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Efficacy of Priming Agents on Seed Germination and Plant Growth Under Salt Stress in *Vigna radiata*

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ABSTRACT

Background: *Vigna radiata* or Mung, an important pulse crop extensively used up worldwide, suffers significant yield loss due to salinity stress. Seed priming technology offers the possibility of sustaining crop yield under abiotic stress by ameliorating stress-induced changes, especially during the germination stage due to highly susceptible behavior.

Methods: The present study aimed to assess the efficacy of selected seed primers on seed germination and plant growth in Mung bean cultivars-Pusa Vishal and Pusa 1431 under salt stress condition. Seeds primed with organic primers such as Cow Urine, Moringa leaf extract (MLE), Neem leaf extract (NLE); chemical primers-(KCl, KNO_3, $MgSO_4$) and hormones-Salicylic acid (SA) and Proline were assessed for various germination, growth and biochemical parameters under salinity stress (150 mM NaCl).

Result: Among the two cultivars, Pusa-1431 exhibited a more positive response towards priming treatments than Pusa-Vishal. In Pusa 1431 seeds primed with NLE 50%, $MgSO_4$ 50 mM and Proline 50 mM and in Pusa Vishal, SA 1mM, Cow Urine 2%, MLE 100% revealed higher germination indices as well as enhanced biochemical parameters.

Key words: Salt stress, Seed germination, Seed priming, *Vigna*.

INTRODUCTION

Vigna radiata, commonly known as green gram, moong, or mung, is a valued legume that forms an integral part of the human diet. It is a rich source of protein, vitamins, minerals and dietary fiber and contains significant amounts of bioactive compounds. *Vigna* crop is also grown under crop rotation regimes for its ability to enhance crop fertility (Singh *et al*., 2016a; Singh and Bell, 2021). Globally 7.3 million ha of agricultural land is under mung bean cultivation producing 5.3 million tons (2015-17), with India and Myanmar each supplying almost 30%, China 16% and Indonesia 5% (Brassica, 2016; Nair *et al*., 2022). In 2022 the global market size of mungbean reached US$ 4,221.5 million (Mung Beans Market: Global industry Trends, Share, Size, Growth, Opportunity and Forecast 2023-2028, Report ID-SR112023A648). Mungbean is grown on an area of 5.13 million hectares in India with the total production of 3.09 million tonnes with a productivity of 601 kg/hectare in 2020-21 (Annual report 2021-22, Directorate of Pulses Development, Ministry of Agriculture and Farmers Welfare, Govt. of India). Mungbean yield potential varies between 2.5-3.0 tonnes/hectare however, the average productivity is strikingly low at 0.5 tonnes/hectare. The reduced productivity is attributed to various biotic stresses such as insect, bacterial and viral infections and abiotic stresses such as salinity, drought, heat stress and waterlogging (Nair *et al.* 2019; Ambreen *et al.* 2021).

Amongst the abiotic stresses, one of the leading factors restricting legume productivity is salinization (Raun *et al.* 2002). It is estimated that NaCl at a concentration of 50mM can cause more than 60% yield loss of *Vigna radiata* (Desai *et al,* 2022). With rapidly increasing salinization of soils due to natural as well as anthropogenic activities, an estimated loss of 50% of cultivable land is expected by 2050 (Saha *et al.* 2010; Hasanuzzaman *et al.* 2013). Salinity stress influences nearly all phases of growth of leguminous plants, most specifically, the germination, vegetative and reproductive phases (Mounia Mansouri and Kheloufi, 2017). Seed germination and seedling emergence stage are highly susceptible to salt. Healthy and higher yield can be assured by enhancing the quality of seed sown and efficient seed germination (Haider and Ur Rehman, 2022). Seed priming is a promising method of enhancing seed germination under abiotic stress (Bose *et al.* 2018). It offers the advantage of overcoming slow and non-uniform germination, low seed vigor, poor crop stand and poor product quality. It also eases germination even under adverse conditions, lifts crop performance and enhances yield potential (Marthandan *et al.* 2020). The technique has emerged as a viable strategy for enhancing crop yield under stressful condition.

