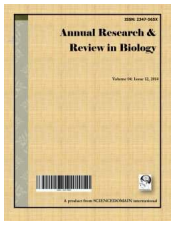

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Effect of Brassinosteroid (Brassinolide) on Seedling Traits, Morphology and Metabolism in Mungbean under Salinity Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SL and AH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SK and RK managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The experiment was conducted in stressed and non stressed condition during *kharif* season 2014 at Department of Plant Physiology, Banaras Hindu University, Varanasi, India. Mungbean seeds were treated with different concentrations of brassinolide (BL) 0.01 mM and 0.05 mM in combination with sodium chloride (NaCl) 100 mM, BL 0.05 mM alone with control. The whole experiment was done in Petriplates and pots in growth chamber and net house, and was laid out in complete randomized design. The results of experiment revealed that plant growth regulator brassinolide (BL 0.05 mM) with salinity stress increased the germination percentage by three folds, root length by two folds, shoot height by two folds, SPAD value by one fold, nitrate reductase activity by seven folds and proline content by one fold when compared with salinity stressed

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condition (NaCl 100 mM) in both HUM-16 and HUM-12 genotypes at 30 days after sowing (DAS). The increase in above parameters was found at par with BL 0.01 mM concentration. Stressed plants exhibited poor growth and biochemical parameters were significantly reduced. However, the follow up application of BL (0.01 mM and 0.05 mM) neutralized the damaging effects of the salinity on the plants.

Keywords: Brassinosteroid; soil salinity; proline content; total chlorophyll content and nitrate reductase activity etc.

1. INTRODUCTION

The current world population of 7.3 billion is expected to reach 8.5 billion by 2030, 9.7 billion in 2050 and 11.2 billion in 2100 [1]. Therefore, providing food and nutritional security to increasing population is a challenge. Food and nutritional security becomes the most important factors with the certainty of changing climate scenario and continuously increasing human population [2]. These twin challenges with respect to increasing population can be addressed by diversifying the global cropping pattern and by promoting food/grain legume crops, generally called pulses in India. Further, Increasing salinization of arable lands is a problem of vital importance to crop production in various parts of the world and especially in irrigated fields of arid and semi-arid regions, leading to a decline in plant growth and yield. An excess of soluble salts causes ion imbalance and hyperosmotic stress, which severely depresses various physiological and biochemical processes in plants [3]. Mungbean is generally known as a salt sensitive crop. A considerable growth reduction in mungbean is due to salt stress. For example, salt stress was found to reduce seed germination, fresh and dry biomass, shoot and root lengths and yield attributes of mungbean [4]. Therefore, Plants were found to be protected either by their own molecular systems or by exogenously applied compounds that mitigate the stress [5]. Thus, the study was planned to evaluate the effect of exogenous application of growth regulators (Brassinosteroids) on mungbean genotype HUM-12 and HUM-16 to reduce salinity stress. In general, HUM-12 and HUM-16 are prominent cultivars adopted by farmers of eastern region of Indo-Gangatic plains of India, but their yield is still low due to soil salinity. Also to validate the significant effect of brassinolide on mungbean, these two genotypes were taken into account.

Brassinosteroids (BRs) are identified to act as antioxidants [6]. BRs are plant hormones with pleiotropic effects, as they regulate various physiological and developmental processes such as growth, seed germination, rhizogenesis and senescence [7]. Brassinosteroids promotes growth, and even induces resistance in plants against salinity [8]. Several workers have demonstrated the protective effects of brassinosteroid pre-treatment against salt stress in many crops [9,10]. Germination of *Eucalyptus camaldulensis* was accelerated in 150 mM salt in presence of 24-epibrassinolide solutions [11]. Brassinosteroids have the ability to counteract the inhibitory effects of salinity on seedling growth. Seed treatment with very dilute solutions of brassinosteroids considerably improved the growth of rice plants in saline media [12]. However, informations regarding use of brassinosteroids in mungbean to mitigate the effect of salinity are meager. Thus, there is need to find out such information regarding neutralization of salinity effects in mungbean with exogenously applied plant growth regulators. Thus, present research work was designed with an objectives to investigate the effects of brassinolide (BL) on seedlings, morphophysiological traits and metabolic activities in mungbean cultivars (HUM-12, HUM-16) under salinity stress.

