

Comparative Studies among Different Genotypes of Soybean (*Glycine max* L.) against Salinity Stress

S. A. Siddiki¹, S. G. Wagh^{1,2*}, R. S. Sul¹, K. R. Pawar¹ and S. N. Harke¹

¹*Mahatma Gandhi Mission's Institute of Biosciences and Technology, N-6 CIDCO, Main Campus, Aurangabad, Maharashtra, 431003, India.*

²*Department of Bio-science and Technology, Agri-Biotech College, VNMK University, Aurangabad, 431003, MS, India.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors SSA, SRS and PKR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WSG and HSN managed the analyses of the study. Author PKR managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i630563

Editor(s):

(1) Dr. Tushar Ranjan, Bihar Agricultural University, India.

Reviewers:

(1) Said A. Saleh, National Research Centre, Egypt.

(2) Aminu Yahaya, Kano State College of Education and Preliminary Studies, Nigeria.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/55505>

Original Research Article

Received 15 January 2020

Accepted 19 March 2020

Published 18 April 2020

ABSTRACT

Soybean (*Glycine max* L.) is a significant legume of food and plays a vital role in human livelihood. It is rich in proteins (40%), which contain major essential amino acids, and edible oil (20%). Salinity stress affects soybean yield 30-80%. Salinity stress significantly reduces net photosynthetic rates, increases energy losses for the mechanism of salt exclusion, substantially decreases nutrient intake and ultimately results in reduced plant growth. Present investigation was conducted to show how morphological and biochemical changes occur due to the stress of salinity on the soybean plant genotypes. Stress with salinity resulted in increased protein and proline content to withstand stress with salinity. Better root length, shoot length fresh weight and dry weight were observed of JS-355 variety. The JS-355 variety demonstrated the better response to all concentrations of salt stress used from 40 mM to 280 mM. As the salt concentration increases, so does the protein and proline concentration. The JS-355 variety showed the better results at all salt concentrations. The highest protein and proline content at high salt concentration was observed in variety JS-355.

*Corresponding author: E-mail: swagh.gene@gmail.com;

Keywords: Salinity; soybean germination; proline; protein; salicylic acid.

1. INTRODUCTION

The world's population continues to grow at an alarming rate and with increasing urbanization; the amount of arable land is diminishing. We need to make arrangements to feed this ever-increasing population, which is invaluable, to protect life on earth. Many efforts are being made in plant research across the globe, not only restricting the improvement of crop production in cereals (staple food), but also focusing on avoiding post-harvest losses in crops due to biotic and abiotic stress, as well as focusing on basic plant science research with other experimental plants and their cell organelles [1-9]. Hence, any effort to feed a single life on earth is more than welcome. In addition, more emphasis must be placed on introducing marginally productive and currently non-arable land into the development of depleting land and water resources. Large areas of formerly arable land are excluded from crop production each year due to an increase in soil salinity [10,11]. A comprehensive understanding of how plants respond to different levels of salinity stress and an integrated approach to combining molecular tools with physiological and biochemical techniques are essential for the development of salt-tolerant plant varieties [12]. Recent research has identified various adaptive responses to salinity stress at molecular, cellular, metabolic, and physiological levels, though mechanisms underlying salinity tolerance are far from fully understood. This paper provides a comprehensive review of major advances in research on biochemical, physiological, and molecular mechanisms that regulate plant adaptation and salinity stress tolerance. Excess NaCl in soil may adversely interrupt cellular homeostasis and uncouple important physiological and biochemical processes. Salinity is considered a significant factor affecting crop production and agricultural sustainability in arid and semiarid regions of the world, decreasing the value and productivity of the affected crop land [12].

Soybean [*Glycine max* (L.) Merrill] is an important legume of food, and plays a vital role in human living. It contains high amounts of proteins (40%) containing major essential amino acids and edible oil (20%) [4]. It is the most important seed legume in the world, contributing to 25% of the world's edible oil, about two-thirds of the world's livestock feeding protein concentrate [13].