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Physiologically seed priming regulates the temperature and moisture content of the seed, bringing it closer to germination without the need for agrochemicals and other labor-intensive and soil-degrading agricultural methods. Both chemical, as well as organic priming agents have yielded positive results. Chemical priming using salts like Sodium Chloride (NaCl), Potassium Chloride (KCl), Potassium Nitrate (KNO_3), Hydrogen Peroxide (H_2O_2) *etc*. are known to be effective due to their involvement in the alleviation of abiotic stresses such as water deficit or salt stress by providing nutrients for growth or modulation of cytoplasmic activities under stress (Miladinov *et al*., 2019; Muñoz-Salinas, 2021). Organic priming agents such as extracts of Moringa leaves are known to contain metabolites like polyphenols, flavonoids and isothiocyanates, which afford defense against pathogens and UV rays (Ambreen *et al*. 2021). Similarly, Neem leaf extract exhibits a synergistic effect on early and uniform seed germination and enhances tolerance to pests and diseases during the early crop stage (Abeysekera, 2021). Cow urine-based priming enhances crop yield and quality as it has nutrients like phosphorus and nitrogen (Satani *et al.* 2021).

Seed priming is an economical and soil-friendly option however, it is not widely used. Limited seed priming options are available to farmers. Organic priming methods with the advantage of having no negative environmental impact need to be explored further. The present study aimed to evaluate the effect of selected seed priming agents (chemical, organic and hormonal) on *Vigna radiata* (mung), under salt stress aiming to provide an efficacious seed priming strategy.

MATERIALS AND METHODS

Plant material

Seeds of *Vigna radiata* (varieties Pusa Vishal and Pusa 1431) were obtained from Seed bank, Indian Agricultural Research Institute (IARI), New Delhi, India. Seeds were surface sterilized with liquid detergent and then thoroughly rinsed with distilled water. For priming treatments, seeds were soaked for 24 hours in various priming agents such as-

- Organic priming-Cow urine (2%), Moringa leaf extract (MLE) (100%), Neem leaf extract (NLE) (50%).
- Chemical priming-NaCl (50 mM), KNO_3 (1 %), $MgSO_4$ (50 mM).
- Hormones-SA (1mM), Proline (50 mM).

The concentrations used in this study were selected from previous reports (Khan *et al.* 2022; Movaghatian and Khorsandi, 2014; Sadeghipour, 2020). After priming the seeds were rinsed with distilled water and air dried at room temperature. Salt stress was given using NaCl (150 mM) to test the efficacy of the priming treatment. For each treatment, at least three replicates were used.

Estimation of germination parameters

Seed germination indices assessed included:

- Germination percentage = $\frac{N_1}{N_2} \times 100$

N1 = Number of germinated seeds.
N2 = Total number of seeds.

- Mean daily germination (MDG) =

$$\frac{\text{Final GP}}{\text{Number of days to final GP}}$$

- Peak value (PV) = $\frac{\text{Final GP}}{\text{Number of days required to reach the peak value of the germination}}$
- Germination value = PV × MDG
- Germination energy percentage =

$$\frac{\text{Number of seeds germinated at 2 days after sowing}}{\text{Total number of the seeds tested}} \times 100$$

- Total biomass of seedling = Dry mass of seedling.

Estimation of growth parameters

- Shoot length was measured from the base of the root hypocotyl transition zone up to the base of the cotyledons.
- Root length was measured from the point below the hypocotyls to the end of the tip of the root.

Biochemical analysis

- Total protein content using Folin-Ciocalteu reagent (Ainsworth and Gillespie 2007).
- Total phenolics content (Singleton *et al.,* 1999).
- α-amylase activity (Bernfeld, 1955).
- DPPH assay (Brand-Williams, 1995).

Statistical analysis

Data expressed as means of three replicates±S.D. Significance ($p<0.05$) was tested using ANOVA and two-tailed t-tests.

