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Impact of salt stress on morphological and biochemical changes in pre flowering stage of *Vigna mungo* (L.) hepper

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Abstract
One of the most crucial limiting factors for crop growth and productivity is salt stress. The morphological, biochemical alterations of the black gram (*Vigna mungo* L.) in response to NaCl stress were studied. The black gram seeds were exposed to different levels of NaCl, (0, 25, 50, 75, 100, and 125mM). The well-developed plants were used for morphological and biochemical parameter observation. With increasing concentration of NaCl treatment, morphological indices such as seedling growth, photosynthetic pigments were reduced, but osmolyte such proline content was elevated. With increasing stress, the buildup of osmolytes rose considerably. Low osmotic potential at the intracellular level affected by NaCl stress was found to be the cause of deterioration in growth and photosynthetic pigments in this study.

Keywords: black gram, NaCl, osmolytes, photosynthetic pigment

Introduction

Plants being sessile in nature are encountered with a variety of environmental stresses, generally classified into abiotic (cold, drought, flooding, heavy metal, salinity, etc.) and biotic (insects, pathogens, etc.). Soil salinity is the most common abiotic stress, and it is a major problem for agriculture, affecting crop growth, production, and productivity (Rahman *et al*., 2017) [39]. Approximately 930 million hectares of arable land worldwide are salt- affected accounting for more than 6% of the total land region, and this proportion is on rise, primarily due to natural and anthropogenic activities (Hasanuzzaman *et al*., 2018) [21].

Currently, the productivity of the crops in the world is declining because of numerous eco-edaphic factors (Hasanuzzaman *et al.*, 2013) [20]. Salinity is one of the significant environmental stresses. It results in a substantial reduction in the germination, plant vigour, and yield of most crops (Shahbaz and Ashraf, 2013) [46]. The arid and semi-arid areas of the world are the most affected. One-fifth of all arable land is affected by salinity, and more land is becoming vulnerable to this threat (Rasool *et al*., 2013) [41]. On the other hand, the world's population has reached around 7.2 billion and is projected to grow to 9.6 billion by 2050. This phenomenal increase in population and livestock necessitates results an increase in crop production of more than 70% over current levels to meet future demands.

Extreme salinity stress causes major changes in plant systems, including water stress, ion toxicity, nutritional imbalances, oxidative stress, and membrane disintegration (Djanaguiraman and Prarad. 2013) [10]. As a result, salinity stress has a major impact on plant growth and future productivity for the production of economic yield (Hessini *et al*., 2015) [23]. Since the photosynthetic potential is directly linked to stomatal and non-stomatal limitations. The salinity stress leads to oxidative stress, because of the phenomenal production of reactive oxygen species (ROS). The deleterious effects of ROS could be mitigated by simulating several metabolites, including phytohormones and changes at the level of endogenous macromolecules (Razzaghi *et al.*, 2014) [42].

Black gram [*Vigna mungo* (L.) Hepper] belongs to Family Papilionaceae, is one of the most highly prized pulses in tropical countries especially in India. Pulses play an important role to meet up the demand of protein for human and livestock. The cheapest source of protein is the pulses that can be considered as the peasant's meat.

Black gram has a surprising range of health benefits, including the ability to increase stamina, protect cardiovascular health, minimize pain and inflammation, improve immunity, support skin health, prevent diabetes, strengthen bones, strengthen the nervous system, and improve digestion. Black gram seed contains a great balance of all nutrients, including proteins (25-26%), carbohydrates (60%), fat (1.5%), minerals, amino acids, and vitamins. This crop comes up reasonably well in drought-prone areas, where other crops invariably fail to grow. Information is lacking regarding to the relative levels of salt tolerance among the existing black gram cultivars. The goal of this study was to see the impact of salt stress on morphological and physiological indicators.

Materials and Methods

Plant material and experimental design

The seeds of Black gram were collected from Vamban Pulse Research Station, Pudukottai, Tamil Nadu, India. The experimental work was conducted at the Botanical garden and Stress physiology laboratory in the Department of Botany, Annamalai University, Chidambaram, Tamil nadu, India. The healthy and homogenous seeds were surface sterilized with 0.01% $HgCl_2$. The plants were raised in standard plastic pots containing a mixture of red soil, sand and farmyard manure in the ratio of 1:2:1 and arranged in a completely randomized block design (CRBD). The plants were allowed to 15 days after sowing with normal watering.

