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Integrated Application of Silicic and Humic Acid Seed Priming for Enhanced Germination and Yield of Lentil (*Lens culinaris* L.)

Deepak Rao[1], Sangita Yadav[1], Ravish Choudhary[1], Dharmendra Singh[2],
Rakesh Bhardwaj[3], Sharmistha Barthakur[4], Shiv Kumar Yadav[1]

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ABSTRACT

Background: Lentil is second most important nutritionally dense pulse crop of India due to its ability to fix nitrogen, helps to maintain soil fertility and increase yield of other crops grown in rotations. Seed priming is a good innovative technology for improvement of emergence and plant stand under various stress conditions and also could enhance various defense mechanisms in seeds against biotic and abiotic stresses. The present study aimed to examine the effect of seed priming with silicic acid (3 mM @18 hr), humic acid (600 ppm @18 hr) and combination of humic and silicic acid (100 ppm + 1 mM @ 16 hr), that may reduce harmful effect of stress at seedling stage and improve early seedling vigor.

Methods: In this field-laboratory investigation during 2022-2023, seed quality and yield quality attributing parameters *i.e.* germination percentage, seed vigor index-I and II, total chlorophyll, emergence percentage, final plant stand, final plant stand emergence, plant height, secondary branches, tertiary branches, number of pod per plant, average number of pod per plant, pod cluster, seed yield per plot and yield per plot were recorded in both laboratory and field conditions.

Result: The results demonstrated a positive effect of seed priming on seed quality parameters against the stress. The highest seed quality and yield quality attributing parameter was recorded in treatment (T_5) combination of humic and silicic acid as compare to control. The chemical priming with combination of humic and silicic acid was found to be most effective treatment to enhance plant stand establishment against stress.

Key words: Germination, Lentil, Seed priming, Stress, Yield attributes.

[1]Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi-110 012, India.
[2]Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi-110 012, India.
[3]ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110 012, India.
[4]ICAR-National Institute of Plant Biotechnology, New Delhi-110 012, India.

Corresponding Author: Sangita Yadav, Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi-110 012, India. Email: sangitaydv@gmail.com

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INTRODUCTION

Lentil is an important cool season food legume of India and positions next to chickpea in production and productivity (Dixit *et al.,* 2017). Productivity of lentil is 1074 kg/ha in the world and 870 kg/ha in India (FAO STAT, 2020). Due to its ability in nitrogen fixation, lentil helps to maintain soil fertility and increase yield of other crops grown in rotations. It is one of the most important grain legumes, mostly grown for its uses as human food and animal feed due to its high nutritious value. Lentil is a nutritionally dense pulse crop with notable concentrations of protein (20-30%), low-digestible carbohydrates (20%), fat (1%), iron (Fe), zinc (Zn) and a range of vitamins. Lentil seeds contain 24-28% protein, 30-40% minerals and 22% vitamins. It is mostly grown in a semi-arid environment and that often faces abiotic stresses (*e.g*., drought and salinity, *etc*) at early seedling growth. Increased abiotic stress is a major threat to pulses production globally due to its high susceptibility (Kaya *et al*., 2006).

Germination is the most sensitive period of plant life cycle (Sinha *et al.,* 2023). Seed germination and subsequent seedling growth are the most important stage for plant stand establishment of crops. Seed germination is delayed or prevented by various abiotic stresses. Thus it is essential to look for ways to overcome problems of early plant establishment. One of the method for increasing germination and seedling quality is seed priming. Seed quality enhancement such as seed coating (Sharam *et al.,* 2019) and seed priming is a seed enhancement technique that causes early seedling emergence through pre-germinative metabolism under salinity stress. It facilitates rapid and uniform germination by reducing mean germination time, enhancing pre-germinative biochemical processes, cell repair etc. There are several reports on effect of seed priming by various chemical agents like ascorbic acid, salicylic acid, KNO_3 (Rabnawaz *et al.,* 2020) toward improving germination, seedling emergence, plant stand establishment, crop growth, nodulation and productivity in quiona (Bhuker *et al.,* 2020) and cowpea (Tetteh *et al .,* 2024) as well as in various crop species (Waqas *et al.,* 2019). Although the chemicals used in seed priming have shown a positive impact but there are concerns their use on the environment. Thus, search for environmentally friendly compounds like humic acid (HA) and silicic acid (SA) are need of the day for sustainable agriculture. Therefore, the purpose of this study was to investigate the effect of chemical priming with silicic acid, humic acid and combination of humic and silicic acid for improvement of lentil seed and yield quality attributes.