2. MATERIALS AND METHODS

The present investigation was conducted with two salinity sensitive mungbean (*vigna radiata* L.) genotypes namely HUM-12 and HUM-16 in net house and growth chamber (i.e. controlled condition) in Complete Randomized Design at Department of Plant Physiology, Banaras Hindu University, Varanasi, India during *kharif* season 2014. The treatments consisting T_0- control, T_1- NaCl (100 mM), T_2- NaCl (100 mM) + BL (0.05 mM), T_3- NaCl (100 mM) + BL (0.01 mM) and T_4- BL (0.05 mM) alone were taken in Petriplates and pots in growth chamber and net house. Soil of pots was sandy loam in texture having pH of 7.11, EC of 0.25 dSm^{-1}, bulk density of 1.49 g cm^{-3} and water holding capacity of 39.46%. Mung bean seeds were washed with distilled water and surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 5 minutes, seeds were then rinsed five to six times with sterile distilled water

and germinated aseptically in Petri plates having sterilized wet filter paper in triplicates. To each petriplate, 0.05 mM of brassinolide (BL), NaCl individually and NaCl in combination with 0.01 mM and 0.05 mM were added. Same treatments were also taken in experimental pots in net house. Observations pertaining to morpho-physiological and biochemical parameters were recorded in all the treatments and genotype-wise at different intervals of 5, 10, 15 and 30 days after sowing (DAS) in the growth chamber and net house. All the growth parameters were recorded and evaluated as per standard procedures. The SPAD value of chlorophyll in the fresh leaf was measured by using the SPAD chlorophyll meter (SPAD-502; Konica, Minolta sensing, Inc., Japan). Proline content was determined by the method of Bates et al. [13]. Sample (0.5 g) was homogenized with 10 ml of 3% (w/v) sulphosalicylic acid and the homogenate was filtered through whatman No.2 filter paper and the supernatant was taken for proline estimation. The reaction mixture was composed of 2 ml of plant extract, 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid and heated in a boiling water bath for 1 hour. The reaction was terminated in an ice bath followed by addition of 4 ml of toluene. The contents were shaken vigorously and then allowed to separate into phases. The chromophase containing upper toulene phase was carefully taken out with the help of a pipette and the absorbance was taken at 528 nm on spectrophotometer. The amount of proline present was quantified with the help of proline standard graph.

Nitrate reductase activity of green leaves was determined according to Jaworski [14]. The fresh leaf samples were cut into small pieces and transferred to plastic vials, containing phosphate buffer (pH 7.5), KNO_3 and isopropanol which were incubated at 30℃ for 2h. After incubation, sulfanilamide and N-1-naphthylethylenediamine hydrochloride solutions were added. The absorbance was read at 540 nm on spectrophotometer.

The data obtained on various parameters was subjected to Analysis of Variance as described by Cochran and Cox [15] to identify the effects of treatments on the basis of critical difference.

3. RESULTS AND DISCUSSION

3.1 Seed Germination

The effects of NaCl on germination of mungbean seeds (*Vigna radiata* L.) are shown in Table 1. Salinity stress delayed germination and significantly reduced the germination percentage. From the table it was observed that the two genotypes HUM-12 and HUM-16 responded similarly to salinity stress. As the concentration of BL increased from 0.01 mM to 0.05 mM, there was corresponding increase in the germination percentage when applied with salinity stress at 5 and 10 DAS and in both the genotypes. The lowest germination percentage was recorded under 100 mM NaCl treatment in both the genotypes. However, the germination percentage in control plants was statistically at par with 0.05 mM BL treatment at 5 and 10 DAS. Thus, it was observed that BL had positive effects on germination and ameliorates the adverse effect of salinity stress in both the genotypes. The germination percentage increased with progressive developmental stage (5 to 10 DAS) except in seed treated with 100 mM NaCl alone. Salinity hampers imbibition of water into seed by alteration in osmotic potential. In contrary to that, application of BL provides potency to plants for germination in adverse situations. This finding was also supported by [16] where he reported that germination rate and the final seed germination percentage decreases with the decrease of the water movement into the seeds during imbibitions, due to imposed salinity which can be mitigated by exogenous application of brassinolides.