After 15 DAS the plants were exposed to salt stress with NaCl at 25mM, 50mM, 75mM, 100mM, and 125mM NaCl, one group of plant was kept as control (no treatment). The plant samples were collected on 30 DAS for morphological and biochemical analysis.

Determination of morphological parameters

After 30 days of growth, 5 plants were randomly uprooted from each treatment, washed thoroughly with distilled water for the determination of NaCl induced damage on morphological parameters *viz* root length, shoot length, fresh weight of root, fresh weight of shoot, dry weight of root, dry weight of shot, number of branches per plant, no of leaves per plant, number of root nodules per plant.

The length between the shoot tip and point of the root-shoot transition region was taken as shoot length (SL). The root length was measured from the root tip and point of root-shoot transition (RL). The root length and shoot length was expressed in centimeters per plant. The root and shoot were separated and oven dried at 80°C for 72 hrs. The dry weight of root and shoot were measured to determine root and shoot dry biomass. Data were also recorded for the number of branches per plant, root nodules number per plant, total number of leaves was counted for each treatment.

Plant pigment analysis

Chlorophyll contents were measured according to Arnon's method (1949) [5]. Fresh leaves were extracted with 80% acetone (V/V), chlorophyll contents were estimated by using a spectrophotometer, expressed in terms of microgram per gram of fresh weight (µg /g FW) The carotenoid content was extracted according to (Kirk and Allen, 1965) [30] and absorbance was measured at 480nm by using a spectrophotometer and expressed in (µg /g FW)

Compatible solutes

Proline

Free proline was estimated by according to method of (Bates *et al*., 1973) [7]. The plant material was homogenized in a 3% aqueous sulfosalicylic acid; the homogenate was centrifuged at 1400 rpm. The supernatant was used to evaluate proline concentration. The reaction combination consists of acid ninhydrin, glacial acetic acid which was boiled at 100°C for 1 hr. After cessation of reaction in an ice bath, the reaction mixture was extracted with toluene and absorbance was read at 520nm using L-proline as a standard.

Protein estimation

The proteins were extracted and estimated by (Lowery *et al*., 1951) [32]. A 0.5g of plant material was macerated in a mortar and pestle with 0.1N trichloroacetic acid followed by centrifugation at 10,000g for 15 minutes. The supernatant was thrown away, and to the pellets, 0.1N NaOH was added followed by centrifugation for 15 minutes. The supernatant was made up to 10ml with 0.1N NaOH. The extract was used for protein valuation using Bovine Serum Albumin (BSA).The absorbance was read at 660nm.

Statistical analysis

The data were analyzed statistically using SPSS software (22.0) followed by one way ANOVA. The obtained data represented are mean values of five replicates (n=5) and (±) standard error (SE). The 0.05% was chosen as significance by Duncan's Multiple Range Test (DMRT).

Results

Effect of salt stress on growth parameters

Growth of black gram genotypes was measured in terms of plant shoot length, root length, plant fresh weight, and dry weight, no of leaves, branches and nodules. Enormous variability in the growth of black gram genotype was observed under various levels of salt treatments. The present investigation, salt stress reduced the growth parameters of *Vigna.mungo*. Our results find out, the reduction of 50% and 56.25% root length and shoot length of *Vigna.mungo* respectively was observed at T5 (125mM NaCl) in comparison to control. Reduction in number of branches and Leaves was observed as 50% and 58.82% at T_5 (125mM NaCl) in comparison to control. Number of root nodules also reduced by 75% as compared to control (Table 1)

Biomass reduction

The reduction in fresh weight of root and shoot observed was 61.54% and 46.69% respectively, at T5 over control in Vigna.mungo plant under NaCl stress. A reduction of 60.53% and 50.31% as compared to control was also observed in dry weight of root and shoot respectively. Salinity treatment showed decline in the biomass production in black gram (Table 1).