MATERIALS AND METHODS

The study was conducted at field and laboratory in the Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi (India) during the 2021-23. The IPL-316 lentil variety was collected from Division of Genetics, ICAR-IARI, New Delhi (India) and was used in the experiment. After standardization with different combinations of humic and silicic acid for various concentrations and durations for seed priming, the final seed priming was carried out with five different treatments *viz*., T_1= Control, T_2= Hydro priming, T_3=, chemical priming with 3 mM silicic acid (18 hr), T_4= 600 ppm humic acid (18 hr) and T_5= combination of 1mM silicic and 100 ppm humic acid (16 hr) were used for evaluation for the yield and yield attributes traits. In laboratory, all the seed quality parameter was observed under normal condition and in field conditions. In field, the experimental treatments were laid out using a randomized block design (RBD) with five replications and the row to row spacing was 45 cm × 15 cm and 50 m^2 was total plot size.

Germination tests (ISTA, 2021)

The seeds were surface sterilized by sodium hypochlorite (1%). The seeds were soaked in solution for specified temperature and duration after drying 50 seeds of lentil were placed equidistantly on top of two layers of moist filter paper in Petri plates and kept them at 20°C. First count was taken at on 5th day, seeds were categorized into normal seedling, abnormal seedling, hard and dead seeds at second count conducted on 10th day. Percentage of normal seedling was used to calculate standard germination in percentage.

Chlorophyll content

The sample were collected from young seedling than take weight of the sample, macerate with 3.0 ml of 80% acetone after transfer into eppendorf tube than centrifuge the sample at 12000 rpm for 7 minutes and finally use ELISA plate reader for taking observation at wavelength *viz*., 470, 645, 652 and 663 nm. (Arnon, 1949).

$$\text{Chl a} = 12.9\ (\text{Ab}663) - 2.69\ (\text{Ab}645) \times \text{V}/1000 \times \text{W}$$

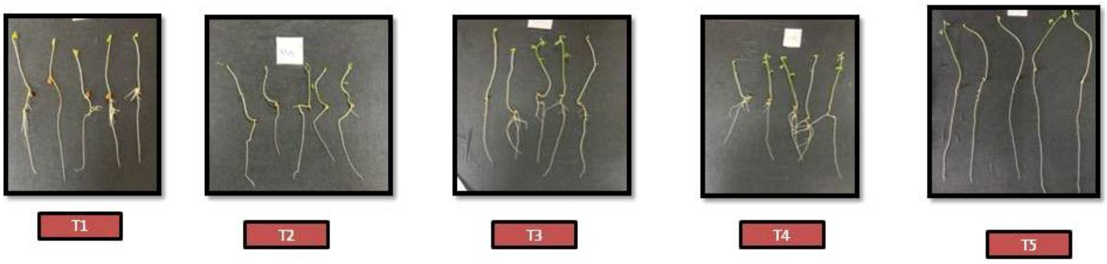
$$\text{Chl b} = 22.9\ (\text{Ab}645) - 4.68\ (\text{Ab}663) \times \text{V}/1000 \times \text{W}$$

Field parameters

The different seed quality attributes parameter was recorded in different intervals of maturity levels. The parameter recorded are final plant stand, final plant stand emergence, primary branches, secondary branches, tertiary branches, number of pod per plot, average number of pod per plot, pod cluster, seed yield per plot (gm), yield per plot (gm), plant height (cm) with the different intervals of vegetative growths. The data was analyzed using the SPSS 16.01 software by using the random block design (RBD) with 5 replications.

RESULTS AND DISCUSSION

Seed priming technique was found to enhance the germination percentage, seed vigor index I & II and physiological parameter to prevent antioxidant damage caused by unfavorable condition, seed priming also repairs the membrane damage occurring during seed development and induces the biochemical changes in seeds such as activation of enzyme involved in cellular metabolism, inhibition metabolism, breaking of dormancy and water imbibitions this facilitating the germination process (Jonhson and Puthur, 2021 and Ajouri *et al.,* 2004). The germination percentage showed an significant impact after seed priming. It increases upto 8.24% when silicic acid was applied at a concentration of 3 mM for 18 hours. Similarly, humic acid at 600 ppm for 18 hours exhibited a significant increase in germination percentage by 5% however, the combination of humic and silicic acid (100 ppm + 1 mM, 16 hr) displayed the most substantial boost in germination percentage,



T1 = Control, T2= Hydropriming @ 18 hr, T3= Silicic acid @3 mM for 18 hr, T4= Humic acid@ 600 ppm for 18 hr, T5 = combination of humic and silicic acid (100 ppm +1 mM for 16 hr).

Fig 1: Seedling length increases after priming with different treatment in lentil IPL-316.

recording a remarkable (9.12%) increase compared to the control and hydropriming (3.2%) under normal condition in laboratory (Fig 1).

