

**Short Communication**

**24-Epibrassinolide Mediated Changes on Germination and Early Seedling Parameters of *Vigna Mungo* (L). Hepper Var. Shekhar-2 under Salinity Stress**

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**ABSTRACT**

Salinity mediated inhibition of seed germination and seedling emergence are the main problems in saline areas. An investigation was carried out to evaluate the effect of four different concentrations ( $10^{-5}$ ,  $10^{-7}$ ,  $10^{-9}$  and  $10^{-11}$  M) of pre-soaked 24-epibrassinolide (24-EBL) on the germination traits associated with seedling emergence in *Vigna mungo* (L). Hepper under salt stress. The results revealed that salinity significantly reduced germination traits especially at higher doses of 16 and 20  $\text{dms}^{-1}$ . Radical length, plumule length, radical fresh weight, plumule fresh weight, germination percentage, seedling length, seedling fresh weight and the seed vigour index also decreased with increasing salinity but seeds primed with 24-EBL alleviated the effect of salinity. Under both stressed and non-stressed conditions,  $10^{-5}$ M 24-EBL was found to be most significant, while  $10^{-11}$  M 24-EBL was least significant.

*Keywords:* *Vigna mungo*, seed priming, salt stress, 24-epibrassinolide, germination traits

**INTRODUCTION**

Salt stress poses a major challenge to agriculture throughout the world by influencing plant growth and greatly reducing crop yield. Salt stress exerts a serious limiting factor for crop growth and production in arid and semi-arid regions. Sodium chloride (NaCl) is the most soluble and widely distributed salt in world (Munns

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& Tester, 2008). Plants vary greatly in their tolerances to salt. However, the performance of crops under saline conditions depends on seed germination, seedling emergence, establishment and also tolerance at late stages of growth. Salinity creates a lot of hardship for seeds in the germination period either by limiting water absorption by seeds (Dodd & Donovan, 1999) or by affecting the mobilisation of stored reserves (Linn & Kao, 1995). Salinity directly affects the organisation or synthesis of proteins in germinating embryos (Ramagopal, 1990). The effect of salt stress on germination percentage, germination rate and seedling growth varies depending on plant species (Ungar, 1996). Plant growth reduction under salt stress is mainly caused by accumulation of salt, an imbalance in the uptake of mineral nutrients and low soil water potential (Hosseini & Thengane, 2007). Ion nutrition gets disrupted due to excessive Na<sup>+</sup> ion accumulation in root surface. Under salt stress, seed germination and early seedling growth are crucial factors limiting crop establishment and yield (Kitajima & Fenner, 2000). Salinity affects the seed germination of pulses like *Glycine max* (Essa, 2002) and *Vigna* spp., (Jabeen et al., 2003). It is well-known that salt stress has a negative impact on seed germination and vigour (Rehman et al., 2000). A low level of salinity inhibits germination by inducing seed dormancy (Khan & Weber, 2008). Salinity causes lower osmotic potential of germination media, and this alters the imbibition of water by seeds (Khan & Weber, 2008), thereby

minimising the utilisation of the reserved food of seeds (Promila & Kumar, 2000) and hormonal imbalance (Khan & Rizvi, 1994).

Brassinosteroids (BRs) are universally occurring plant polyhydroxysteroids (Noguchi et al., 1999). BRs play a substantial role in many developmental processes of plants such as seed germination, root growth and flowering (Sasse, 2003). Khripach et al. (1999) reported that in plants, BRs have growth promoting and other regulatory properties. Bajgua and Hayat (2009) reported that BRs also participate in plant response to biotic and abiotic stresses like salinity, cold and drought stress. BR-regulated stress responses occur through induction of protein synthesis, activation or suppression of key enzymatic reactions and the production of various chemical defense compounds (Bajgua & Hayat, 2009). Exogenous application of BRs promoted seed germination and seedling growth of *Brassica napus* and *Arabidopsis thaliana* under salt stress (Kagale et al., 2007). Seed priming is an effective technique for increasing seed germination and seedling growth of many crops under stressful conditions (Farooq et al., 2006; Bajehbaj, 2010).