Table 1: Effect of NaCl on morphological parameters such as Root length (cm plant^{-1}), Shoot length (cm plant^{-1}), Fresh Weight (gram plant^{-1}), Dry Weight (gram plant^{-1}), No. of leaves, No. of branches, and No. of root nodules plant^{1} after 30DAS.

Treatments (NaCl)	Root length	Shoot length	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	No. of branches	No. of leaves	No. of root nodules
Control(T_0)	20±1.141	16±3.53	9.53±0.01	1.61±0.01	1.3±0.07	0.38±0.01	6±0.70	17±1.41	20±4.74
T_1 (25mM)	17±2.12	14±2.12	9.46±0.03	1.49±0.04	1.07±0.01	0.3±0.07	5±0.35	15±2.82	15±6.32
T_2(50mM)	16±2.82	12±1.41	8.9±0.47	1.39±0.06	1±0.21	0.27±0.02	5±0.42	15±2.12	12±6.32
T_3(75mM)	15±3.53	10±0.70	7.48±0.06	1.2±0.31	0.77±0.02	0.23±0.02	4±0.56	14±3.53	8±3.16
T_4(100mM)	14±0.70	9±2.12	6.72±0.07	1.17±0.07	0.72±0.04	0.2±0.03	3±0.21	9±2.12	8±4.74
T_5(125mM)	10±1.41	7±0.70	5.08±0.01	0.8±0.31	0.5±1.41	0.15±0.07	3±0.49	7±1.41	5±3.16
F value	7.819*	5.980*	0.024	1.193	2.051	3.048*	3.999*	6.382*	4.236*

(*) values significant at p≤ 0.05 and (±) S.E of n=5

Effect of salt stress on photosynthetic pigment

Under varying NaCl concentration, pigment composition-chlorophyll a, b and carotenoid content of *Vigna mungo* showed significant reduction in all the treatments as compared to control. The results showed that the photosynthetic pigment of was decreased by increasing salt stress. The NaCl lead reduction in chlorophyll a, chlorophyll b and carotenoid constituents observed was 71.86%, 72.04% and 67.73% respectively at T5 (125mM) compared to control plant (Table 2)

Proline

Proline accumulation in the black gram genotype increased significantly with increasing levels of NaCl treatments. Maximum increase was observed at 125 mM NaCl treatment (Table 2).

The results of the present research show 112.68% increase in the concentration of proline at T5 (125mM) NaCl Concentration compared to control.

Total protein

Total protein content of shoot reduced at all the varying NaCl concentrations (T_1-T_5). A reduction of 59.43% was observed at T_5 in the total protein of shoot as compared to control plant (Table 2).

Table 2: Impact of NaCl on Biochemical parameters (chlorophyll a, chlorophyll b, carotenoid, proline and Total protein content) on different doses of NaCl after 30 Days of Sowing (DAS).

Variety black gram (ADT-5)	Treatments NaCl (mM)	Chl a (µ g^{-1} FW)	Chl b (µ g^{-1} FW)	Carotenoid (µ g^{-1} FW)	Proline (µ g^{-1} FW)	Total Protein (µ g^{-1} FW)
	T_0 (control)	821 ± 2.12^a	658 ± 2.12^a	471 ±0.002	2941±0.001^a	3638 ± 1.414^b
	T_1 (25mM NaCl)	720 ± 0..70^b	646 ± 3.53^b	356 ±0.003^b	3472±0.002^b	2942 ± 0.707^a
	T_2(50mM NaCl)	582 ± 3.53^c	314 ± 0.70^c	468 ±0.001^a	4416 ± 0.002^c	2516 ± 3.536^c
	T_3(75mM NaCl)	491 ± 4.24^d	271 ± 1.41^d	259 ±0.004^c	5115 ± 0.002^d	2132 ± 2.121^d
	T_4(100mM NaCl)	315± 2.12^e	221± 0.70^e	159 ±0.001^d	5716 ± 0.001^e	1842 ± 2.828^e
	T_5(125mM NaCl)	231 ± 2.82^f	184 ± 3.53^f	152 ±0.004^d	6255 ± 0.003^f	1476 ± 0.707^f

Data are mean of five replicates SE at 0.05 levels (Tukey's test). Different letters above bars indicate significant differences between control and salt treatment in presence of different doses of salt stress. Analysis of variance was done using type statistica, ANOVA.