Soybean also has the ability to mitigate the nutritional situation, enhance other crop productivity, and protect the environment from agricultural chemicals allelopathy tendencies [14,15]. Soybean improve the soil health and fertility by fixing nitrogen through biological nitrogen fixation in soil which is carried out by symbiotic nitrogen fixing bacteria residing in the root nodule of soybeans [16].

One of the biggest problems with soybean crops is high sensitivity to soil and water salinity. Results indicated that salinity has the effect of osmotic and ionic stress on plant growth and development. Because of accumulated salts in soil under salt stress, the plant apparently wilts while soil salts such as Na⁺ and Cl⁻ disrupt normal growth and plant development [17]. Increased salinity has resulted in lower chlorophyll content in soybean leaves [18,19]. Plant salt stress is a condition in which the excessive salts in the soil solution cause plant growth inhibition or plant death. No toxic substance on a world scale restricts plant growth more than the salt of sea. Salt stress poses a growing threat to agricultural plants. Among the different sources of soil salinity, irrigation, combined with poor drainage, is the most serious, since it represents losses of once productive agricultural land [20]. Salinity is a major biotic stress that restricts plant growth and productivity in many parts of the world as a result of increasing use of poor water quality for irrigation and soil salinisation. Plant adaptation or salinity stress tolerance involves complex physiological characteristics, metabolic pathways and molecular or gene networks [21]. Salinity is vitally important for today's agriculture, as rapid population growth has made salinity-oriented problems urgent, particularly in the developing world and consequently increased demand for agricultural products. Salinity is one of the most significant abiotic factors that restrict crop production from marginal agricultural soils.

Soil salinity is one of the major biotic stresses causing significant reduction in soybean plant growth parameters, photosynthetic pigments, and yield components. The present study aims to improve soybean production under saline conditions and attempts to elucidate possible plant tolerance mechanisms by using three different treatments (Gibberellic acid, Paclobutrazol and Zinc sulphate). The magnitude of reduction is increased by increasing salinity

levels [22]. Accumulated salts in soil under the NaCl salt stress condition make the plants appear to wane, while soil salts such as Na⁺ and Cl⁻ interfere with normal plant growth and development [20]. The use of high amounts of fertilizers, rising tables of water and the use of saline irrigation water cause salinity in the soil. Studies of the physiology of crop plants under saline conditions should be carried out in the field soils with combined natural salts [23]. The study therefore aimed at investigating the Comparative Studies among Different Genotypes of Soybean (*Glycine max* L.) against salinity stress.

2. MATERIALS AND METHODS

2.1 Collection of Sample and Plant Material

The certified seeds of eleven varieties of soybean namely (MAUS-158, MAUS-162, MAUS-57, PUSA-20, Mahabeej, and Js-355) were collected from National seed corporation Aurangabad. And mahabeej privet limited Akola. In a separate beaker, take 6-8 seeds for each cultivar and wash with distilled water. The seed was treated with 0.1% HgCl₂ for 2-3 min for surface sterilization, as previously explained by Pawar et al. [24]. Move the seed into a fresh beaker and wash it 3 times with distilled water to remove trace of Mercuric Chloride. And planting is given the different salt concentration (40 mM, 100 mM, 160 mM, 220 mM & 280 mM) and the controlled is used as distilled water.

2.2 Analysis of Growth

The increase in shoot and root length was observed in centimetres on the 11 day of germination. And seedlings have been divided into cotyledon, shooting, and root. The fresh weights of the plant components are measured on the electronic balance immediately.

2.3 Biochemical Parameter

2.3.1 Protein

The theory behind the Lowry method of determining protein concentrations is the reactivity of the peptide nitrogen with the copper ions under alkaline conditions and the subsequent reduction of the Folin-Ciocalteu phosphomolybdic-phosphotungstic acid by the copper-catalyzed oxidation of aromatic acids to heteropolymolybdenum blue. After the 11 days of germination. The germinated

seeds were crushed for 2-3 min at 10,000 rpm in 1 M tris buffer (pH 7.0), and centrifuge and filter paper to filter through. After that, the sample was diluted and used as unknown with distilled after and the normal solution taken as BSA (Bovine Serum Albumin).