Black gram (*Vigna mungo* L. Hepper) is grown in the tropical and subtropical regions of the Indian sub-continent for its protein-rich edible dry seeds. It is a source of nutritionally rich protein that complements cereals to provide a balanced diet. Limited genetic variations in black gram germplasm slows the development of breeding varieties

and provides resistance to abiotic and biotic stresses. Therefore, increasing attention is being focussed on enhancing production of black gram by using phytohormones to overcome its limitations. The present research was undertaken to study the effect of 24-EBL on salinity induced changes in seed germination of *Vigna mungo*.

## MATERIALS AND METHOD

Certified seeds of black gram (*Vigna mungo* L. Hepper var. Shekhar-2) were procured from CCS Haryana Agriculture University, Hisar, India. The plant growth regulator, 24-EBL, was purchased from Sigma Aldrich Ltd., New Delhi, India. The experiment was performed in the research laboratory of the Botany Department, Kurukshetra University, Kurukshetra. Uniform seeds were surface sterilised with 10% hypochloride for 2 to 3 min to avoid fungal infection and then rinsed with distilled water. The seeds were soaked at 25°C for 4 h in  $10^{-5}$ ,  $10^{-7}$ ,  $10^{-9}$  and  $10^{-11}$  M 24-EBL. Distilled water was used as the control treatment. The salinity treatments were given up to five levels (0, 8, 12, 16 and 20  $\text{dsm}^{-1}$ ). A total of 10 seeds were taken in each Petri dish containing a double layer of sterile filter paper and then given the same appropriate amount of distilled water (as control) and different salinity solutions. Seeds were regularly checked for seven days and the germinated seeds were counted. After emergence of radical about 2 mm in length, each seed was considered as germinated. At the end of the

test the seed germination percentage (SGP) was determined.

$$\text{Germination percentage (GP)} = \frac{\text{Number of germinated seeds}}{\text{Total no. seeds}} \times 100$$

After the seventh day, plumule length (PL), plumule fresh weight (PFW), radicle length (RL), radicle fresh weight (RFW), seedling length (SL), seedling fresh weight (SFW) and seedling vigour index (SVI) was determined. Plumule length (PL) and radicle length (RL) were measured using a metre scale. The seedlings were blotted using blotting paper to remove adhering water and plumule fresh weight (PFW), radicle fresh weight (RFW) and seedling fresh weight (SFW) of each plant were weighed on an electronic balance to record the respective fresh mass. The seedling vigour index (SVI) was calculated based on the work by Abdul-Baki and Anderson (1970) as given below.

$$\text{Seed vigour index (SVI)} = \frac{\text{Germination \%} \times \text{Seedling length}}{100}$$

Analysis of variance was based on ANOVA procedure using the SAS software. Two-way ANNOVA range tests at the 5% probability level were used to estimate the differences among the means of the different treatments.

## RESULTS

### Radical Length

In the present study radical length decreased progressively with increase in salinity (Table 1). Salinity decreased radical length by about 18%, 31%, 43% and 54% at  $S_1$ ,

S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> levels of salinity, respectively compared to the control. Application of 10<sup>-5</sup> M EBL increased radical length by 39.4%, 28.7%, 21.5% and 15.5%, respectively at S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> levels of salinity corresponding to their controls. Other concentrations of 24-EBL like 10<sup>-7</sup>, 10<sup>-9</sup> and 10<sup>-11</sup> M also increased radical length but the magnitude was less compared to when 10<sup>-5</sup> M concentration was used.