Discussion

Growth parameters

Soil salinity is one among the abiotic factors limiting the yield and productivity of crops all over the world. Intensive agriculture practices, poor water management, long periods of hot and dry season and high levels of evapotranspiration lead to salinization of agricultural land. The values for all growth biomarkers (root and shoot lengths, fresh and dry masses, number of leaves and branches, number of root nodules of plant) decreased significantly in the plants exposed to salt stress. Moreover the maximum reduction was observed at 125mM NaCl. Our results showing maximum reduction in growth parameters occurred at 125mM NaCl (T_5) concentration compared to control. This reduction in root and shoot length are in agreement with Kandil *et al*. (2012) [27], Velmani *et al*., (2015) [57]. Salinity reduced root length and the number of lateral roots in mung bean according to Haleem and Mohmmad (2007) [17].

According to Al-Mutawa (2003) [2] higher salinity reduces the length of radicle in chickpea. The reduction in root and shoot length varies and depends on the genotype and the time period at which plant is exposed to stress (Misra and Dwivedi, 2004) [34]. A decrease in root and shoot growth under saline environment caused reduced total plant growth (Sehrawat *et al*., 2013) [44]. The number of branches per plant was higher in control condition but decreased with increasing salt stress. These findings are similar to those of Karim *et al* (2001) [28]. Salingpa *et al*., (2018) [43] observed that number of leaves decreased considerably by salinity in green gram plants. The reduction in growth is a result of physiological responses including modification of water status, mineral nutrition, ion balance and photosynthetic effectiveness (Zahra *et al*., 2020) [62]. This reduced growth under salinity stress is either due to osmotic or ionic effects; inhibition of cell division and cell elongation process associated with the growth of the seedling and decrease in plastic extensibility of the growing cell walls (Veeranagamallaiah *et al*., 2007) [56]. Salt stress caused low intra-cellular water potential and water scarcity around the root zone due to which roots failed to absorb sufficient water and nutrients for adequate plant growth (Voss *et al*., 2013) [59]. Growth inhibition under salt stress may be due to the diversion of energy from growth to maintenance (Greenway and Gibbs, 2003) [15].

Biomass reduction

From 50mM NaCl concentration on words, the effect of salt stress on fresh weight and dry weight was apparent. At a concentration of 125mM NaCl, the fresh weight and dry weight assigned to root and shoot dropped considerably. Salinity diminished both fresh weight and dry weight according to Parida and Das (2005) [38]. Our findings are consistent with those of Mohmed and Kramany (2005) [35] in the Mung bean. Reduced water intake by seedlings in saline circumstances to increase the osmotic potential may explain the loss in fresh weight (Aloui *et al*., 2014) [3]. Hasan *et al*., 2017 [19] also reported such reduction in black gram and mung bean. Farooq *et al*., 2020[11] also observed similar results in Cowpea. Root fresh weight varies more in Mung bean genotypes, according to Kandil *et al*., (2012) [27], due to differences in salt concentration. The decrease in the rate of photosynthesis explains the harmful response of biomass to increasing salinity stress. The suppression of conserved food hydrolysis and translocation to growing shoots may be the cause of the decline in dry matter production at high saline levels (Xu *et al*., 2008) [61]. According to Velmani *et al*., (2015) [57], the action of NaCl produces a reduction in root dry weight as salt concentration increases when compared to control. This is supported by Shakeel and Mansoor's (2012) [47] findings, as well as El Kafafi., *et al* (2015) [18] who found that salinity retarded root dry weight considerably, with the roots being more affected than the shoots.