Table 1. Effect of brassinolide on germination percentage of mungbean (*Vigna radiata* L.) genotypes under induced salinity at 5 and 10 DAS

Treatments	HUM-12		HUM-16	
	5 DAS	10 DAS	5 DAS	10 DAS
T_0 (Control)	86.67	86.67	83.33	90.00
T_1 (100 mM NaCl)	50.00	43.33	36.67	20.00
T_2 (100 mM NaCl + 0.05 mM BL)	70.33	73.00	73.33	73.33
T_3 (100 mM NaCl + 0.01 mM BL)	53.33	60.00	63.33	70.00
T_4 (0.05 mM BL)	83.33	86.67	86.67	93.33
SEm±	**0.471**	**05.16**	**03.33**	**04.94**
CD 5%	**14.85**	**16.27**	**10.50**	**15.58**

3.2 Roots Length

Data recorded at various periods of growth as influenced by treatments are presented in Table 2. It was observed that the root length increased with the crop age and conspicuous increase was observed between 15 and 30 DAS in BL treated genotypes. The highest radicle length was found in control plants of both the genotypes as compared to other treatments at 5 DAS. whereas, at subsequent stages, the highest root length was recorded with 0.05 mM BL treatment. Similar trend of data was recorded in Hum-16 genotype. Salt stress significantly decreased radicle length. Therefore, the lowest root length was found under 100 Mm NaCl alone as compared with other treatments at all the growth stages. Application of 0.05 mM BL induced positive effects on root lengths under 100 mM NaCl which was statistically at par with application of 0.01 mM BL at 30 DAS. Also, in both treatments (0.01 and 0.05 mM) BL the root lengths were found significantly higher than 100 mM NaCl treatment in HUM-12 and HUM-16. However, highest root length was recorded under 0.05 mM BL which was statistically at par with control (T_0) at all growth stages except 5 DAS.

The reduction in root development may be due to reduction in water and nutrient uptake caused by the decrease in external osmotic potential as osmotic shock is produced, which reduces water and nutrient uptake to a great extent. Further, it might be due to large amount of absorption of Na^+ ions which would result in limited assimilation, distribution and transport of other important mineral nutrients. This increase in Na^+ ions may induces nutrient imbalance in plant organs. A sort of competition occurs between Na^+ ions with other mineral nutrients like K^+, Mg^+, Ca^+ [17,18]. In nutshell, the reduction in root development may be due to toxic effects of the higher NaCl concentration as well as unbalanced nutrient uptake by the seedlings [19]. Our results are in agreement with that of [20] who showed marked reduction in growth parameters of tomato plants under salt stress.

3.3 Shoot Height

The data pertaining to shoot height (cm) at different growth stages at periodic intervals are summarized in Table 3. Shoot height is an important parameter reflecting the vertical growth of a plant. In general, shoot height increases with the age of plant. From table it was observed that under salinity stress there was significant reduction in shoot height noticed at all growth stages in both the genotypes. Salinity of 100 mM NaCl drastically reduced the plant height as compared with other treatments. The different BL concentrations significantly increased the shoot height at different growth stages. The lowest shoot height was recorded in NaCl treated plants in comparison with all other treatments. However, the highest shoot height was found in 0.05 mM BL treatment as compared with other treatments and was found statistically at par with control (T_0) at all stages. Seeds treated with 0.05 mM BL showed positive effects on shoot height and further it decreased the adverse effect of salinity stress more than 0.01 mM BL in both the genotypes. BRs are known to improve the growth of roots and shoots in various plants [2,9].

3.4 SPAD Value

SPAD value is an indicator of chlorophyll content in plants. A significant decrease in SPAD value was noticed under 100 mM NaCl treatment as compared with other treatments at 10, 15 and 30 DAS in both the genotypes. There was a drastic reduction in SPAD value at 30 DAS in NaCl treatment when compared with 15 DAS in both the genotypes. The highest SPAD reading was recorded in 0.05 mM BL treatment compared with other treatments at 30 DAS.