Table 1  
*Pre-soaking effect of 24-EBL on radical length, plumule length, radical fresh weight and plumule fresh weight under salt stress*

Treatment (M)	Radicle	Plumule	Radicle	Plumule
	Length (cm)		Fresh Weight (gm)	
Control	10.63 ± 0.88	7.16 ± 0.19	0.118 ± 0.016	0.086 ± 0.004
EBL 10 <sup>-5</sup>	11.56 ± 0.24	7.73 ± 0.14	0.132 ± 0.071	0.102 ± 0.006
EBL 10 <sup>-7</sup>	11.46 ± 0.26	7.60 ± 0.21	0.128 ± 0.028	0.098 ± 0.005
EBL 10 <sup>-9</sup>	11.20 ± 0.23	7.53 ± 0.14	0.126 ± 0.026	0.095 ± 0.004
EBL 10 <sup>-11</sup>	11.00 ± 0.25	7.26 ± 0.17	0.121 ± 0.013	0.089 ± 0.002
8 dsm <sup>-1</sup>	08.70 ± 0.11	5.90 ± 0.11	0.091 ± 0.025	0.068 ± 0.001
8 dsm <sup>-1</sup> + EBL 10 <sup>-5</sup>	12.13 ± 0.14	8.33 ± 0.88	0.148 ± 0.038	0.107 ± 0.004
8 dsm <sup>-1</sup> + EBL 10 <sup>-7</sup>	11.83 ± 0.91	8.10 ± 0.17	0.140 ± 0.035	0.106 ± 0.002
8 dsm <sup>-1</sup> + EBL 10 <sup>-9</sup>	11.50 ± 0.57	7.46 ± 0.14	0.130 ± 0.040	0.096 ± 0.003
8 dsm <sup>-1</sup> + EBL 10 <sup>-11</sup>	11.30 ± 0.83	6.80 ± 0.19	0.125 ± 0.046	0.081 ± 0.002
12 dsm <sup>-1</sup>	7.30 ± 0.11	3.93 ± 0.12	0.065 ± 0.005	0.047 ± 0.001
12 dsm <sup>-1</sup> + EBL 10 <sup>-5</sup>	9.40 ± 0.15	5.26 ± 0.81	0.091 ± 0.008	0.068 ± 0.001
12 dsm <sup>-1</sup> + EBL 10 <sup>-7</sup>	8.89 ± 0.85	4.83 ± 0.89	0.084 ± 0.004	0.066 ± 0.001
12 dsm <sup>-1</sup> + EBL 10 <sup>-9</sup>	8.70 ± 0.11	4.53 ± 0.15	0.082 ± 0.006	0.054 ± 0.003
12 dsm <sup>-1</sup> + EBL 10 <sup>-11</sup>	8.10 ± 0.86	4.33 ± 0.85	0.073 ± 0.004	0.048 ± 0.002
16 dsm <sup>-1</sup>	6.03 ± 0.17	2.63 ± 0.81	0.050 ± 0.002	0.024 ± 0.001
16 dsm <sup>-1</sup> + EBL 10 <sup>-5</sup>	7.33 ± 0.16	3.33 ± 0.14	0.065 ± 0.005	0.031 ± 0.002
16 dsm <sup>-1</sup> + EBL 10 <sup>-7</sup>	6.83 ± 0.20	3.26 ± 0.12	0.063 ± 0.004	0.030 ± 0.004
16 dsm <sup>-1</sup> + EBL 10 <sup>-9</sup>	6.46 ± 0.78	3.20 ± 0.18	0.058 ± 0.003	0.028 ± 0.002
16 dsm <sup>-1</sup> + EBL 10	6.16 ± 0.14	2.90 ± 0.13	0.052 ± 0.002	0.025 ± 0.001
20 dsm <sup>-1</sup>	4.90 ± 0.11	1.70 ± 0.05	0.038 ± 0.002	0.016 ± 0.002
20 dsm <sup>-1</sup> + EBL 10 <sup>-5</sup>	5.66 ± 0.88	2.03 ± 0.08	0.045 ± 0.004	0.020 ± 0.003
20 dsm <sup>-1</sup> + EBL 10 <sup>-7</sup>	5.53 ± 0.12	1.96 ± 0.12	0.043 ± 0.002	0.018 ± 0.001
20 dsm <sup>-1</sup> + EBL 10 <sup>-9</sup>	5.36 ± 0.84	1.86 ± 0.04	0.039 ± 0.003	0.017 ± 0.002
20 dsm <sup>-1</sup> + EBL 10 <sup>-11</sup>	5.03 ± 0.12	1.73 ± 0.09	0.038 ± 0.003	0.016 ± 0.001
F value				
Salinity	849.915	1970.2952	845.049	1307.367
Treatment	238.294	57.308	47.045	56.675
Salinity* Treatment	35.118	7.644	6.809	8.528