Photosynthetic pigments

Chlorophyll loss in salt-stressed plants has long been reflected as an indicator of oxidative stress (Smirnoff, 1996) [50]. In Our results pigment content in black gram was greatly affected by NaCl at T_5 (125mM) stage. Reduction in chlorophyll content at 100mM NaCl was also observed in mung bean (Baghel *et al*., 2012) [6].The results are consistent with Turan *et al*. (2007) [55] *P. vulgaris* L. Taffouo., *et al* (2010) [53] *Vigna subterranean*. Similar reduction in chl a and chl b contents were also observed by Rangaraj *et al*.,

2021 [40] in different varieties of green gram and black gram. Chlorophyll a and chlorophyll b content decreased under stress conditions in *Vigna radiata* (Suleiman *et al*., 2021) [52]. Reduction in chlorophyll content occurs as a result of water imbalance under salt stress (Chandrasekaran *et al*., 2019) [8] Na^+concentration increases in leaves under salt stress, which increases oxidative stress. The increased oxidative stress leads to chlorophyll loss by the enzyme chlorophyllase and consequently overall decrease of chlorophyll content Gulmezoglu and Daghan (2017) [16]. In our results significant reduction in carotenoid concentration occurred at 125mM (T_5) NaCl concentration. Gadallah (1999) [12] on P. vulgaris L. and Singh *et al*. (2008) [49] on maize and wheat genotypes reported similar findings on reduction in carotenoid concentration at 125mM NaCl concentration. Carotenoids are recognized to serve as photosynthetic light gatherers, triplet chlorophyll quenchers, and O2 quenchers. Because carotenoids are antioxidants, they can protect plants from the effects of reactive oxygen species (Verma and Mishra, 2005) [58]. Furthermore, salt stress reduces membrane fluidity and selectivity by causing chlorophyll degradation and membrane lipid peroxidation.

Protein and proline

Reduction in protein content was observed above 50mM salt stress. Chen *et al*. (2007) [9] observed that exposing *Vigna unguiculata* (L.) plants to a salt treatment using 75 mM sodium chloride at the age of 14 days lowered the plant's soluble protein level. Our findings are in agreement with the Jaleel *et al*. (2008) [25], who studied *Catharnathus roseus* (L.), and Khosravinejad *et al*. (2009) [29], who studied *Hordeum vulgare* L., Plant seedlings treated with sodium chloride, had decreased protein content. Salinity adversely affected the protein metabolism. Protein degradation under saline environment have been reported due to decrease in protein synthesis, accelerated proteolysis, decrease in the availability of amino acids and denaturation of enzymes involved in protein synthesis (Weimberg *et al*, 1982) [60]. It was observed that the protein content of black gram decreased with increasing concentration of NaCl (Kumar *et al*, 1996) [31].) Black gram, a salt sensitive species showed a reduced protein content under salt stress (Altuntas *et al*., 2020) [4]. Plant proteins that accumulate in saline conditions may provide a sort of nitrogen storage that can be used later (Nelson 1994) [37]. They also play an osmotic-adjustment role in plants. Disruption of protein synthesis by elevated concentration of Na^+ appears to be an important cause of damage by NaCl. Salt stress also increases the production of ROS and causes damage to Proteins. Autophagy might be responsible for degrading oxidized proteins under salt stress. The accumulation of compatible solutes may help to maintain the relatively high water content obligatory for plant growth and cellular functions. Stress leads to an increase in proline content in black gram. Similar results were also observed by Altuntas *et al*., 2020[4] in maize crop. In response to salinity, plants make new amino acids that help them to grow and develop under saline conditions (Goudarzi and Pakniyat, 2009) [14]. Among those amino acids, proline is known to build up widely in higher plants and accumulates in large quantities in response to salinity to protect the cell and minimize the salt induced damage (Shafi *et al*., 2011; Iqbal *et al*., 2019) [45, 24]. Accumulation of proline under salt stress have also been reported in *D.superbus* (Ma *et al*., 2017) [33], *Brassica. Juncea* (Ahmad *et al*., 2015) [1]. Proline being antioxidant and helps in ROS quenching, thus it protects the cell from oxidative damage (Jogaiah *et al*., 2013) [26].

It has been reported that under salt stress Proline act as a source of energy i.e. C and N.

Conclusion

In the present experiment it was observed that salinity decreases morphological and biochemical parameters in *Vigna mungo* at the 30th day due to a decrease in osmotic potential and an increase in the toxicity of ions. However physiological system regenerates the osmotic potential by increasing the synthesis of proline. The concentration of proline increases even at higher doses of NaCl. It regulates osmosis and cytoplasmic viscosity, acts as an osmoprotectant, stabilizes proteins and macro molecules, and thus acts as a major source of energy.

