2) data demonstrated that lentil varieties as well as different pre-sowing seed treatments significantly ($p \le 0.05$) influenced seed viability and first count of seed germination individually or in different combinations. Individual effects of variety indicated that KLB303 $(V₂)$ had higher seed viability (89.17%) compared to lower seed viability (88.46) in K-75 (V_1). Among different pre-sowing seed treatments, T_{12} (Coating of hydro primed seeds with Drought Alleviating Bacteria + BioNPK) resulted in highest seed viability and first count of seed germination (97.31 and 77.00%) followed by T_6 (Nutripriming II: ZnSO_4 0.3% + MnSO4 0.5% solutions) (95.19 and 74.81%) against the minimum seed viability and first count of seed germination (81.50 and 53.75%) recorded in T_1 (control), respectively. Among different interaction treatments, $V_2 \times T_{12}$ recorded the resulted in highest seed viability and first count (97.75 and 77.50%) followed by $V_1 \times T_{12}$, $V_2 \times T_6$ and $V_1 \times T_6$ against the least values of these attributes in $V_1 \times T_1$ (81.00 and 54.50).

Analyzed data regarding final germination percent (FGP) and germination speed (GS) indicate significant variations ($p \le 0.05$) due to variety as well as pre-sowing seed treatment (table 3). Individual effects of treatments showed that variety KLB303 $(V₂)$ produced higher FGP and GS (87.88 and 25.29) compared to a lesser value of these attributes (87.12 and 24.59) recorded with K-75 (V_1) . Coating of hydroprimed seeds with Drought Alleviating Bacteria + BioNPK (T_{12}) maintained its superiority by producing highest values of FGP and GS (96.00 and 32.31),

respectively followed by T6 (Nutripriming II: $ZnSO₄ 0.3%$ + MnSO4 0.5% solutions) with FGP and GS values of 94.25 and 29.95 compared to a minimum FGP and GS values of 94.25 and 29.95 80.50 and 18.90 observed with $T₁$ (control). Perusal of the interaction data of the two factors clarified that $V_2 \times T_{12}$ resulted in highest FGP as well as GS (96.38 and 32.89) followed by $V_1 \times T_{12}$ (95.63 and 31.73), $V_2 \times T_6$ (94.75 and 30.49) and $V_1 \times T_6$ (93.75 and 29.41) against compared to the minimum FGP and GS values 79.63 and 18.51 recorded with $V_1 \times T_1$ followed by $V_1 \times T_2 (80.25)$ and 18.91).

Analysis of variation in root and shoot length due to various treatments implies a significant variation ($p \le 0.05$) and KLB303 (V_2) produced higher root and shoot length (7.26 and 8.63cm) compared to a lesser value of root and shoot length (7.01 and 8.38cm) of K-75 (V₁). Pre-sowing seed treatment T_{12} (coating of hydroprimed seeds with Drought Alleviating Bacteria + BioNPK)also registered the highest root and shoot length (9.53 and 11.16cm) followed byT6 (Nutripriming II: $ZnSO₄ 0.3%$ + MnSO4 0.5% solutions) with recorded values of 9.09 and 10.52cm in contrast to the minimum root and shoot length of 4.69 and 6.73cm in control (T_1) . Information regarding the fusion of pre-sowing seed treatments and variety indicate that $V_1 \times T_{12}$ resulted in highest root and shoot length (9.74 and 11.37cm) followed by $V_1 \times T_{12}$ (9.31 and 10.96cm), $V_2 \times T_6$ (9.18 and 10.66cm) and $V_1 \times T_6$ (9.00 and 10.38cm) against the minimum root and shoot length of 4.30 and 6.65m recorded with $V_1 \times T_1$.

Facts regarding seedling length and dry weight as influenced by variety and pre-sowing seed treatment designate a noticeable alteration ($p \le 0.05$) and variety KLB303 (V₂) recorded greater seedling length (15.89cm) compared to a lesser value of seedling length (15.39) of K-75 (V₁). However, seedling dry weight did not differ markedly due to various treatments. So fa as the individual effects of pre-sowing seed treatment is concerned, T_{12} (Coating of hydroprimed seeds with Drought Alleviating Bacteria + BioNPK) produced the seedlings with highest seedling length and dry weight (20.69cn and 0.091g) with second highest values of these attributes (19.61cm and 0.090g) resulted by T_c (Nutripriming II: ZnSO₄ 0.3% + MnSO4 0.5% solutions) in contradiction with the lowest values of seedling length (11.41cm) and dry weight (0.060) recorded in control (T_1) . Interaction of variety and pre-sowing seed treatment (V×T) revealed that $V_2 \times T_{12}$ recorded the highest seedling length and dry weight (21.10cm and 0.093g) seconded by $V_1 \times T_{12}$ with their absolute values of 20.27cm and 0.090gfollowed by $V_2 \times T_6$ and $V_1 \times T_6$ against the least values of these attributes in $V_1 \times T_1$ (10.95cm and 0.060g).

