International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 24-08-2021, Accepted: 08-09-2021, Published: 24-09-2021
Volume 6, Issue 5, 2021, Page No. 830-835

Salinity induced effects on the morphological and biochemical parameters of *Vigna radiata* L. Co (Gg) 7 variety

Zahoor Ahmad Kutay, A Venkatesan*
Department of Botany, Annamalai University, Tamil Nadu, India

Abstract
Salinity is a major abiotic stress which limits crop production, especially in arid and semi- arid regions of the world. The objective of this research was to examine the effects of increasing NaCl concentration on the morphological and biochemical parameters of *Vigna radiata* L. NaCl caused decrease in root and shoot length, fresh and dry weight (biomass), number of branches, leaves and root nodules. NaCl significantly reduced chlorophyll a and chlorophyll b content at higher concentrations (75 -100mM) and an enhanced reduction was seen at 125mM NaCl. However an increase in proline content of root and shoot was observed at 125mM NaCl when compared to control.

Keywords: biomass, crop production, proline, salinity, *Vigna radiata*

Introduction

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. Green gram (*Vigna radiata* L. Wilczek) is an important leguminous crop mainly cultivated in tropical, subtropical and temperate zones of Asia including India, Bangladesh, Pakistan, Myanmar, Indonesia, Philippines, Sri Lanka, Nepal, China, Korea and Japan (Rahim *et al*., 2010) [38]. India is the world's largest producer as well as consumer of green gram. Indian farmers have covered 130.04 lakh ha as on 6th September 2019. Around 30.48 lakh ha land was covered under green gram and a production of 2.37 million tons was observed (DES, Green gram outlook, September 2019) [15]. The daily requirement of pulses per adult is about 80g but in India only 47g is available per adult (Nutrition Nectar for India Pulses and Grains Association, October 2020) [35]. But this is again diminishing as the arable land is getting reduced by increasing salinity. Salinity stress is a serious problem in arid and semi-arid tropics and in the Indo-Gangetic plains in irrigated areas. It is recognized as major constraint in the production of this crop where 50 mM NaCl can cause yield losses ≥70% (Hasanuzzaman *et al*., 2013) [20]. The arable land is continuously transforming into saline (1-3% per year) either due to natural salinity or due to human interference which accounts nearly 20% of the irrigated agricultural land (Velmurugan *et al*., 2020) [51]. Soil salinity has become a major agricultural problem of the modern world (Noreen *et al*., 2021) [34]. Liang *et al*., (2018) [26] reported that if the salinization to agricultural lands continues at the current rate then the yield of important crops may fall around 50%. Plants in saline soils not only suffer from high sodium level, but also affected by some degree of hypoxia by the action of Na^+ ions because when they occupy the cation exchange complex of clay particles, makes the soil more compact, thereby hampering soil aeration. Sodium ions in saline soils are toxic to plants because of their adverse effects on potassium ion nutrition, photosynthesis, metabolism and result in ionic imbalance (Lambridge and Godwin, 2007; Isayenkov and Maathuis, 2019) [25, 22]. Salt stress inflicts considerable adverse effects on morphological and biochemical parameters of green gram (Zhang and Dai, 2019) [57]. However, the intensity of adverse and injurious effects of salinity stress depends upon the nature of plant species, concentration and duration of salt stress, plant developmental stage and mode of salt application to the crop. Salt stress affects plant growth in a bi-phasic manner causing osmotic stress followed by specific ion toxicity (Hanin *et al.* 2016) [18]. The present study was conducted to evaluate the effects of NaCl on the morphological and biochemical parameters of green gram (*Vigna radiata* L.) CO (Gg) 7 variety.

Materials and Methods

Healthy seeds of *Vigna radiata*, CO (Gg) 7 variety, were collected from the TNAU, Coimbatore, Tamil Nadu, India, were used in this study. The seeds were surface sterilized using 0.001% $HgCl_2$ for 3 minutes and were then thoroughly rinsed three times with distilled water followed by overnight soaking. These seeds were then sown in the polythene bags filled with soil in the ratio of 1:2:1 (Sand: Red soil: Farmyard manure) in the garden of Botany department, Annamalai University, Tamil Nadu, India. In order to screen the plants for salt tolerance, varying concentration of NaCl (25mM, 50mM, 75mM, 100mM and 125mM) was applied after 15 days of sowing in the soil. Without NaCl treatment was taken as control. The whole culture treatment was arranged in a Completely Randomized Block (CRB) design. Plants were taken into six groups (T_0-T_5) with five replicates (n=5) each group. Analysis was done after 30 days of sowing and the experimental work was carried out in the stress physiology laboratory of the above said department.