Table 2. Effect of brassinolide on length of roots (cm) in mungbean (*Vigna radiata* L.) genotype HUM-12 and HUM-16 under induced salinity at different stages of growth

Treatments	HUM-12				HUM-16			
	5 DAS	10 DAS	15 DAS	30 DAS	5 DAS	10 DAS	15 DAS	30 DAS
T_0 (Control)	0.90	6.37	10.07	10.93	0.73	6.30	9.70	10.73
T_1 (100 mM NaCl)	0.30	3.10	4.47	5.07	0.33	3.53	4.93	4.23
T_2 (100 mM NaCl + 0.05 mM BL)	0.50	4.53	5.70	6.97	0.43	4.73	7.67	9.73
T_3 (100 mM NaCl + 0.01 mM BL)	0.37	3.17	4.67	7.03	0.40	3.80	6.70	6.73
T_4 (0.05 mM BL)	0.77	7.43	10.90	12.20	1.00	7.93	10.23	11.77
SEm±	**0.07**	**0.39**	**0.43**	**0.33**	**0.09**	**0.37**	**0.45**	**0.38**
CD 5%	**0.21**	**1.22**	**1.36**	**1.06**	**0.29**	**1.18**	**1.42**	**1.21**

Seeds treated with 0.05 or 0.01 mM BL+100 mM NaCl showed less decrease in chlorophyll content as compared with NaCl alone. The increase in SPAD value in BL treated plants might be due to increase in chlorophyll content and expression of regulatory gene involved in defense mechanism [10].

Salinity induced decrease in chlorophyll content may be either due to inhibition of new chlorophyll biosynthesis or accelerated degradation of existing chlorophyll molecules Also, it may be due to decrease in biosynthesis of d-aminolevulinic acid and protochlorophyllide reductase complex [21]. The BL induced transcription and/or translation of the enzymes involved in chlorophyll biosynthesis associated with a decrease in the level of catabolizing enzymes [11].

3.5 Nitrate Reductase (NR) Activity

Data presented in Table 5 revealed that the maximum enzyme activity was noted in the plants raised from seed soaked with 0.05 mM BL. In general, plants raised in salinity stressed soil showed inverse relationship between enzyme activity and NaCl treatment. The least NR activities were noted in the plants exposed to 100 mM NaCl in both the genotypes at all stages in comparison with other treatments. The NR activities at 0.05 mM BL+100 mM NaCl was found statistically at par with those of control and 0.01 mM BL+100 mM NaCl at all stages in both the genotypes. However, the values measured at 0.05 mM BL were significantly higher than other treatments at 30 DAS. Data from Table 5 showed that BL treated plant completely or partially ameliorated the damaging effect of salinity. Comparison among the treatments clearly showed significantly increased NR activity in plants treated with BL @ 0.05 mM as compared to control and others.

Decrease in NR activity under salinity stress might be due to metabolic disfunctioning of this enzyme [22] Excess of Na^+ ions may restricts the uptake of NO_3^- ions from the soil. The observed decrease in NR activity is an impact of salinity on the activity of plasma membrane proton pump and the membrane fluidity [23] restricting the uptake of nitrate, the inducer and the substrate of NR [22], salt stress resulted in distorting the structure and fluidity of membrane and adversely affecting the inbuilt ATPase activity [24,25]. Because of a lower flux of Nitrate from soil there is reduction in gene expression and NR-protein synthesis [26]. However, treatment with BL elevated the activity of NR, by speeding up the assimilation of CO_2 [27] and increasing the concentration of NO_3 by acting at the membrane level [28]. There could be an additional impact of BL on the expression of specific genes [29] detoxifying the Na^+ ions externally or partitioning it internally in vacuoles. Moreover, BRs are also involved in translation and/or transcription that may generate a significant impact on the activity of the enzyme to facilitate the uptake of NO_3^- [30].