Mean ± SE was calculated for three replicates. Values with NS are not significantly different at p < 0.05

### Plumule Length

Similar to radical length, plumule length also decreased with increasing salinity. Plumule length experienced the highest decrement at the salinity level of 20 dsm<sup>-1</sup>. The 24-EBL at 10<sup>-5</sup> M mitigated salt stress by enhancement of plumule length by 41.1%, 33.8%, 26.6% and 19.4% at S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> levels, respectively corresponding to their controls.

### Radical Fresh Weight

Salinity decreased radical fresh weight by 22.8%, 44.9%, 57.6% and 67.7% at S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> levels, respectively compared to their controls (Table 1). In this study, we found that radical fresh weight of black gram was reduced in the presence of NaCl as a result of salt osmotic effects, which reduced water availability. Seeds of black gram primed with 24-EBL increased the radical fresh weight of the salt-stressed seedlings. The most effective concentration was 10<sup>-5</sup> M, which increased 62.6%, 40%, 30% and 18.2% radical fresh weight at S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> levels, respectively corresponding to their controls.

### Plumule Fresh Weight

Reduction of about 20.9%, 45.3%, 72%, 81.3% by salinity at S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub>, respectively in fresh weight of plumule was observed in the present investigation. The effect of salt stress was diminished by seeds primed with different concentrations of 24-

EBL. The most effective concentration was 10<sup>-5</sup> M, which alleviated salt stress as seen in plumule fresh weight of black gram by 57.3%, 44.6%, 29.1% and 25% at S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> levels, respectively corresponding to their controls.

### Germination Percentage

Germination percentage was decreased by salinity levels and the percentage of decrement was 13.1%, 27%, 34%, and 48.2% at S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> levels of salinity, respectively compared to non-stressed conditions (Table 2). In the present study, 24-EBL significantly increased germination percentage under salt-stressed and non-stressed conditions. The most effective concentration of 24-EBL was found to be that of 10<sup>-5</sup> M, which increased germination percentage by about 15.9%, 32.2%, 36.8% and 53.2% at 8, 12, 16 and 20 dsm<sup>-1</sup> levels of salinity, respectively corresponding to their controls.

### Seedling Length

Seedling length of *Vigna mungo* decreased with an increase in salinity level. Data presented in Table 2 showed the effect of seed pre-treatment by different concentrations of 24-EBL on seedling length during stress. Seeds pre-soaked with 24-EBL alleviated the seedling length in both stress and non-stress conditions, whereby a concentration of 10<sup>-5</sup> M increased seedling length at S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> levels by 40.1%, 30.5%, 23% and 16.6% over controls.

Table 2

*Pre-soaking effect of 24 EBL on germination, seedling length, seedling fresh weight and seed vigour index under salt stress*