Morphological Attributes

Determination of root length and shoot length

The green gram plants were uprooted, washed with tap water and taken to the lab immediately to determine the mor phological traits. The root length was measured below the point of root-shoot transition to the farthest point of root tip. The length between shoot tip and point of the root shoot transition region was taken as shoot length. The values were expressed in cm plant^{-1}.

Determination of number of branches and leaves
Branches and leaves were counted manually and recorded numerically.

Determination of fresh weight and dry weight
The plant roots and shoots were washed with the tap water and blotted gently with tissue paper to remove moisture. The root and shoot fresh weights were taken by using an electronic balance (Model – DS-852J Series) and subsequently the plants were dried at 70^{0}C in a hot air oven for 48 hours. After drying, their weight was measured and the material was kept in same oven until the constant dry weight obtained. The values of both fresh and dry weight were recorded and expressed as g plant^{-1}.

Determination of chlorophyll and carotenoid contents
A fresh leaf tissues of 500mg were ground in a pre-chilled mortar by adding 10ml of 80% acetone(v:v). After complete extraction, the mixture was centrifuged at 800 rpm for 15 minutes. Further, the extraction was repeated again by discarding the supernatant. Finally, the supernatant collected was made up to a final volume of 10ml with 80% acetone. The absorbance was read at 645, 663 and 480nm using spectrophotometer (Systronic India Limited, Gujarat, India, UV-VIS Model-118). The chlorophyll and carotenoid contents obtained were calculated followed by coefficients of Arnon (1949) [3] and the values are expressed in mg g^{-1} Fr. Wt.

Determination of proline content
For determination of proline concentration the samples were extracted and quantified in systronic India Limited, Gujarat, India, 'UV–VIS, 118- Model spectrophotometer at 520 nm', as described by Bates *et al.*, 1973 [6].

Protein estimation
The proteins were extracted and estimated by (Lowery *et al.*, 1951) [27] method. 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.

Stastical analysis
The data were analyzed statistically using SPSS software (version 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 and Discussion
In the current study sodium chloride stress caused a drastic reduction of plant growth attributes like root length, shoot length, and biomass production. Saline soils and saline irrigations constitute a serious production problem for vegetable crops as these are known to suppress plant growth (Turan *et al*., 2007) [48]. The present study demonstrates that salinity, particularly when applied at high levels (75 and 100 mM NaCl), adversely affected the growth of green gram plants.

Growth attributes
NaCl stress in varying concentration caused a significant reduction in growth related characters like root length and shoot length, fresh weight and dry weight, number of branches and Leaves in green gram plants. A reduction of 60.0% and 44.44% of root length and shoot length respectively was observed at T_5 (125mM NaCl) in comparison to control. Reduction in number of branches and Leaves was also observed as 50% in comparison to control. Number of root nodules also reduced by 77.27% as compared to control (Table 1). Salinity stress causes a significant reduction in mungbean yield (Saha *et al*., 2010) [42] through decline in seed germination, root and shoot lengths and varies with different genotypes (Misra and Dwivedi, 2004) [30]. 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) [53]. Seedling growth shoot height and root length were the most sensitive to salt stress (Saddiq *et al*., 2019) [41]. A decrease in root and shoot growth under saline environment caused reduced total plant growth (Sehrawat *et al*., 2013) [44]. Growth inhibition under salt stress may be due to the diversion of energy from growth to maintenance (Greenway and Gibbs, 2003) [16]. Significant reduction was observed in plant height and root length with increasing salt stress in different crops (EL Sabagh *et al*., 2015) [10]. Similar results were also recorded by Qados (2011) [37] and Velmani *et al*., 2015 [50] in *vigna* species. Hasan *et al*., 2017 [19] also reported such reduction in black gram and mungbean. Farooq *et al.*, 2020 [12] also observed similar results in Cowpea.