Furthermore, the combination of humic and silicic acid (100 ppm + 1 mM, 16 hr) showed the highest (26.32%) increase in seed vigor index-I followed by (21.55%) with silicic acid at 3 mM and treatment with humic acid at 600 ppm exhibited a (20%) increases, (4%) after hydropriming compared to the control. Similar trend were observed in seed vigor index-II, where the combination of humic and silicic acid showed the most prominent increase of (26.32%), followed by silicic acid at (22.18%) and humic acid at (16.92%), after hydropriming (4%) when compared to the control (Table 1). All the priming treatments improved the radicle length highest root length was observed combination of humic and silicic acid (100 ppm + 1 mM, 16 hr) treatment (Table 1). The highest levels of total chlorophyll content were seen in treatment (T$_5$). In normal conditions, the total chlorophyll content was increases 25.03 mg/g FW in control, 36.03 mg/g FW with hydropriming @ 18 hr, 37.48 mg/g FW with silcic acid @ 3 mM 18 hr, 41.11 mg/g FW after humic acid @ 600 ppm 18 hr, 42.20 mg/g FW with combination of humic and silicic acid @ 100 ppm + 1 mM 16 hr. Chloroplasts are especially sensitive to stress and it has been demonstrated that salinity, drought, heat stress adversely impacts photosynthetic pigments in various pulse crops. This degradation of chlorophyll is thought to be linked to the generation of reactive oxygen species (ROS), which reduces the rate of photosynthesis and enhances cellular respiration. Seed priming preserves the photosynthesis machinery and enhances the production of photosynthetic pigment under stress conditions (El-Badri *et al*., 2021). Several reports indicate that priming increases Chl *a*, Chl *b* and carotenoid contents compared to unprimed plants (Latef and Tran, 2016; Huang *et al.,* 2020). Conversely, seed priming with silicic acid, humic acid and the combination of both significantly augmented the levels of photosynthetic pigments. The application of different concentrations of silicic and humic acid through chemical priming offered protection to chloroplast membranes against photo-oxidation, thereby creating an environment help full to efficient photosynthetic function under oxidative stress (Mathur and Roy, 2020).

Plant height is one of the most important parameter which was greatly influenced by different crop management practices like seed priming. Maximum plant height (49.13 cm) was observed with the combination of humic and silicic acid across all crop growth stages (Table 2). This was followed by treatment with silicic acid at 3 mM (45.27 cm) and humic acid at 600 ppm (45.27 cm). The hydropriming and control exhibited the lowest plant height of 43.07 cm and 43.02 cm, respectively, among all treatments. The increase in plant height could be due to proper plant stand establishment that enhanced the uptake of nutrients which improved plant growth and promote cell division.

The highest emergence percentage was observed after 10 days of sowing. The treatment involving the combination of humic and silicic acid resulted in significantly higher emergence percentage of 92% (Table 2). This was followed by humic acid at 90%, silicic acid at 87%, hydropriming at 89% and the control at 78%. Similarly, the final plant stand after 30 days of sowing indicated that the highest final plant stand was achieved with the treatment involving the combination of humic and silicic acid (18) out of 20 seeds. The silicic acid treatment resulted in a final plant stand of (16), followed by humic acid at (15), hydropriming at (14) and the lowest stand recorded in the control at (11). The final plant stand emergence was notably high in the combination of humic and silicic acid, reaching 84% followed by silicic acid (T$_3$) at 80%, humic acid (T$_4$) at 79%, hydropriming (73%) and the lowest in the control (T$_1$) at 59%. Seed priming helps in early seedling growth by expediting the pre-occurrence of metabolic events necessary for seed germination and hence, reduces the time-gap between seed sowing and seedling emergence, improves tillering, primary and secondary branches also improve plant height and grain yield.

All the treatments showed significant difference on number of branches at all the growth stages of observations

Table 1: Effect on seed quality parameter and physiological parameter at laboratory conditions in lentil var IPL-316.

Treatments	Germination (%)	Seedling vigour index-I	Seedling vigour index-II	Chlorophyll mg/m FW
Control	88^{b} (70)	2345^{c}	6.05^{c}	25.03^{c}
H.P @ 18 hr	92^{ab} (74)	2445^{b}	6.46^{b}	36.03^{b}
SA @ 3 mM 18 hr	92^{ab} (74)	2479^{ab}	6.77^{ab}	37.48^{ab}
HA @ 600 ppm 18 hr	92^{ab} (74)	2561^{ab}	6.81^{ab}	41.11^{ab}
HA+SA100 ppm+1 mM 16 hr	94^{a} (76)	2787^{a}	6.94^{a}	42.2^{a}
Mean	92 (74)	2523	6.61	36.37
			P=0.05	
CD (V)	2.34	135	0.98	2.14
CD (T)	2.12	120	0.85	2.05
CD (V*T)	2.65	145	1.12	2.23

The interaction between the varieties and treatments.* Significant differences ($p<0.05$).

Table 2: Effects on seed quality and yield quality attributes under field conditions.