RESULTS AND DISCUSSION

Effect on seed germination parameters

Both Pusa Vishal and Pusa 1431 showed enhanced seed germination under different priming treatments (Fig 1). In Pusa Vishal the highest germination of 80% was realized in SA (1 mM), Cow Urine (2%) and MLE (100%). The highest mean daily germination of 13.3 was seen in SA (1 mM), Cow Urine (2%) and MLE (100%) while the highest peak value of 40 was obtained in NaCl (100 mM), SA (1mM), Cow Urine (2%) and MLE (100%). In Pusa 1431 the highest germination of 100% was seen in Proline (50 mM) and NLE (50%). The mean daily germination varied from 11.6-16.6 different priming agents. The highest mean daily germination of 16.6 was seen in Proline (50 mM) and NLE (50%) and the highest peak value of 50 was seen in Proline (50 mM).

In Pusa Vishal, germination energy percentage varied from 10-90% in different priming agents. The highest germination energy percentage of 90% was seen in SA (1 mM), Cow Urine (2%) and MLE (100%). In Pusa 1431, germination energy percentage varied from 50-100% in different priming agents with an overall average of 84.4%.

Highest germination energy percentage 100% was seen in SA (100 mM), Proline (50mM) and MgSO4 50mM. In Pusa Vishal, the highest germination value of 532 was seen in SA (1 mM), Cow Urine (2%) and MLE (100%) (Fig 2). In Pusa 1431, the highest germination value of 830 was seen in Proline (50 mM). The exogenous application of SA is known to assist seed germination and seedling establishment under salt stress (Anaya *et al.* 2018). Under

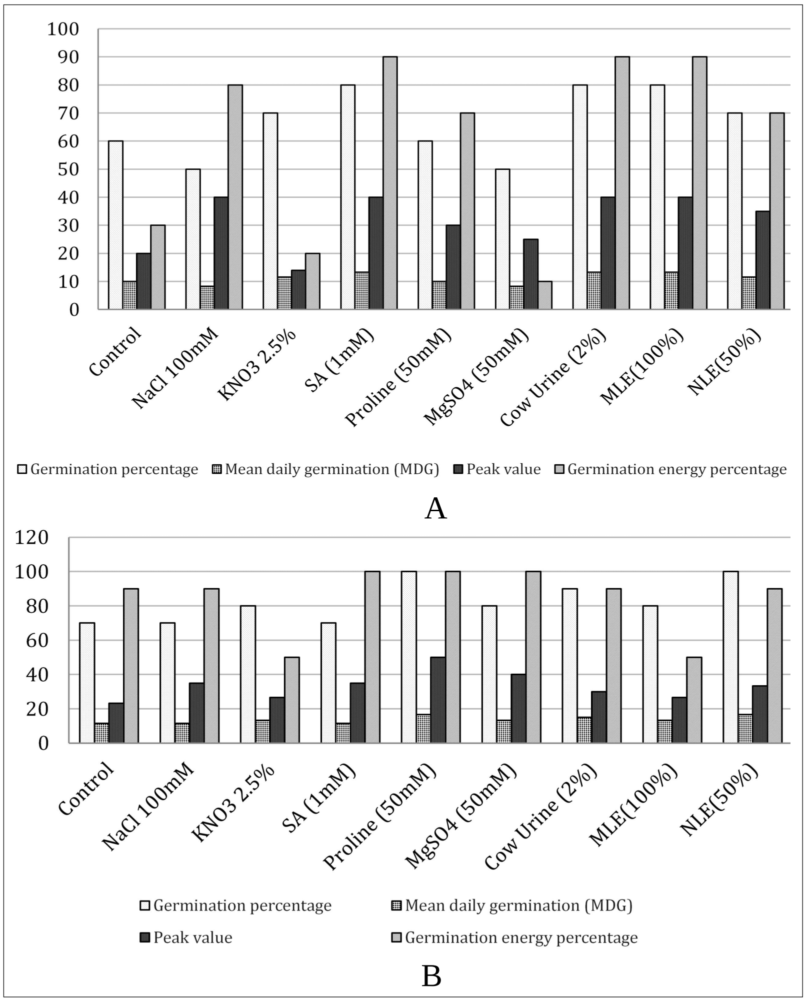

Fig 1: Effect of seed priming on seed germination parameters in A. Pusa Vishal and B. Pusa1431.