Reduction in number of branches in mungbean was recorded 41.21% and 57.50% under 90 and 120 mM NaCl, respectively by Hasan *et al*., 2017 [19]. The number of branches plant^{-1} was higher in control condition that decreased with imposing salt stress reported by Mohamed and Kramany (2005) [32]. Salingpa *et al*., 2018 [43] observed that number of leaves was 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) [55].

Table 1: Impact of varrying NaCl concentration on morphological parameters of *Vigna radiata* L. Values shown are mean ± S.E for five replicate experiments.

Treatments	Root length (cm plant^{-1})	Shoot length (cm plant^{-1})	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	No. of branches plant^{-1}	No. of Leaves plant^{-1}	No. of root nodules plant^{-1}
Control	15±1.73	18±1.73	1.39±0.011	0.41±0.017	9.71±0.017	1.71±0.017	6±1.732	18±1.732	22±1.154
T_1 (25 mM)	14±1.73	17±1.73	1.23±0.011	0.36±0.017	9.56±0.011	1.58±0.011	6±1.154	16±1.154	18±1.154

T_2 (50 mM)	12±1.73	15±1.73	1.08±0.01	0.29±0.011	9.16±0.017	1.41±0.028	5±1.732	16±0.577	16±1.732
T_3 (75 mM)	9±1.73	14±1.73	0.86±0.017	0.25±0.023	8.53±0.017	1.33±0.011	4±1.154	14±1.154	10±0.577
T_4 (100mm)	7±1.73	12±1.73	0.75±0.02	0.21±0.028	7.01±0.023	1.22±0.028	3±0.577	12±1.732	8±1.154
T_5 (125 mM)	6±1.73	10±1.73	0.61±0.02	0.17±0.017	6.63±0.011	0.95±0.017	3±1.154	9±0.577	5±0.577
F(Value)	3.982*	6.164*	0.0809	1.0573	0.0023	0.1046	9.836*	1.748	0.5352

(*) Values Significant at p≤ 0.05 and (±) SE.

Biomass reduction

The reduction in fresh weight of root and shoot observed was 56.42% and 31.72% respectively, at T_5 over control in green gram plant under NaCl stress. A reduction of 58.54% and 44.44% as compared to control was also observed in dry weight of root and shoot respectively. Salinity treatment showed decline in the biomass production in green gram (Table 1). Our results were also according to Farooq *et al*., 2020 [12] who observed that salt stress induced considerable reduction in biomass of the crop plants. The reduction in biomass of plants due to NaCl stress results by upsurged uptake of Na^+ ions, with concurrent decline in the amount of K^+ uptake by the plants (Noreen *et al*., 2021) [34]. The accumulation of Na^+ ions in the rhizosphere results osmotic stress lead hinder in water and mineral absorption from the soil (Zeeshan *et al*., 2020) [56]. This reduction in water availability of cells causes loss in turgidity that results low performance of physio-biochemical processes. Since, enzymes normally function in their aqueous environment (Vighi *et al*., 2017) [52]. Together the effects of these limitations degrade photosynthetic machinery and ultimately reduced growth and development (Choi *et al*., 2014) [9]. The increasing water salinity level had an impact on the dry matter of green gram. The dry matter production was significantly affected by irrigation water salinity. The highest dry matter was obtained from the control treatment, irrigated with fresh water. An increase in irrigation water salinity caused a significant decline in dry matter weight. Differences in dry matter weight between the treatments increased and became more pronounced at later stages of crop growth. Reduction in weights with increasing salinity may be due to limited supply of metabolites to young growing tissues, because metabolic production is significantly perturbed at high salt stress, either due to the low water uptake or toxic effect of NaCl (Akram *et al*., 2010) [1]. Similar results were also observed by Zahra *et al*., 2020 [55] and Saddiq *et al*., 2019 [41] who confirmed that salinity caused a considerable reduction in fresh and dry weight of plants.