3.6 Leaf Proline Content

The increase in proline is a common plant stress response. The level of proline exhibited an increase in relation to NaCl stress (Table 6). Seeds treated with 0.05 mM BL had higher proline content as compared with control. However, in association with NaCl, BL further increased the quantity of proline content as compared to NaCl alone. The maximum accumulation of proline was found in the plants which were subjected to 100 Mm NaCl + 0.05 mM BL in comparison with other treatments in both the genotypes. Whereas, the lowest proline content was recorded in control plants. Application of BL alone increase proline content

Table 3. Effect of brassinolide on shoot height (cm) of mungbean (*Vigna radiata* L.) genotype HUM-12 and HUM-16 under induced salinity at different stages of growth

Treatments	HUM-12				HUM-16			
	5 DAS	10 DAS	15 DAS	30 DAS	5 DAS	10 DAS	15 DAS	30 DAS
T_0 (Control)	1.70	9.10	12.07	14.63	2.10	8.90	11.63	13.40
T_1 (100 mM NaCl)	1.17	6.03	9.13	8.60	1.17	6.10	8.57	10.63
T_2 (100 mM NaCl + 0.05 mM BL)	1.43	7.60	10.20	12.20	1.43	7.60	10.23	12.40
T_3 (100 mM NaCl + 0.01 mM BL)	1.30	6.80	8.70	10.77	1.37	7.10	8.57	11.43
T_4 (0.05 mM BL)	2.23	9.47	13.50	15.50	2.17	8.70	13.00	15.53
SEm±	**0.21**	**0.42**	**0.34**	**0.41**	**0.23**	**0.58**	**0.33**	**0.37**
CD 5%	**0.66**	**1.31**	**1.08**	**1.29**	**0.72**	**1.83**	**1.05**	**1.17**

Table 4. Effect of brassinolide on total chlorophyll content (SPAD unit) of mungbean (*Vigna radiata* L.) genotype HUM-12 and HUM-16 under induced salinity at different stages of growth

Treatments	HUM-12				HUM-16			
	5 DAS	10 DAS	15 DAS	30 DAS	5 DAS	10 DAS	15 DAS	30 DAS
T_0 (Control)	10.267	21.833	23.267	24.600	11.933	22.367	23.367	24.400
T_1 (100 mM NaCl)	8.633	18.133	19.633	20.033	8.500	17.600	18.167	19.933
T_2 (100 mM NaCl + 0.05 mM BL)	12.533	21.500	23.333	24.200	12.700	22.233	23.000	24.167
T_3 (100 mM NaCl + 0.01 mM BL)	11.533	21.733	21.333	23.233	13.667	23.100	23.533	22.767
T_4 (0.05 mM BL)	13.300	22.500	24.067	27.033	13.800	23.467	24.300	26.667
SEm±	**0.192**	**0.248**	**0.565**	**0.705**	**0.152**	**0.121**	**0.257**	**0.262**
CD 5%	**0.605**	**0.780**	**1.780**	**2.222**	**0.479**	**0.382**	**0.810**	**0.827**

Table 5. Effect of brassinolide on nitrate reductase activity (n Mol NO_2 released g^{-1} fresh leaf tissue h^{-1}) of mungbean (*Vigna radiata* L.) genotype HUM-12 and HUM-16 under induced salinity at different stages of growth

Treatments	HUM-12				HUM-16			
	5 DAS	10 DAS	15 DAS	30 DAS	5 DAS	10 DAS	15 DAS	30 DAS
T_0: (Control)	7.48	8.54	8.78	9.55	7.58	8.14	8.55	9.11
T_1: (100 mM NaCl)	3.46	4.92	3.23	2.00	3.84	2.37	2.61	1.30
T_2: (100 mM NaCl + 0.05 mM BL)	7.23	8.15	8.77	9.16	7.63	8.92	8.64	8.77
T_3: (100 mM NaCl + 0.01 mM BL)	7.03	8.00	8.53	9.45	7.61	9.32	9.05	9.79
T_4: (0.05 mM BL)	8.04	8.64	8.02	11.73	8.14	9.50	9.81	12.58
SEm±	**0.40**	**0.41**	**0.57**	**0.58**	**0.10**	**0. 14**	**0.15**	**0.32**
CD 5%	**1.33**	**1.37**	**1.91**	**1.93**	**0.33**	**0.47**	**0.51**	**1.07**