2.3.2 Proline

The extracts were made of 0.5 gm of plant material in 10 ml of 3% Sulfosalicylic acid. Filtered through Whatman No.2 filter paper. 0.5 ml of filtrate is taken in a test tube and in a sequence 2ml of glacial acetic acid and 2ml of acid Ninhydrin have been added. The test tube was heated for 1hr in the boiling water bath, by placing the tubes in the ice bath, the reaction is stopped. Added 4 ml of toluene to the reaction mixture and stir well for 20 to 30sec. The layer of Toluene is separated and the room temperature is warm. The intensity of red colors is measured at 520 nm [25].

2.3.3 Salicylic acid

SA easily forms complexes with minute traces of ferric salts and is a violet color complex that can be determined at 540 nm due to the formation of a ligand and the extinction of the ferric complex. In this study for the detection of SA leaf samples, this principle was adopted crushed in frozen liquid nitrogen and ground to powder. Samples were left to thaw at room temperature. SA extraction into the optimized solvent by varying solvent volumes in all experiments for 50 mg and 100 mg of tissue. Samples were well swirled in the solvent followed by 10,000 g of centrifugation for 10 min for SA measurement the supernatant was stored on ice.

3. RESULTS

At room temperature the seed is sown in a cocopit. And planting is given the different salt concentration (40 mM, 100 mM, 160 mM, 220 mM & 280 mM) and the controlled is used as distilled water. Total plant germinates after the 11 days and calculates the morphological parameter such as shoot length, root length, leaf no, fresh weight & dry weight. The following data show how morphological changes occur on soybean plant genotypes due to salinity stress.

3.1 Morphological Character

3.1.1 Root length

The results indicated that the root length of the seedling decreased linearly as the salt

concentrations increased (40 to 280 mM) due to the salinity stress of all soybean cultivators. The average root length decreased over all varieties respectively from 10.65 cm and 4.22 cm for 0 mM (the control) and 8.62 cm and 0.72 cm for 280 mM NaCl (Table 1). Variety JS-355 shows the highest root length 9.80 cm at low salt concentration 40mM, MAHABEEJ shows root length decrease of 5.37 cm. At concentration MAUS-158 of 100mM show higher root length of 9.15 cm. MAUS162, MAUS57 & JS-355 show an average length of 4.6 cm to 5.7 cm. But the root length was observed to be more reduced in MAHABEEJ being 2,525 cm. At 160 mM Js-355 the root length is 8.80 cm higher. Also MAUS57 show better root length 6.97cm. At 220 mM JS-355 show higher root length 6.00cm. Also MAUS57 & PUSA20 show better result with concern 280 mM. MAUS57 show a higher root length (8.62 cm) at 160 mM Js-355 respectively show a higher root length (8.80 cm).

3.1.2 Shoot length

The results indicated that the shooting length of the seedling decreased linearly as the salt concentrations increased (40 to 280 mM) due to the salinity stress of all soybean growers. The average shoot length decreased over all varieties respectively from 30.10 cm and 11.42 cm for 0 mM (the control) and 17.35 cm and 3.35 cm for 280 mM NaCl (Table 1). Variety MAUS162 shows the highest shoot length of 27.50 cm at low salt concentration 40mM, and MAHABEEJ shows the reduction in shoot length of 9.17 cm. JS-355 show higher shoot length at 100mM concentration 25.32 cm. MAUS162, MAUS57&JS-355 show an average 11.4cm-25.32 cm long. But in MAHABEEJ it was observed that there is more reduction in shoot length is 3.35 Cm. At 160 mM Js-355 display 24.35 cm of higher shoot length. Also MAUS57 shows better length of shoot 14.27 cm. At 220 mM JS-355 show the higher shoot length 17.35 cm. also PUSA20 & JS-355 show better results at 280 mM concentration. JS-355 show higher shoot length (17.35 cm) at 100 mM Js-355 show higher shoot length (25.32 cm) respectively.