References

1. Ahmad P, Hashem A, Abd-Allah EF, Alqarawi AA, John R, Egamberdieva D *et al*. Role of Trichoderma harzianum in mitigating NaCl stress in Indian mustard (*Brassica juncea L*) through antioxidative defense system. Frontiers in plant science,2015:6:868.
2. Al-Mutawa MM. Effect of salinity on germination and seedling growth of chickpea (*Cicer arietinum* L.) genotypes. International Journal of Agriculture and Biology,2003:5(3):226-229.
3. Aloui H, Souguir M, Latique S, Hannachi C. Germination and growth in control and primed seeds of pepper as affected by salt stress. Cercetari agronomice în Moldova,2014:47(3):83-95.
4. Altuntaş C, Demiralay M, Muslu AS, Terzi R. Proline-stimulated signaling primarily targets the chlorophyll degradation pathway and photosynthesis associated processes to cope with short-term water deficit in maize. Photosynthesis research,2020:144(1):35-48.
5. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology,1949:24(1):1.
6. Baghel P, Hemantranjan A, Singh R. Physiological performance of mungbean (*Vigna radiata* L.) under salinity conditions. Journal of Biotechnology and Crop Science,2012:1(1):47-53
7. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and soil,1973:39(1):205-207.
8. Chandrasekaran M, Chanratana M, Kim K, Seshadri S, Sa T. Impact of arbuscular mycorrhizal fungi on photosynthesis, water status, and gas exchange of plants under salt stress-a meta-analysis. Frontiers in plant science,2019:10:457.
9. Chen C, Tao C, Peng H, Ding Y. Genetic analysis of salt stress responses in asparagus bean (*Vigna unguiculata* (L.) ssp. sesquipedalis Verdc.). Journal of Heredity,2007:98(7):655-665.
10. Djanaguiraman M, Prasad PV. Effects of salinity on ion transport, water relations and oxidative damage. In Ecophysiology and responses of plants under salt stress. Springer, New York, NY, 2013, 89-11.
11. Farooq M, Rehman A, Al-Alawi AK, Al-Busaidi WM, Lee DJ. Integrated use of seed priming and biochar improves salt tolerance in cowpea. Scientia Horticulturae,2020:272:109507.