Table 6. Effect of brassinolide on proline content (µg g^{-1}) of mungbean (*Vigna radiata* L.) genotype HUM-12 and HUM-16 under induced salinity at different stages of growth

Treatments	HUM-12				HUM-16			
	5 DAS	10 DAS	15 DAS	30 DAS	5 DAS	10 DAS	15 DAS	30 DAS
T0: (Control)	0.054	0.058	0.064	0.070	0.052	0.067	0.062	0.079
T1: (100 mM NaCl)	0.074	0.084	0.096	0.128	0.072	0.081	0.086	0.092
T2: (100 mM NaCl + 0.05 mM BL)	0.086	0.097	0.148	0.180	0.078	0.089	0.098	0.190
T3: (100 mM NaCl + 0.01 mM BL)	0.076	0.087	0.098	0.136	0.078	0.089	0.091	0.098
T4: (0.05 mM BL)	0.066	0.068	0.072	0.080	0.061	0.072	0.080	0.082
SEm±	**0.002**	**0.002**	**0.003**	**0.003**	**0.002**	**0.002**	**0.002**	**0.003**
CD 5%	**0.006**	**0.008**	**0.009**	**0.010**	**0.006**	**0.007**	**0.006**	**0.009**

to some extent but it was found statistically at par with control plants. Comparison among different treatments clearly showed significantly increased proline content in plants treated with BL. The data indicates that BL ameliorated the adverse effect of salinity by production of osmolyte (proline) in both the genotypes.

The application of BL enhanced the proline content in combination with salinity stress which reveals that it plays an important role in osmotic adjustments. These adjustments might be due to expression of genes responsible for biosynthesis of proline. The increased proline content in plant provides protection to the sub cellular structures by reducing the oxidative damage caused due to

free radicals produced in response to salinity stress [31].

4. CONCLUSION

From this present study, it is possible to conclude that the application of NaCl adversely affected the growth and biochemical parameters of mungbean plant as compared to control. Based on the results obtained in this study, it was concluded that BL play an important role in the protection of mungbean plants against salinity stress by increasing germination percentage, root length, shoot length, SPAD values, Nitrate reductase activity and proline content with BL alone followed by 0.05 mM BL in combination with sailinity stressed condition in both HUM-12 and HUM-16 genotypes. Application of 0.05 and 0.01 mM BL in combination with salt also enhance growth and metabolism of mungbean as compared to 100 mM NaCl.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. UN DESA Report. World Population Prospects: The 2015 Revision.
2. Fariduddin Q, Yusuf M, Ahmad I, Ahmad A. Brassinosteroids and their role in response of plants to abiotic stresses. Biol Plantarum. 2014;58:9-17.
3. Munns R. Comparative physiology of salt and water stress. Plant Cell. Environment. 2002;25:239-250.
4. Ahmed S. Effect of soil salinity on the yield and yield components of mungbean. Pakistan. J. Bot. 2009;41:263-268.
5. Ingram J, Bartels D. The molecular basis of dehydration tolerance in plants. Plant Physiol. 1996;47:377-403.
6. Haubrick LL, Assmann SM. Brassinosteroids and plant function: Some clues, more puzzles. Plant Cell Environ. 2006;29:446-457.
7. Deng Z, Zhang X, Tang W, Oses-Prieto JA, Suzuki N, Gendron JM, Chen H, Guan S, Chalkey RJ, Peterman TK, Burlingame AL, Wang ZY. A proteomics study of brassinosteroid response in *Arabidopsis.* Mol Cell Proteomics, 2007; 6(12): 2058-2071.
8. Zeng H, Tang Q and Hua X. Arabidopsis brassinosteroid mutants *det2-1* and *bin2-1* display altered salt tolerance. Journal of Plant Growth Regulator. 2010;29:44-52.
9. Zhou XY, Song L, Xue HW. Brassinosteroids regulate the differential growth of Arabidopsis hypocotyls through auxin signaling components IAA19 and ARF7. Mol Plant. 2013;6:887-904.
10. Xia XJ, Wang YJ, Zhou YH, Tao Y, Mao WH, Shi K, Asami T, Chen Z, Yu JQ. Reactive oxygen species are involved in brassinosteroid-induced stress tolerance in cucumber. Plant Physiol. 2009;150:801–814.
11. Bajguz A. Metabolism of brassinosteroids in plants. Plant Physiol Biochem. 2007;45:95-107.
12. Rao SSR, Vardhini B, Sujatha V, Anuradha S. Brassinosteroids- a new class of phytohormones. Curr. Sci. 2002;82:1239–1245.
13. Bates LS, Waldren RP, Teare LD. Rapid determination of free proline for water stress studies. Plant and Soil. 1973;23: 205-207.
14. Jaworski EG. Nitrate reductase assay in intact plant tissues. Biochem Biophys Res Co. 1971;43:1274-1279.
15. Cochran WC, Cox CM. Experimental design. John Wiley Publishers, New York. 1963;31.
16. Welbaum GE, Tissaoui T, Bradford KJ. Water relations of seed development and germination muskmelon (*Cucmis melo* L.): Sensetivity of germination to water potential and abscisic acid durning development. Plant Physiol. 1990;92:1029-1037.
17. Yao L, Wu Z, Zheng Y, Kaleem I, Li C. Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. Europe J Soil Biol. 2010;46:49-54.
18. Zhao H, Wu L, Chai T, Zhang Y, Tan J, Ma S. The effects of copper, manganese and zinc on plant growth and elemental accumulation in the manganese-hyperaccumulator *Phytolacca americana.* J Plant Physiol. 2012;169:1243–1252.
19. Datta JK, Nag S, Banerjee A, Mondal NK. Impact of salt stress on five varieties of wheat *Triticum aestivum* L. cultivars under laboratory condition. Journal of Applied