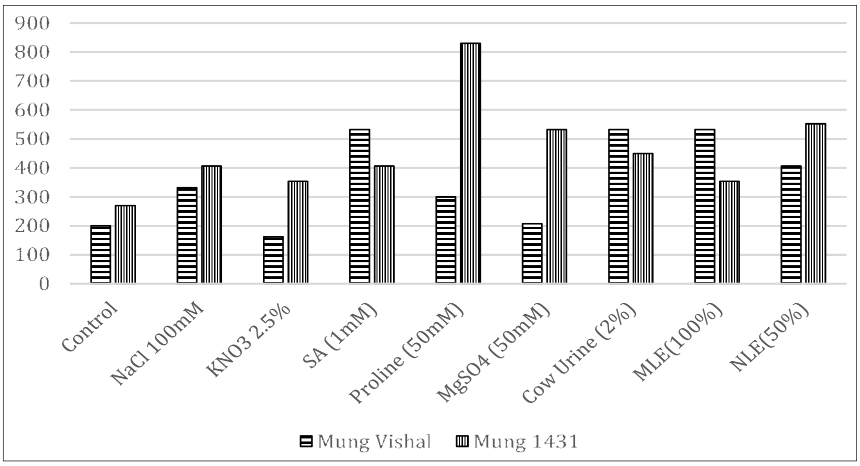

Fig 2: Effect of seed priming on seed germination value of Pusa Vishal and Pusa 1431.

salinity stress, SA reduces oxidative damage and enhances seed germination by assisting the synthesis of proteins crucial for germination and the mobilization of seed proteins gathered during seed maturation. Additionally, SA also dynamically activates the biosynthesis of various enzymes involved in metabolic pathways like the glyoxylate cycle, the pentose phosphate pathway, glycolysis and gluconeogenesis, indicating that SA stimulates the release from a quiescence stage to the establishment of energetic seedling (Rivas- San Vicente *et al.* 2011). Amongst the organic primers seeds primed with leaf extracts of Moringa and Neem displayed a higher germination percentage under saline conditions. The use of MLE and NLE for priming is advantageous as these are sustainable and economical. Moringa leaf extract can limit the accumulation of harmful ions effectively and reduce the creation of ROS, which makes the plant endure unfavorable saline conditions (Ahmed *et al.* 2021). Similarly, Cow Urine is known to positively influence seed physiological parameters because it contains physiologically active substances such as growth regulators, nutrients and trace elements (Ambika and Balakrishnan, 2015).

Effect on growth parameters

No significant increase was noted in shoot length and fresh and dry weight of seedlings in treated plants as compared to the control in both varieties. However, an increase in root length was seen in $MgSO_4$ (50mM) and MLE (100%) in Pusa Vishal and Proline (50 mM) in Pusa 1431 (Table1).

Biochemical analysis

No significant increase in protein content was observed in treated seeds as compared to control in most of the treatments in Pusa Vishal and Pusa 1431 (Fig 3).

In Pusa Vishal, a significant increase was noted in amylase activity was noted in seeds treated with MLE (100%) (5.09±1.8 µg maltose equivalent/gm fresh wt.) as compared to control (2.4 µg maltose equivalent/gm. fresh wt.) (Fig 4). In M-1431 the highest amylase activity was observed to be 5.75±1.5 µg maltose equivalent/gm. fresh wt. in KNO3 (2.5%) and the least was observed to be 2.62±0.009 µg maltose equivalent/gm fresh wt. in cow Urine (2%).