12. Gadallah MAA. Effects of proline and glycinebetaine on *Vicia faba* responses to salt stress. Biologia plantarum,1999:42(2):249-257.
13. Gama PBS, Inanaga S, Tanaka K, Nakazawa R. Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. African Journal of biotechnology, 2007, 6(2).
14. Goudarzi M, Pakniyat H. Salinity causes increase in proline and protein contents and peroxidase activity in wheat cultivars. Journal of Applied Sciences,2009:9(2):348-353.
15. Greenway H, Gibbs J. Mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. Functional plant biology,2003:30(10):999-1036.
16. Gulmezoglu N, Daghan H. The interactive effects of phosphorus and salt on growth, water potential and phosphorus uptake in green beans. Appl. Ecol. Environ. Res,2017:15(3):1831-1842.
17. Haleem M.A. Mohammed. (2007). Physiological aspects of mungbean plant (Vigna radiata L. Wilczek) in response to salt stress and gibberellic acid treatment. Res J Agr Biol Sci, 3, 200-213.
18. Hasan Elkafafi S. Characterization and evaluation of some mung bean genotypes for salt tolerance. World Applied Sciences Journal,2015:33(3):360-370.
19. Hasan M, Cheng Y, Kanwar MK, Chu XY, Ahammed GJ, Qi ZY. Responses of plant proteins to heavy metal stress-a review. Frontiers in plant science,2017:8:1492.
20. Hasanuzzaman M, Nahar K, Fujita M, Ahmad P, Chandna R, Prasad MNV *et al*. Enhancing plant productivity under salt stress: relevance of poly-omics. Salt Stress in Plants, 2013, 113-156.
21. Hasanuzzaman M, Oku H, Nahar K, Bhuyan MB, Al Mahmud J, Baluska, F *et al*. Nitric oxide-induced salt stress tolerance in plants: ROS metabolism, signaling, and molecular interactions. Plant Biotechnology Reports,2018:12(2):77-92.
22. Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. Plant cellular and molecular responses to high salinity. Annual review of plant biology,2000:51(1):463-499.
23. Hessini K, Ferchichi S, Ben Youssef S, Werner KH, Cruz C, Gandour M. How does salinity duration affect growth and productivity of cultivated barley? Agronomy Journal,2015:107(1):174-180.
24. Iqbal S, Basra SM, Afzal I, Wahid A, Saddiq MS, Hafeez MB *et al*. Yield potential and salt tolerance of quinoa on salt-degraded soils of Pakistan. Journal of Agronomy and Crop Science,2019:205(1):13-21.
25. Jaleel CA, Gopi R, Kishorekumar A, Manivannan P, Sankar B, Panneerselvam R. Interactive effects of triadimefon and salt stress on antioxidative status and ajmalicine accumulation in *Catharanthus roseus*. Acta Physiologiae Plantarum,2008:30(3):287-292.
26. Jogaiah S, Govind SR, Tran LSP. Systems biology-based approaches toward understanding drought tolerance in food crops. Critical reviews in biotechnology,2013:33(1):23-39.
27. Kandil AA, Arafa AA, Sharief AE, Ramadan AN. Genotypic differences between two mungbean varieties in response to salt stress at seedling stage. International Journal of Agriculture Sciences,2012:4(7):278.
28. Karim MA, Raptan PK, Hamid A, Khaliq QA, Solaiman ARM, Ahmed JU. Salinity tolerance of blackgram and mungbean: II. Mineral ions accumulation in different plant parts. Korean Journal of Crop Science,2001:46(5):387-394.
29. Khosravinejad F, Heydari R, Farboodnia T. Effect of salinity on organic solutes contents in barley. Pakistan journal of biological sciences: PJBS,2009:12(2):158-162.
30. Kirk JTO, Allen RL. Dependence of chloroplast pigment synthesis on protein synthesis: effect of actidione. Biochemical and Biophysical Research Communications,1965:21(6):523-530.
31. Kumar SA, Muthukumarasamy M, Panneerselvam R. Nitrogen metabolism in black gram under NaCl stress. J. indian bot. Soc,1996:75:69-71.
32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of biological chemistry,1951:193:265-275.
33. Ma X, Zheng J, Zhang X, Hu Q, Qian R. Salicylic acid alleviates the adverse effects of salt stress on *Dianthus superbus* (Caryophyllaceae) by activating photosynthesis, protecting morphological structure, and enhancing the antioxidant system. Frontiers in plant science,2017:8:600.
34. Misra N, Dwivedi UN. Genotypic difference in salinity tolerance of green gram cultivars. Plant Science,2004:166(5):1135-1142.
35. Mohamed MH, El Kramany MF. Salinity tolerance of some mungbean varieties. J Appl Sci Res,2005:1:78-84.
36. Mohammed AHMA. Physiological aspects of mungbean plant (*Vigna radiata* L. Wilczek) in response to salt stress and gibberellic acid treatment. Res J Agr Biol Sci,2007:3:200-213.
37. Nelson N. A photometric adaptation of the Somogyi method for the determination of glucose. Journal of biological chemistry,1944:153(2):375-380.
38. Parida AK, Das AB. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and environmental safety,2005:60(3):324-349.
39. Rahman A, Nahar K, Al Mahmud J, Hasanuzzaman M, Hossain MS, Fujita M. Salt stress tolerance in rice: Emerging role of exogenous phytoprotectants. Advances in international rice research, 2017, 139-174.
40. Rangaraj M, Muthswamy S, Kumarasamy P. Journal of Crop and Applied Sciences. Journal of Crop and Applied Sciences,2021:1(2):71-75.
41. Rasool S, Ahmad A, Siddiqi TO, Ahmad P. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. Acta physiologiae plantarum,2013:35(4):1039-1050.
42. Razzaghi F, Jacobsen SE, Jensen CR, Andersen MN. Ionic and photosynthetic homeostasis in quinoa challenged by salinity and drought–mechanisms of tolerance. Functional plant biology,2014:42(2):136-148.
43. Salingpa TW, Lal EP, Shukla PK. Effect of foliar application of salicylic acid on growth, yield, physiological and biochemical characteristics of mung bean (*Vigna radiat*a L.) under salt stress. Journal of Pharmacognosy and Phytochemistry,2018:7(6):857-1860.