Photosynthetic pigments

Under varrying NaCl concentration, data on pigment composition- chlorophyll a, b and carotenoids of green gram plants showed maximum reduction in all pigment constituents compared to control. The NaCl lead reduction in chlorophyll a, chlorophyll b and carotenoid constituents observed was 62.64%, 57.08% and 64.64% respectively (Table 2). When three species of *Vigna (V. radiata, V. mungo,* and *V. unguiculata)* were subjected to varied doses of NaCl (50, 75, 100, 125, and 150 mM), reduction in chlorophyll content was observed (Arulbalachandran *et al*., 2009) [4]. A great reduction in chlorophyll content was observed in salt sensitive plants (Shahid *et al*., 2020) [46]. Chlorophyll a and chlorophyll b content decreased under stress conditions in *Vigna radiata* (Suleiman *et al*., 2021) [47]. Similar reduction in chl a and chl b contents were also observed by Rangaraj *et al*., 2021 [39] in different varieties of green gram and black gram. Reduction in chlorophyll content occurs as a result of water imbalance under salt stress (Chandrasekaran *et al*., 2019) [7].

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) [17]. Chlorophyll content in Mungbean plants was greatly affected by NaCl. Reduction in chlorophyll content on 100mM NaCl was also observed in mungbean (Baghel *et al*., 2012) [5]. According to Altuntas *et al*., 2020 [2], proline elevation in crop plants causes chlorophyll reduction.

Table 2: Impact of varrying NaCl concentration on biochemical studies of *Vigna radiata* L. Values shown are mean ± S.E for five replicate experiments. (mg g^{-1} Fr. Wt.)

Treatments	CHL A	CHL B	Carotenoid	Root proline	Shoot proline	Root protein	Shoot protein
Control	0.712±0.001	0.678±0.001	0.461±0.001	2.653±0.001	2.612±0.001	3.211±0.001	3.713±0.001
T_1 (25 mM)	0.645±0.002	0.659±0.002	0.406±0.001	3.231±0.001	2.936±0.002	2.513±0.001	3.034±0.002
T_2 (50 mM)	0.596±0.003	0.413±0.001	0.375±0.002	3.456±0.003	3.356±0.001	2.316±0.001	2.716±0.001
T_3 (75 mM)	0.503±0.001	0.361±0.001	0.277±0.003	4.243±0.001	4.173±0.001	1.632±0.001	2.232±0.001
T_4 (100 mM)	0.341±0.001	0.310±0.002	0.203±0.002	4.901±0.002	4.762±0.002	1.392±0.002	1.963±0.001
T_5 (125 mM)	0.266±0.001	0.291±0.001	0.163±0.001	5.236±0.001	5.036±0.002	1.183±0.001	1.512±0.002
F(Value)	0.0024	0.0023	0.0072	8.488*	8.988*	0.0001	0.0017

(*) Values Significant at p≤0.05 and (±) SE.

Protein and proline content

Total protein content of root and shoot reduced in all the varying NaCl concentrations (T_1 – T_5). A reduction of 63.16% and 59.28% was observed at T_5 in the total protein of root and shoot as compared to control. But an increasing trend in the proline content was observed along the varying NaCl concentration. Proline content of root and shoot got increased by 97.36% and 92.80% respectively at T_5 as compared to control (Table 2). Similar results were also observed by Altuntas *et al*., 2020 [2] 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, 21].The salt treatments induced an increase in proline concentration in lentil plants.

Proline accumulation under salinity may contribute to osmotic adjustment, protecting cell structure and function and/or may serve as metabolic or energetic reserve in plants (Madan *et al.,* 1994; Nirmada *et al*., 2013) [28, 33]. Proline level in roots and shoots increased in mungbean cultivar 'T 44' subjected to NaCl stress at seedling stage (Misra and Gupta, 2006) [31]. It was reported that salt stress results in extensive proline accumulation in chickpea (Eyidogan and Öz, 2007) [11]. Rasool *et al* (2012) [40] reported that proline content increased with the increase in salt concentration.

Reduction in protein content under salinity have been well documented by various workers in different crop plants like peas (Uprety and Sarin, 1975) [49], Pigeon Pea (Gill and Sharma, 1993) [13], *Phaseolus vulgaris* (Younis *et al*, 1993) [54], *Crotalaria striata* (Chandrashekar and Sandhya Rani, 1996) [8]. 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 (poljakoff - Mayber, 1982) [36]. It was observed that the protein content of black gram decreased with increasing concentration of NaCl (Kumar *et al*, 1996) [24]. However, the protein content slightly increased in the two salt resistant varieties of barley, wheat and sunflower (El-Waheed *et al*, 2015) [29]. Green gram, a salt sensitive species showed a reduced protein content under salt stress (Altuntas *et al*., 2020) [2].