Variation in total phenolic content (TPC) was noted in primed seeds as compared to control (Fig 5). In Pusa Vishal, a significantly higher TPC (197.78±30.47 µg GAE equivalent/

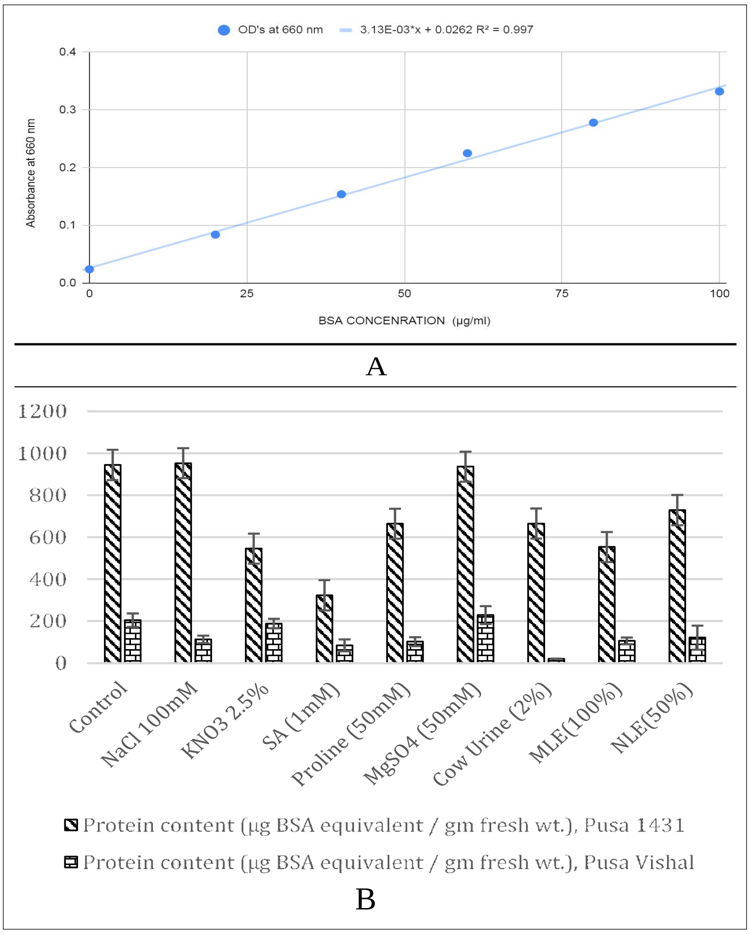

Fig 3: A. Standard curve of BSA; B. Effect of seed priming on total protein content in Pusa Vishal and Pusa 1431.

gm fresh wt.) was seen in MLE (100%) as compared to control (70.05±16.65 µg GAE equivalent/gm fresh wt.). In Pusa 1431, TPC in control was observed to be 82.4±22.7 (µg GAE equivalent/gm fresh wt.). The highest phenolics content of 142.6±38 µg GAE equivalent/gm fresh wt. was seen in Proline (50 mM) and the least (17.4±0.5 µg GAE equivalent/gm fresh wt.) was observed in NaCl (100 mM).

A significant increase in antioxidant activity was noted in primed seeds (Fig 6). In Pusa Vishal, the % reduction in control was observed to be 49.5±1.1%. The highest %