44. Sehrawat N, Jaiwal PK, Yadav M, Bhat KV, Sairam RK. Salinity stress restraining mungbean (*Vigna radiata* (L.) Wilczek) production: gateway for genetic improvement. International Journal of Agriculture and Crop Sciences,2013:6(9):505.
45. Shafi Mohammad, Bakht Jehan, Khan MJ, Khan MA, Raziuddin M. Role of abscisic acid and proline in salinity tolerance of wheat genotypes. Pakistan Journal of Botany,2011:43(2):1111-1118.
46. Shahbaz M, Ashraf M. Improving salinity tolerance in cereals. Critical reviews in plant sciences,2013:32(4):237-249.
47. Shakeel SADAF, Mansoor S. Salicylic acid prevents the damaging action of salt in mung bean [(*Vigna radiata* L.) Wilczek] seedlings. Pak. J. Bot,2012:44(2):559-562.
48. Sheokand S, Dhandi S, Swaraj K. Studies on nodule functioning and hydrogen peroxide scavenging enzymes under salt stress in chickpea nodules. Plant physiology and biochemistry (Paris),1995:33(5):561-566.
49. Singh AK, Singh RA, Kumar S. Influence of salinity on seedling growth and metabolism in maize genoytypes. Indian J Plant Physiol,2008:13:95-99.
50. Smirnoff N. Botanical briefing: the function and metabolism of ascorbic acid in plants. Annals of botany,1996:78(6):661-669.
51. Su GX, Bai X. Contribution of putrescine degradation to proline accumulation in soybean leaves under salinity. Biologia plantarum,2008:52(4):796-799.
52. Suleiman MAQ, Al-Hamdani KM. Effect of growth factors on mineral, proline and protein accumulation in mungbean (V*igna radiata* L.) Under salt stress. plant cell biotechnology and molecular biology, 2021, 98-109.
53. Taffouo VD, Wamba OF, Youmbi E, Nono GV, Akoa A. Growth, yield, water status and ionic distribution response of three bambara groundnut (*Vigna subterranea* (L.) Verdc.) land races grown under saline conditions. International Journal of Botany,2010:6(1):53-58.
54. Tawfik KM. Evaluating the use of rhizobacterin on cowpea plants grown under salt stress. Research Journal of Agriculture and Biological Sciences,2008:4(1):26-33.
55. Turan MA, Turkmen N, Taban N. Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of lentil plants. Journal of Agronomy, 2007.
56. Veeranagamallaiah G, Chandraobulreddy P, Jyothsnakumari G, Sudhakar C. Glutamine synthetase expression and pyrroline-5-carboxylate reductase activity influence proline accumulation in two cultivars of foxtail millet (*Setaria italica* L.) with differential salt sensitivity. Environmental and Experimental Botany,2007:60(2):239-244.
57. Velmani S, Murugesan S, Arulbalachandran D. Growth and biochemical characteristics of black gram (*Vigna mungo* (L.) Hepper) under NaCl salinity. International Journal of Current Trends in Research,2015:4:13-17.
58. Verma S, Mishra SN. Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system. Journal of plant physiology,2005:162(6):669-677.
59. Voss I, Sunil B, Scheibe R, Raghavendra AS. Emerging concept for the role of photorespiration as an important part of abiotic stress response. Plant biology,2013:15(4):713-722.
60. Weimberg, Ralph, H. R. Lerner, and A. Poljakoff-Mayber. "A relationship between potassium and proline accumulation in salt-stressed Sorghum bicolor." Physiologia plantarum 55, no. 1 (1982): 5-10.
61. Xu X, Xu H, Wang Y, Wang X, Qiu Y, Xu B. The effect of salt stress on the chlorophyll level of the main sand-binding plants in the shelterbelt along the Tarim Desert Highway. Chinese Science Bulletin,2008:53(2):109-111.
62. Zahra N, Raza ZA, Mahmood S. Effect of salinity stress on various growth and physiological attributes of two contrasting maize genotypes. Brazilian Archives of Biology and Technology, 2020, 63.