Treatments	Plant height (cm)	Primary branches	Secondary branches	Tertiary branches	Total no of pods/plant	Avg no of pods/plant	Pod cluster	Seed yield per plant (kg)	Yield per plot (kg)
Control	43.20^{b}	3.60^{b}	20.13^{c}	18.07^{c}	196.60^{b}	1.77^{c}	2.00^{ab}	0.02^{b}	0.11^{c}
HP @ 18 hr	43.07^{b}	4.33^{ab}	25.13^{b}	25.93^{ab}	199.27^{b}	1.96^{b}	2.00^{b}	0.03^{b}	0.17^{b}
SA @ 3 mM 18 hr	47.67^{ab}	4.13^{ab}	23.87^{b}	28.00^{ab}	232.93 ^{ab}	1.90^{ab}	2.20^{a}	0.05^{ab}	0.34^{ab}
HA @ 600 ppm 18 hr	45.27^{ab}	4.20^{ab}	29.60^{ab}	23.47^{b}	227.13^{ab}	1.97^{ab}	2.07^{ab}	0.04^{ab}	0.18^{b}
HA+SA100 ppm+1 mM 16 hr	49.13^{a}	4.80^{a}	30.93^{a}	28.13^{a}	247.33^{a}	1.99^{a}	2.27^{a}	0.06^{a}	0.43^{a}
Mean	43.67	4.01	23.53	24.72	196.6	1.93	2.15	0.04	0.25
P=0.05									
CD (Field parameter)	2.87	0.37	5.11	5.7	25.07	0.043	0.15	0.006	1.49
CD (Treatment)	2.39	0.3	4.28	4.74	20.89	0.035	0.13	0.004	0.88
CD (FP*T)	3.44	0.45	6.16	6.83	30.05	0.052	0.18	0.007	1.27

The interaction between the varieties and treatments. *Significant differences ($p<0.05$).

with respect to control. Statistically significant more number of branches has observed with seed priming with the combination of humic and silicic acid 100 ppm (4.80 branches per plant), followed by seed priming with humic acid (4.20) over rest of the treatments. For the number of primary branches (Table 2) the treatment involving the combination of humic and silicic acid demonstrated the highest count at 4.80, followed by humic acid at 4.20, silicic acid at 4.13, hydropriming at 4.33 and the control at 3.60. Similarly, the secondary and tertiary numbers of branches were highest in the combination treatment (30.93, 27.13), followed by humic acid (29.60 and 23.47), silicic acid (23.87 and 28.00), hydropriming (25.13 and 25.93) and the control (20.13 and 18.07).

The number of pods per plot and the average number of pods per plot were significantly higher in the treatment involving the combination of humic and silicic acid (247.33 and 1.99), followed by silicic acid (232.93 and 1.90), humic acid (227.13 and 1.97), hydropriming (199.27 and 1.96) and the control (196.60 and 1.77). This increase might be caused by the metabolic improvements with priming that contributed to better germination, growth and yield performance. The number of pod clusters (Table 2) also exhibited a significant increase in the treatment involving the combination of humic and silicic acid (2.27), followed by silicic acid (2.20), humic acid (2.07), hydropriming (2.00) and the lowest count observed in the control (2.00). Seed priming has been observed to support the partitioning of dry matter into the developing pods boosting the yield (Kaur *et al*., 2015).

Moreover, seed yield per plot (kg) and overall yield per plot (kg) were significantly higher in the treatment involving the combination of humic and silicic acid (0.06 and 0.43), followed by silicic acid treatment (0.05 and 0.34), humic acid (0.04 and 0.18), hydropriming (0.03 and 0.17), while the yield was recorded lowest significantly in the control (0.02 and 0.11). High activities of invertases and sucrose synthase (SS) in pod wall of primed plants could result in more availability of hexoses as well as sugar nucleotides for starch synthesis in pod wall that can be utilized for seed filling, seed setting, increases the size of the seed and maintain the shape of seeds (Haider *et al.,* 2020 and Kaur *et al.,* 2005). Thus, increase in yield could be due to the collaborative action of vigorous seedling growth and enhanced net assimilation due to increased chlorophyll content, resulting in a increased number of branches and pods.

CONCLUSION

Thus, seed priming with 100 ppm humic acid and 1 mM silicic acid for 16 hr can significantly improve germination, plant stand establishment, physiological traits, pods per plot, plant growth and overall seed yield attributes compared to other priming treatment and control. The technique is an environmental friendly method to improve plant stand establishment and yield of lentil. The integrated application of silicic and humic acid seed priming emerges as a promising strategy to enhance germination, stress tolerance, nutrient uptake and ultimately, the yield of lentil crops. Further research and field trials may be warranted to explore the scalability and adaptability of this approach across different lentil varieties and environmental conditions.

Conflict of interest

All authors declared that there is no conflict of interest.

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