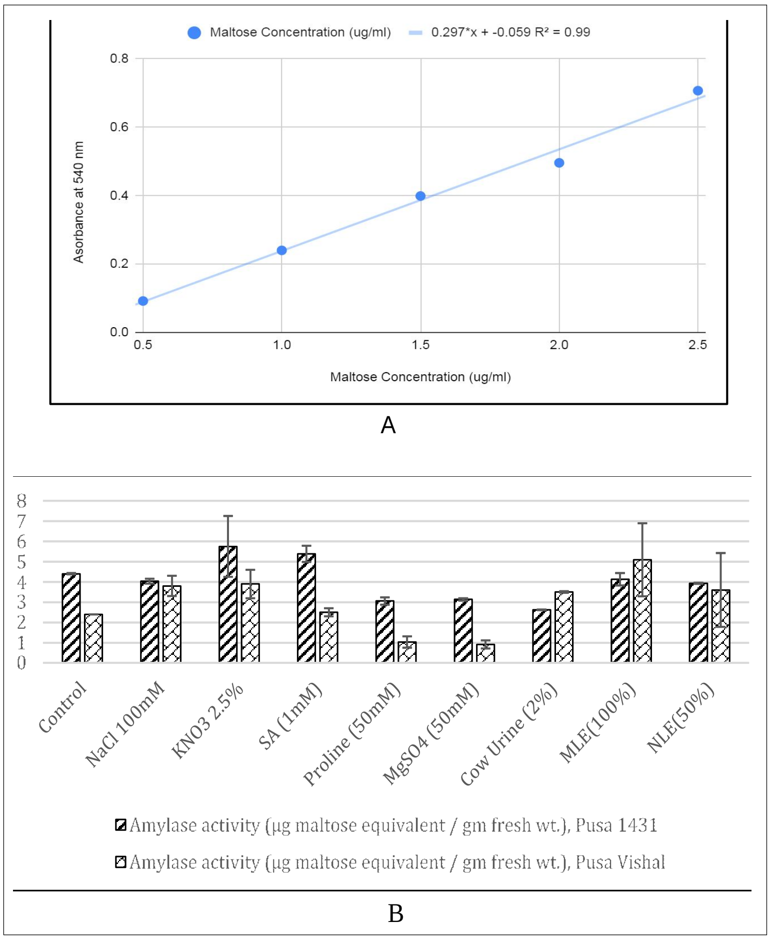

Fig 4: A. Standard curve of maltose; B. Effect of seed priming on amylase activity in Pusa Vishal and Pusa 1431

Table 1: Effect of seed priming treatments on the shoot and root length after one week in Pusa Vishal and Pusa 1431.

Treatment	Root length		Shoot length		Average fresh weight		Average dry weight	
	(M-Vishal)	(M-1431)	(M-Vishal)	(M-1431)	M-Vishal	(M-1431)	M-Vishal	(M-1431)
Control	5.8±1	5.3±1.25	11.5±0.5	10.9±5.9	0.255±0.007	0.2±0.065	0.03±0.0025	0.03±0.006
NaCl 100 mM	4.8±0.4	0.3±0.2	2.1±0.5	5.93±0.35	0.1±0.06	0.1±0.04	0.03±0.006	0.04±0.01
KNO3 2.5%	2.9±1.15	4.7±0.3	1.65±0.2	5.0±0.7	0.2±0.025	0.2±0.04	0.03±0.01	0.05±0.02
SA (1mM)	3.8±0.3	5.9±0.4	2.3±0.6	5.1±0.56	0.11±0.055	0.2±0.03	0.03±0.01	0.04±0.01
Proline (50 mM)	3.3±0.93	9.46±0.5	6.95±2.8	11.3±1.35	0.2±0.07	0.25±0.05	0.025±0.004	0.05±0.008
MgSO4 (50 mM)	9.45±0.78	5.5±0.45	9.67±1.92	3.5±0.5	0.2±0.07	0.2±0.04	0.025±0.004	0.04±0.002
Cow Urine (2%)	2.1±0.65	4.74±1.4	1.8±0.8	4.08±0.7	0.2±0.07	0.1±0.04	0.035±0.01	0.04±0.004
MLE (100%)	7.5±0.8	5.4±0.5	6.12±0.25	9.36±1.09	0.25±0.08	0.2±0.04	0.02±0.004	0.04±0.006
NLE (50%)	2.3±0.6	1.5±0.57	1.2±0.2	1.75±0.86	0.1±0.05	0.1±0.07	0.02±0.002	0.04±0.006

Values represent Mean±S.D.