Treatment (M)	Germination (%)	Seedling Length (cm)	Seedling Fresh Weight (gm)	Seed Vigour Index
Control	90.66 ± 0.33	17.80 ± 1.25	0.205 ± 0.032	17.21 ± 1.14
EBL 10 <sup>-5</sup>	100.0 ± 000	19.30 ± 1.18	0.235 ± 0.037	19.3 ± 1.22
EBL 10 <sup>-7</sup>	100.0 ± 000	19.06 ± 1.26	0.224 ± 0.075	19.06 ± 1.18
EBL 10 <sup>-9</sup>	100.0 ± 000	18.73 ± 1.19	0.221 ± 0.038	18.73 ± 1.16
EBL 10 <sup>-11</sup>	90.66 ± 0.35	18.20 ± 0.90	0.211 ± 0.037	17.64 ± 1.12
8 dsm <sup>-1</sup>	80.33 ± 0.38	14.60 ± 0.78	0.159 ± 0.037	12.16 ± 0.92
8 dsm <sup>-1</sup> + EBL 10 <sup>-5</sup>	90.66 ± 0.42	20.46 ± 0.92	0.256 ± 0.032	19.78 ± 1.12
8 dsm <sup>-1</sup> + EBL 10 <sup>-7</sup>	90.33 ± 0.47	19.93 ± 0.83	0.247 ± 0.057	18.6 ± 1.21
8 dsm <sup>-1</sup> + EBL 10 <sup>-9</sup>	90.00 ± 0.57	18.96 ± 0.97	0.226 ± 0.059	17.08 ± 1.12
8 dsm <sup>-1</sup> + EBL 10 <sup>-11</sup>	80.66 ± 0.36	17.60 ± 0.10	0.206 ± 0.023	15.47 ± 1.19
12 dsm <sup>-1</sup>	70.00 ± 0.39	11.23 ± 0.77	0.113 ± 0.035	7.86 ± 0.69
12 dsm <sup>-1</sup> + EBL 10 <sup>-5</sup>	90.00 ± 0.43	14.66 ± 0.71	0.160 ± 0.020	13.20 ± 0.87
12 dsm <sup>-1</sup> + EBL 10 <sup>-7</sup>	80.66 ± 0.45	13.66 ± 0.97	0.150 ± 0.033	11.85 ± 0.76
12 dsm <sup>-1</sup> + EBL 10 <sup>-9</sup>	80.33 ± 0.33	13.20 ± 0.86	0.137 ± 0.043	11.01 ± 0.81
12 dsm <sup>-1</sup> + EBL 10 <sup>-11</sup>	70.33 ± 0.57	12.46 ± 0.99	0.122 ± 0.055	09.15 ± 0.59
16 dsm <sup>-1</sup>	60.33 ± 0.55	08.66 ± 0.62	0.075 ± 0.008	05.47 ± 0.45
16 dsm <sup>-1</sup> + EBL 10 <sup>-5</sup>	80.66 ± 0.57	10.66 ± 0.93	0.096 ± 0.004	09.25 ± 0.58
16 dsm <sup>-1</sup> + EBL 10 <sup>-7</sup>	80.33 ± 0.38	10.10 ± 0.95	0.094 ± 0.007	08.40 ± 0.49
16 dsm <sup>-1</sup> + EBL 10 <sup>-9</sup>	70.35 ± 0.31	09.66 ± 0.75	0.086 ± 0.005	07.08 ± 0.46
16 dsm <sup>-1</sup> + EBL 10 <sup>-11</sup>	70.00 ± 0.33	09.06 ± 0.67	0.077 ± 0.006	06.34 ± 0.52
20 dsm <sup>-1</sup>	50.00 ± 0.57	6.60 ± 0.350	0.054 ± 0.004	03.29 ± 0.37
20 dsm <sup>-1</sup> + EBL 10 <sup>-5</sup>	70.66 ± 0.38	7.70 ± 0.590	0.065 ± 0.008	05.90 ± 0.43
20 dsm <sup>-1</sup> + EBL 10 <sup>-7</sup>	70.35 ± 0.33	7.46 ± 0.480	0.062 ± 0.009	05.47 ± 0.39
20 dsm <sup>-1</sup> + EBL 10 <sup>-9</sup>	60.00 ± 0.53	7.23 ± 0.390	0.056 ± 0.006	04.33 ± 0.40
20 dsm <sup>-1</sup> + EBL 10 <sup>-11</sup>	50.33 ± 0.88	6.76 ± 0.280	0.054 ± 0.004	03.62 ± 0.36
F Value				
Salinity	29.951	3643.340	1856.474	573.078
Treatment	12.345	161.837	91.521	47.834
Salinity*Treatment	1.393 N.S.	17.012	12.584	2.535