Conclusion

Environmental stresses especially NaCl stress has a negative influence on the growth and productivity of agricultural crops. In this study, NaCl stress showed a negative impact on growth parameters, biomass production and pigment composition of *Vigna radiata* L. Consequently, soil salinity significantly inhibited the growth and resulted in a decrease of dry matter of green gram. However, NaCl caused significant increase in proline content of green gram plants.

References

1. Akram M, Ashraf MY, Ahmad R, Waraich EA, Iqbal J *et al*. Screening for salt tolerance in maize (*Zea mays* L.) hybrids at an early seedling stage. Pak. J. Bot,2010:42(1):141-154.
2. 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.
3. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant physiology, 1949, 24(1), 1.
4. Arulbalachandran D, Ganesh KS, Subramani A. Changes in metabolites and antioxidant enzyme activity of three *Vigna* species induced by NaCl stress. American-Eurasian Journal of Agronomy,2009:2(2):109-116.
5. 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.
6. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and soil,1973:39(1):205-207.
7. 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.
8. Chandrashekar KR, Sandhyarani S. Salinity induced chemical changes *in Crotalaria striata* DC plants. Indian Journal of Plant Physiology,1996;1:44-8.
9. Choi WG, Toyota M, Kim SH, Hilleary R, Gilroy S. Salt stress-induced Ca_2^+ waves are associated with rapid, long-distance root-to-shoot signaling in plants. Proceedings of the National Academy of Sciences,2014:111(17):6497-6502.
10. El-sabagh A, Sorour S, Ueda A, Saneoka H. Evaluation of salinity stress effects on seed yield and quality of three soybean cultivars. Azarian Journal of Agriculture,2015:2:138-141.
11. Eyidogan F, Öz MT. Effect of salinity on antioxidant responses of chickpea seedlings. Acta Physiologiae Plantarum,2007:29(5):485-93.
12. 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.
13. Gill KS, Sharma PC. Mechanism of salt injury at seedling and vegetative growth stages in *Cajanus cajan* (L.) Millsp. Plant Physiology And Biochemistry-New Delhi,1993;20:49.
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):348-53.
15. Green gram outlook- September. Directorate of Economics and Statistics. Agricultural Market Intelligence Centre, PJTSAU, 2019, 1-3.
16. 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.
17. 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):1831-42.
18. Hanin M, Ebel C, Ngom M, Laplaze L, Masmoudi K. New insights on plant salt tolerance mechanisms and their potential use for breeding. Frontiers in Plant Science,2016:7:1787.
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. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In Ecophysiology and responses of plants under salt stress. Springer, New York, NY, 2013, 25-87.
21. 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.