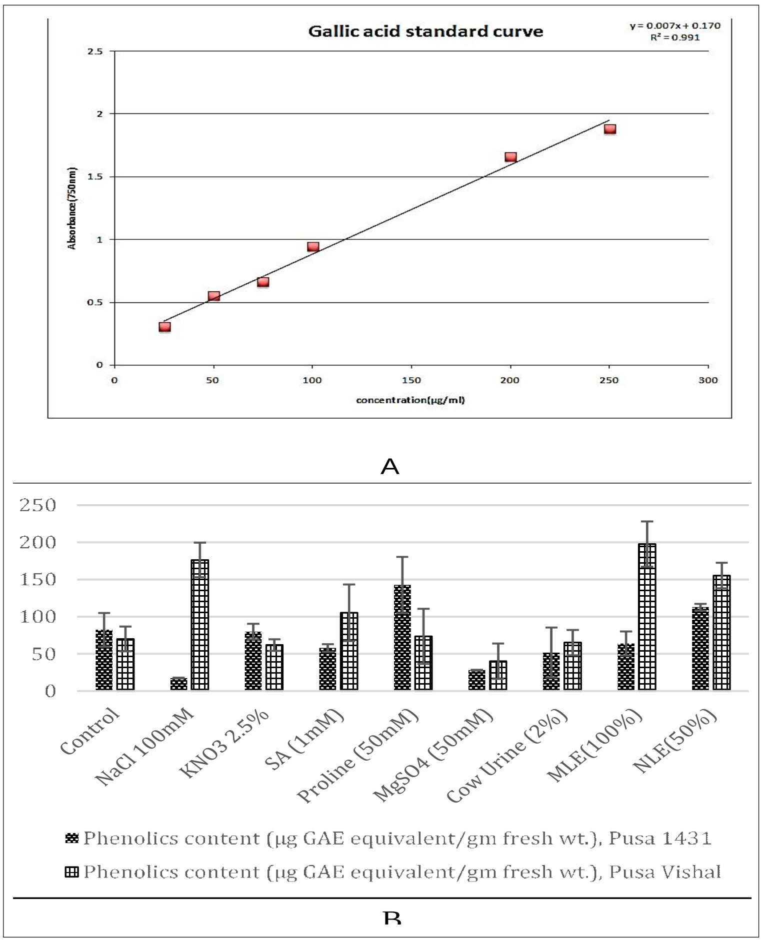

Fig 5: A: Gallic acid standard curve; B: Effect of seed priming on total phenolics content in Pusa Vishal and Pusa1431.

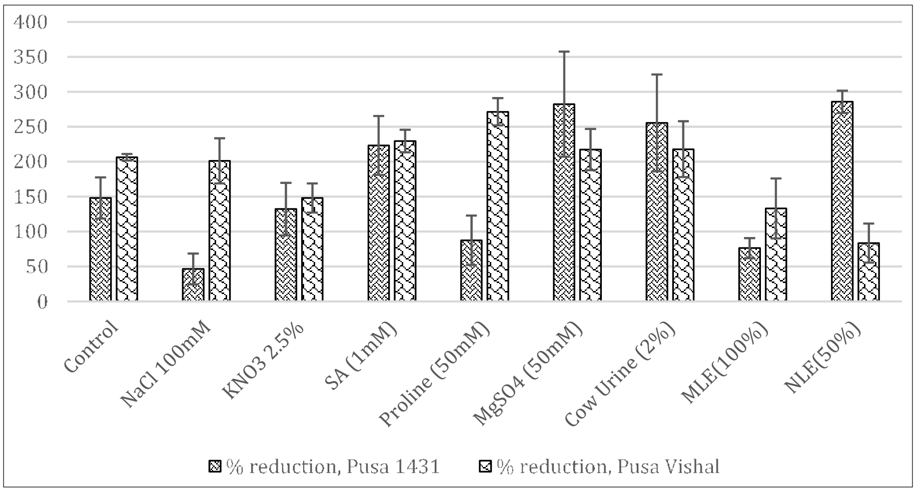

Fig 6: Effect of seed priming on DPPH activity in Pusa Vishal and Pusa1431.

reduction was observed in 50mM Proline (65.14±4.%). In Pusa 1431, the % reduction was the highest in NLE (50%) (68.58±3.8%) as compared to control (39.6±1.8%).

CONCLUSION

V. radiata cultivars Pusa Vishal and Pusa 1431 seeds can be successfully primed for improved germination and field performance. Variation in responses of the two cultivars was noted with Pusa 1431 being more responsive towards priming treatments as compared to the cultivar Pusa Vishal. In the cultivar, Pusa 1431 the use of Proline 50mM and NLE (50%) is recommended for seed priming to enhance seed germination and biochemical parameters under a salt stress ecosystem. Similarly, for the cultivar Pusa Vishal the use of the priming method involving soaking of seeds in 1 mM Salicylic acid, Cow Urine (2%) and MLE (100%) to improve the seed germination under 150mM salt stress, is most amenable.

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Conflict of interest: None.

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