Examination of the data regarding seedling vigorindices (SVI-I and SVI-II) as influenced by variety and pre-sowing treatment revealed that variety KLB303 $(V₂)$ recorded higher SVI-I and SVI-II (1409.65 and 6.58) compared to a weaker SVIs recorded with K-75 (V_1) Coating of hydro primed seeds with Drought Alleviating Bacteria + BioNPK (T_{12}) maintained its superiority by producing higher SVIs (1987.61 and 8.93), respectively followed by T6 (Nutripriming II: $ZnSO_a 0.3%$ + MnSO4 0.5% solutions) with SVIs values of 1848.75 and 8.37 compared to a minimum SVIs values of 919.54and 4.69 observed with T_1 (control). Scrutiny data regarding $V\times T$ interactions clarified that $V_1 \times T_2$ resulted in highest SVIs (2035.54 and 9.06) followed by $\rm V_1\!\times\!T_{12}$ (1939.69 and 8.80), $\rm V_2\!\times\!T_6$ (1880.91 and 8.48) and $\rm V_1\!\times\!T_6$ (1816.58 and 8.25) compared to the minimum SVIs values 871.17 and 4.54 recorded with $V_1 \times T_1$ followed by $V_1 \times T_2$ (904.47 and 4.66), respectively.

Discussion

Seed size and density is related to the number of stored proteins, the quality of mRNA built up during embryo maturation, and the hormonal constitution of seed and are key determinant of successful germination and seed vigour [18][19]. Differences in seed germination attributes between the two varieties in the present study may be attributed to the difference in their seed size as KLB303 (V2) is relatively a larger seed than K-75 (V1). It has been claimed that larger seeds increase germination with high vigor, survival rate and competitively superior seedlings compared with plants produced from small seeds [20]. Seed size is an important component in plant growth and development because the likelihood of dispersal, germination, and survival can all depend on seed size. It was further reported that the final germination percentage (FGP) of Senna depended on seed size with large seeds having highest germination percentage [18]. Seed size is a plastic trait of plants that directly affects seed germination and seedling recruitment [21]. Recent studies also indicated that larger seeds had a higher germination rate, germination index, vigor index, and seedling biomass than small seeds [22][19].

The present study reports that hydropriming significantly improved the FGP, SVI and other germination attributes of lentil varieties. Seed priming, where seeds are hydrated to activate metabolism without actual germination followed by drying increases the germination, stand establishment and stress tolerance in different crops [7][23]. Pre-sowing hydro-priming of seed facilitates the softening of seed coat and the biological process required for germination thereby resulting in early germination [24]. Additionally, it also attenuates initial imbibition variations between the plants, resulting in more uniform germination [25]. Our results are in parallel with those obtained on different crops [4][5][7][8]. In rainfed areas, seed germination and plant growth are often constrained by adverse conditions in the field. The magnitude of the beneficial response of hydro-priming was more evident under limited soil moisture after sowing, whereas the beneficial effect of seed hydropriming was masked when rainfall followed sowing [26].

Findings of the present study that nutripriming (T_4-T_6) has greater effects on seed germination attributes compared to hydropriming is supported by earlier researchers [27][28]. When seeds are primed with micronutrients, they may swiftly absorb water, restart metabolism and start germination thereby improving stand establishment, enhanced resistance to pests and drought and eventually higher yield [29]. The improved germination potential of nutriprimed seeds may be due to the involvement of mineral elements in activating many enzymes needed for key metabolism related to germination like respiration [30]. During seed priming, the supplied -nutrients are eficiently transported to the inner regions of the seed from the husk, increasing its availability to the region where it is required the most [31]