Mean ± SE was calculated for three replicates. Values with NS are not significantly different at p<0.05

### Seedling Fresh Weight

Table 2 showed the effect of 24-EBL on seedling fresh weight of black gram during salinity stress. Seedling fresh weight decreased by 22.4%, 44.8%, 63.4% and

76% at four different salinity levels (8 dsm<sup>-1</sup>, 12 dsm<sup>-1</sup>, 16 dsm<sup>-1</sup> and 20 dsm<sup>-1</sup>), respectively corresponding to their controls. The concentration, 10<sup>-5</sup> M 24-EBL, was found to be the most effective concentration

in increasing seedling fresh weight by 61%, 41.5%, 28% and 20% at S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> salinity levels, respectively relative to their controls in the present study.

### Seed Vigour Index (SVI)

Enhanced salinity significantly decreased seed vigour in the present investigation. Reduction in seed vigour was expected because seedling length and germination percentage were decreased by salinity stress. Salinity decreased the SVI by 29.3%, 60.1%, 68.2% and 80.8% as compared to the control. Best results were obtained using a concentration of 24-EBL (10<sup>-5</sup> M) in alleviation of salt stress (Table-2).

### DISCUSSION

The seeds treated with 24-EBL enhanced radical length at all the salinity levels compared to the untreated seeds. High salinity may slow down the uptake of water by the plants, resulting in inhibition of root elongation (Werner et al., 1995). Similarly, HBR alleviated salt-stress mediated inhibition of root elongation in barley (Marakli et al., 2014). This might be attributed to enhancement at the level of nucleic acid and soluble proteins by BRs, ultimately promoting growth (Anuradha & Rao, 2001). Similar results were obtained by Jaleel et al. (2007) in *Catharanthus roseus*, where root length was affected by different salinity levels.

Some growth parameters such as root fresh weight and shoot fresh weight of *Pisum sativum* was decreased by NaCl

(Yildirim et al., 2008). Diminution in shoot growth and fresh weight of barley was caused by a salt concentration of 100 mM (Demirkiran et al., 2013). During seed germination water entry occurred through aquaporins. Salinity reduced the radical fresh weight because NaCl is an inhibitor of aquaporin-mediated root water transport (Martínez-Ballesta et al., 2006).

Jamil et al. (2007) also reported reduction in fresh weight of radish plants under salt stress. Our results were also corroborated by Rahim et al. (2012), who reported the negative impact of increasing salinity on the germination of barley.

Our results were in conformity with the germination of *Eucalyptus camaldulensis* seeds under salt stress by 24-EBL (Sasse et al., 1995). The 24-EBL significantly reduced the dormant period of embryos and increased germination percentage in cherry plum and sloe, as reported by Pugachev et al. (2000). Brassinosteroids enhanced seed germination by increasing the growth potential of tobacco seedling embryos (Leubner & Metzger, 2001). Abscisic acid-mediated inhibition of germination in *Arabidopsis* was overcome by brassinosteroids (Steber & McCourt, 2001).

Reduction in seedling length may have been due to the negative effects of NaCl ion toxicity (Mashadi et al., 1991). Jeannette et al. (2002) reported that increasing salinity reduced the seedling growth of *Phaseolus* spp. Enhanced salinity significantly decreased seed vigour in the present investigation. Reduction in seed vigour due to salinity was expected

because seedling length and germination percentage was decreased by salinity stress (Segatoleslami, 2010).

## CONCLUSION

From this study, it can be concluded that higher doses of salt drastically can inhibit germination traits in black gram. Seeds primed with 24-EBL ( $10^{-5}$  M) overcame the deleterious effects of salinity stress on the parameters investigated. Application of 24-EBL significantly improved all parameters of seedling length, fresh weight and seedling vigour index, thus improving the tolerance of black gram to salt stress. Our results are expected to contribute to information on alleviation of salt stress in different legumes.

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