Sciences and Environmental Management. 2009;13:93-97.
20. Manaa A, Ahmed HB, Valot B, Bouchet JP, Aschi-Smiti S, Causse M, Faurobert M. Salt and genotype impact on plant physiology and root proteome variations in tomato. Exp Bot. 2011;62:2797-2813.
21. Iyengar ERR, Reddy MP. Photosynthesis in highly salt-tolerant plants. In: Pessaraki M, (Ed). Handbook of Photosynthesis. Marcel Dekker, New York. 1996;897–909.
22. Panda SK, Choudhury S. Changes in nitrate reductase activity and oxidative stress response in the moss *Polytrichum commune* subjected to chromium, copper and zinc phytotoxicity. Braz J Plant Physiol. 2005;17:192-197.
23. Campbell HW. Nitrate reductase structure, function and regulation bridging the gap between biochemistry and physiology. Ann Rev Plant Physiol Plant Mol Biol. 1999;50:277-303.
24. Feng G, Zhang FS, Li XL, Tian CY, Tang C, Rengel Z. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza. 2002;12:185–190.
25. Munns R. Genes and salt tolerance together: Bringing them together. New Phytol. 2005;167:645–663.
26. Wang R, Tischner R, Rodrigo AG, Hoffman M, Xing X, Chen M, Coruzzi G, Crawford NM. Genomic analysis of the nitrate response using a nitrate reductase-null mutant of arabidopsis. Plant Physiol. 2004;136:2512–2522.
27. Yu JQ, Huang LF, Hu WH, Zhou YH, Mao WH, Ye SF, Nogues S. A role of brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. J. Exp. Bot. 2004;55:1135–1143.
28. Mai Y, Lin S, Zeng X, Ran R. Effect of brassinolide on nitrate reductase activity in rice seedlings. Plant Physiol. Commun. 1989;2:50–52.
29. Khripach VA, Zhabinskii VN, Groot AE. Brassinosteroids: A new class of plant hormones. Acad. Press, San Diego; 1999.
30. Khripach V, Zhabinskii V, Groot AD. Twenty years of brassinosteroids: Steroidal plant hormones warrant better crops for the XXI century. Annal Bot. 2000;86:441-447.
31. Ozdemir F, Bor M, Demiral T, Turkan I. Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. Plant Growth Regul. 2004;42:203-211.

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