Seed priming with living bacterial inoculum is termed as biopriming that involves the application of plant growthpromoting rhizobacteria [32] that increases speed of germination and ensures rapid, uniform and high establishment of crops. Among the pre-sowing techniques, bio-priming has emerged out to be the most simple, economical, and eco-friendly delivery system of beneficial microorganisms in the agroecosystem [33]. Seed biopriming allows the bacteria to enter/adhere the seeds and also acclimatization of bacteria in the prevalent conditions. Bio-NPK is a blend of beneficial microorganisms capable of ixing atmospheric nitrogen, solubilizing phosphate and mobilizing potash into an available

form for crops whereas Biogrow is contorium of diverse bacterial strains known for their ability in phosphorus solubilization, IAA production, and siderophore synthesis. Biophos also contains PSB and other beneficial microorganisms. A greater improvement in seed germination potential due to hydro-priming followed by seed coating with bioinoculants may be attributed a fast water and oxygen uptake that quickly initiate germination related metabolic activities whereas coating of seeds with different bioinoculants might have increased the availability of major nutrients that additionally supported the germination process. through improving the availability of N, P and K to the germinating seeds [34]. The increase in speed of germination through the mediation nitrogen can be attributed to the fact that the faster initiation of metabolic activities in the seed [35]. Nitrogen may also promote seed germination through its function as a signaling molecule [36]. Phosphorus content in seeds can directly affect the seed germination, seedling vigor and crop establishment as it plays a crucial role in providing energy for chemical reactions [37]. Increase in root length, shoot length and seedling length due to recommended doses of NPK may be due to improved quality of seed, nitrogen and mineral content in the seed [8]. Utilization of PGPMs as seed biopriming methodologies has demonstrated potential in accelerating seed germination, enhancing seed vigor, and ensure consistent seedling emergence [38]. The introduction of KSB led to marked enhancements in seed germination, root and shoot elongation, and grain yield, discernibly surpassing the performance exhibited by the non-inoculated control group[39]. It has been reported that freshly harvested seeds of P-solubilizing bacteria (PSB) and ZnSO4 treatment recorded higher germination percentage, root length, shoot length, and seedling vigor index [40]. Also, germination and viability can be improved through seed priming with *Azatobacter* biopriming[41].

Conclusion

This research underscores the intricate interplay between lentil varieties and seed priming techniques in inluencing seed quality under sub-optimal (rainfed) conditions. Variety KLB-303being a bold seed consistently exhibited superior seed quality attributes compared to K-75, emphasizing the role of genetic selection. The priming treatment involving the coating of hydroprimed seeds with Drought Alleviating Bacteria + BioNPK and nutripriming with 0.3% ZnSO₄ + 0.5% MnSO₄ solution emerged as robust strategies for enhancing seed quality parameters.

Therefore, from the findings, it may be recommended that the utilization of either BioNPK+Drought alleviating bacteria or $ZnSO₄ + MnSO₄$ as seed priming may be practiced to enhance seed germination potential under sub-optimal (rainfed) conditions.

Future prospects

The research on various pre-sowing seed priming methods and their effects on lentil seed quality traits holds significant promise for the future of lentil agriculture. By continuing to refine and optimize these seed priming techniques, we can potentially revolutionize the lentil farming industry, leading to the development of high-yielding, stress-tolerant lentil varieties. This research can play a pivotal role in addressing environmental stressors, such as drought, cold, and diseases, which commonly affect lentil crops. Furthermore, delving deeper into the molecular mechanisms underlying seed priming can provide valuable insights, potentially unlocking new avenues for improving seed quality and crop performance.

Field applications and scalability are essential steps for realworld implementation, benefiting lentil production and the livelihoods of farmers. Assessing the long-term effects on seed quality and crop yields will provide valuable insights for sustainable agriculture. Finally, knowledge dissemination and international collaboration can facilitate the global adoption of these practices, contributing to enhanced food and nutritional security, particularly in regions where lentils are a staple crop.

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Declaration of Competing Interest

The authors affirm that there are no identifiable conflicting inancial interests or personal afiliations that could be perceived as exerting influence over the findings presented in this paper.

Table 3: Effect of pre sowing seed priming techniques on seed quality of lentil produced under sub-optimal conditions

Table 4: Effect of pre sowing seed priming techniques on seed quality of lentil produced under sub-optimal conditions

Table 5: Effect of pre sowing seed priming techniques on seed quality of lentil produced under sub-optimal conditions

Table 6: Effect of pre sowing seed priming techniques on seed quality of lentil produced under sub-optimal conditions

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