3.1.3 Number of leaves

Due to salinity stress, the results indicated that the root length of the seedling decreased linearly as the salt concentrations increased (40 to 280 mM) in the number of leaves of all soybean growers. The average number of leaves over all varieties decreased from 3 and 1 for 0 mM (the control) and from concentrations of 5 and 0.75

for 280 mM NaCl respectively (Table 1). Variety MAUS-158 MAUS162&PUSA20 showed the highest number of leaves 2.7 & MAHABEEJ showing the reduction in root length of 0.50 cm at low salt concentration 40 mM. At JS-355 concentration of 100 mM, higher leaf number 3.00. MAUS-158, MAUS162&PUSA20 shows the 4.6-5.7 average number of leaves. But there is more decrease in MAHABEEJ's No. of leaves is 1.0. At 160 Mm, MAUS57&JS-355 shows 2.50 higher leaf No. MAUS-158&MAUS162 also show better leaf no. 2.75. At 220 mM JS-355, 3.75. reduction in shows the higher leaves no of the leaves in MAHABEEJ, 1.25 is observed. At 280mM concen also MAUS162 shows the better result. MAUS162 show a higher number of leaves (5.00) at 160 mM PUSA20, respectively.

3.1.4 Fresh weight

Reduction in Fresh Weight of all soybean growers was observed due to salinity stress, the results indicated that fresh seedling weight decreased linearly as salt concentrations (40 to 280 mM) increased. The average Fresh Weight was observed for all varieties from 0.55 gm and 1.25 gm for 0 mM (the control) and 0.75 gm and 0.65 gm for 280 mM NaCl concentration respectively (Table 1). The MAUS-158&MAUS57 variety shows the highest Fresh Weight 1.40 gm & 1.50 gm. MAHABEEJ at low salt concentration 40 mM showing the reduction in Fresh Weight 0.23 gm. Fresh Weight 0.95 gm higher at 100 mM concentration MAUS162 show, MAUS-158, MAUS57&JS-355 show average 0.83 gm -0.92 gm Fresh Weight. But Fresh Weight was observed to have more reduction in PUSA20 is 0.44 gm. Fresh Weight 0.84gm higher at 160 Mm MAUS-158&MAUS57 show. More Fresh Weight 0.74 gm show also MAUS162&PUSA20. The higher Fresh Weight 0.76gm shows at 220 mM JS-355. Reduction of Fresh Weight in MAHABEEJ is 0.23 gm was observed. In MAHABEEJ higher fresh weight was observed at 280 mM concentration MAUS-158 show 0.75 gm & reduction in fresh weight is 0.15 gm respectively.

3.1.5 Dry weight

Dry Weight reduction of all soybean growers was observed due to salinity stress, the results showed that seedling dry weight decreased linearly as salt concentrations increased (40 to 280 mM). The average Dry Weight decreased over all varieties respectively from 0.35 gm and 0.10 gm for 0 mM (the control) and 0.14 gm and 0.01 gm for 280 Mm NaCl (Table 1). Variety

MAUS-158, MAUS162 & MAUS57 show the Dry Weight 0.12 gm & MAHABEEJ show the reduction in Dry Weight 0.03 gm at a low salt concentration of 40 mM. In PUSA20is 0.6 gm at 160 Mm MAUS-158&MAUS162show higher Dry Weight 0.10 gm is observed at 100mM concentration MAHABEEJ show Dry Weightbut there is more reduction in Dry Weight. Also show MAUS-158&MAUS162 better Dry Weight 0.09gm. The higher Dry Weight 0.14 gm shows at 220 mM MAUS-158. Dryweight reduction was observed to 0.01gm in MAHABEEJ. Also JS-355 shows a better result at concentration of 280 mM MAUS162 show higher (0.11 gm) and higher Dry Weight (1.14 gm) at 280mM MAUS-158, respectively.