22. Isayenkov SV, Maathuis FJ. Plant salinity stress: many unanswered questions remain. Frontiers in Plant Science, 2019:10:80.
23. Kumar BS, Prakash M, Narayanan S, Gokulakrishnan J. Breeding for salinity tolerance in mungbean. APCBEE Procedia,2012:4:30-35.
24. Kumar SA, Muthukumarasamy M, Panneerselvam R. Nitrogen metabolism in black gram under NaCl stress. J. indian bot. Soc,1996;75:69-71.
25. Lambridge CJ, Godwin ID. Mungbean. In: Kole C, editor. Genome mapping and molecular breeding in plants, Pulses, sugar, and tuber crops. Heidelberg: Springer Verlag,2007:3:69-90.
26. Liang W, Ma X, Wan P, Liu L. Plant salt-tolerance mechanism: A review. Biochemical and Biophysical Research Communications,2018:495(1):286-291.
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of biological chemistry,1951:193:265-275.
28. Madan S, Nainawatee HS, Jain S, Jain RK, Malik MS, Chowdhury JB. Leaf position-dependent changes in proline, pyrroline-5-carboxylate reductase activity and water relations under salt-stress in genetically stable salt-tolerant somaclones of Brassica juncea L. Plant and soil,1994:163(2):151-6.
29. Abd El-Wahed MH, Sabagh AE, Mohammed H, Ueda A, Saneoka H, Barutçular C. Evaluation of barley productivity and water use efficiency under saline water irrigation in arid region. Int. J. Agr. Crop. Sci,2015:8:765-73.
30. Misra N, Dwivedi UN. Genotypic difference in salinity tolerance of green gram cultivars. Plant Science,2004:166(5):1135-1142.
31. Misra N, Gupta AK. Interactive effects of sodium and calcium on proline metabolism in salt tolerant green gram cultivar. Am. J. Plant Physiol,2006:1(1):1-12.
32. Mohamed MH, El Kramany MF. Salinity tolerance of some mungbean varieties. J Appl Sci Res,2005:1:78-84.
33. Nirmada S, Bhat KV, Sairam RK, Toomoka N, Kaga A, Shu Y *et al*. Diversity analysis and confirmation of intra-specific hybrids for salt tolerance in mungbean (*Vigna radiata* L. Wilczek). International Journal of Integrative Biology,2013:14(2):65-73.
34. Noreen S, Sultan M, Akhter MS, Shah KH, Ummara U, Manzoor H *et al*. Foliar fertigation of ascorbic acid and zinc improves growth, antioxidant enzyme activity and harvest index in barley (*Hordeum vulgare* L.) grown under salt stress. Plant Physiology and Biochemistry,2021:158:244-254.
35. Nutrition Nectar for India Pulses and Grains Association, 2020. http://poshan.outlookindia.com.
36. Poljakoff-Mayber A. Biochemical and physiological responses of higher plants to salinity stress. Biosaline research. A look to the future. Plenum Press, New York, NY, 1982, 245-70.
37. Qados AMA. Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). Journal of the Saudi Society of Agricultural Sciences,2011:10(1):7-15.
38. Rahim MA, Mia AA, Mahmud F, Zeba N, Afrin KS. Genetic variability, character association and genetic divergence in Mungbean (*Vigna radiata* L. Wilczek). Plant Omics,2010:3(1):1-6.
39. Rangaraj M, Muthswamy S, Kumarasamy P. Optimization Effect of salinity and drought stress in the chlorophyll content of *Vigna mungo* and *Vigna radiata* genotypes. Journal of Crop and Applied Sciences,2021:1(2):71-75.
40. Rasool S, Ahmad A, Siddiqi TO. Differential response of chickpea genotypes under salt stress. J Funct Environ Bot,2012:2(1):59-64.
41. Saddiq MS, Iqbal S, Afzal I, Ibrahim AM, Bakhtavar MA, Hafeez MB *et al*. Mitigation of salinity stress in wheat (*Triticum aestivum* L.) seedlings through physiological seed enhancements. Journal of Plant Nutrition,2019:42(10):1192-1204.
42. Saha P, Chatterjee P, Biswas AK. NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek), 2010.
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 radiata* L.) under salt stress. Journal of Pharmacognosy and Phytochemistry,2018:7(6):1857-1860.
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 MO, Bakht JE, 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):1111-18.
46. Shahid MA, Sarkhosh A, Khan N, Balal RM, Ali S, Rossi L et al. Insights into the physiological and biochemical impacts of salt stress on plant growth and development. Agronomy,2020:10(7):938.
47. Suleiman MAQ, Al-Hamdani KM. Effect of growth factors on mineral, proline and protein accumulation in mungbean (*Vigna radiata* L.) Under salt stress. plant cell biotechnology and molecular biology, 2021, 98-109.
48. 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.
49. Uprety DC, Sarin MN. Physiological studies on salt tolerance in Pisum sativum (-L.). II. Mechanism of salt action during germination. Acta Agron Budap, 1975.
50. 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.
51. Velmurugan A, Swarnam P, Subramani T, Meena B, Kaledhonkar MJ. Water demand and salinity. In Desalination-Challenges and Opportunities. Intech Open, 2020.
52. Vighi IL, Benitez LC, Amaral MN, Moraes GP, Auler PA, Rodrigues GS *et al*. Functional characterization of the antioxidant enzymes in rice plants exposed to salinity stress. *Biologia plantarum*,2017:61(3):540-550.
53. 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.

54. Younis ME, Abbas MA, Shukry WM. Effects of salinity on growth and metabolism of *Phaseolus vulgaris*. Biologia plantarum,1993:35(3):417-24.
55. 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.
56. Zeeshan M, Lu M, Sehar S, Holford P, Wu F. Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance. Agronomy,2020:10(1):127.
57. Zhang Q, Dai W. Plant response to salinity stress. In Stress Physiology of Woody Plants. CRC Press, 2019, 155-173.