Table 1. Effect of morphological parameter in different varieties of soybean

	MAUS-158	MAUS162	MAUS-57	PUSA-20	Mahabeej	Js-355
Root length						
0 mM	9.525	8.050	0.050	4.775	4.225	10.650
40 mM	9.425	7.000	6.525	9.025	5.375	9.800
100 mM	9.150	4.625	4.750	5.000	2.525	5.775
160 mM	4.175	4.375	6.975	3.400	2.075	8.800
220 mM	3.175	3.850	4.050	4.250	3.175	6.00
280 mM	3.225	3.250	8.625	3.050	0.725	4.125
CD	0.256	0.901	1.234	1.653	0.879	1.848
SE	0.086	0.301	0.412	0.552	0.294	0.617
Shoot length						
0 mM	23.950	30.100	29.350	26.350	11.425	27.100
40 mM	22.175	27.500	12.500	17.525	9.175	24.175
100 mM	22.300	25.300	11.400	14.475	7.300	25.325
160 mM	20.625	19.650	14.275	18.350	5.350	24.350
220 mM	15.125	17.125	12.125	17.225	5.500	23.625
280 mM	9.650	9.200	7.950	12.350	3.350	17.350
CD	1.120	1.015	2.455	2.928	0.426	0.870
SE	0.374	0.339	0.820	0.978	0.142	0.291
No. of leaves						
0 mM	4.250	4.250	3.500	4.250	1.500	3.500
40 mM	3.500	3.500	2.500	3.500	0.500	2.750
100 mM	2.750	2.750	2.250	2.750	1.000	3.000
160 mM	2.00	2.00	2.500	2.00	0.750	2.500
220 mM	2.750	2.750	2.250	2.750	1.250	3.750
280 mM	1.500	5.00	1.750	1.500	0.750	1.750
CD	1.332	-	-	1.332	-	-
SE	0.445	0.866	0.460	0.445	0.339	0.445
Fresh weight						
0 mM	1.258	1.258	1.438	0.642	0.550	1.148
40 mM	1.050	0.940	1.040	0.848	0.233	0.945
100 mM	0.928	0.958	0.848	0.443	0.543	0.835
160 mM	0.838	0.740	0.840	0.735	0.230	0.875
220 mM	0.730	0.725	0.625	0.720	0.235	0.763
280 mM	0.750	0.448	0.543	0.555	0.158	0.658
CD	0.035	0.031	0.028	0.027	0.030	0.022
SE	0.012	0.010	0.009	0.009	0.010	0.007
Dry weight						
0 mM	0.100	0.093	0.045	0.035	0.036	0.065
40 mM	0.100	0.113	0.110	0.062	0.073	0.035
100 mM	0.100	0.113	0.069	0.066	0.118	0.116
160 mM	0.106	0.105	0.096	0.046	0.046	0.047
220 mM	0.087	0.106	0.064	0.094	0.074	0.054
280 mM	0.144	0.113	0.087	0.084	0.054	0.012
CD	0.004	0.003	0.004	0.003	0.014	0.004
SE	0.001	0.001	0.001	0.001	0.005	0.001

3.2 Biochemical Parameter

3.2.1 Protein

Increase in the protein content of all soybean cultivators under salinity stress was observed. The results showed that the seedling protein content increased linearly as the salt concentrations increased (40 to 280 mM). The average protein content for all varieties increased from 2.8 µg/ml - 3.4 µg/ml for 0 mM (control) and 3.3 µg/ml - 4.3 µg/ml for 280 mM NaCl concentration respectively. The variety MAUS-158MAUS162&PUSA20 show an average protein content of 3.1 µg/ml - 3.7 µg/ml at low salt concentration 40 mM. MAUS57 shows reduction of 2.78 µg/ml of protein content. The higher protein content in MAHABEEJ&JS-355 is 4.3 µg/ml & 4.6 g/ml. At a concentration of 100 mM MAUS162, MAUS57, PUSA20&JS-355 having an average concentration of 3.3 µg/ml - 3.7 µg/ml. And MAUS-158 & MAHABEEJ shows a 2.65 µg/ml - 2.8 µg/ml lower protein concentration. Higher protein concentration at 160 Mm MAHABEEJ&JS-355 shows 5.25 µg/ml & 4.78 µg/ml. Show higher protein concentrations at 220 Mm MAHABEEJ&JS-355.

3.2.2 Proline

Increase in the proline content of all soybean growers was observed under the stress of salinity. As the salt concentrations increased (40 to 280 mM), the results indicated that Proline content seedling increased linearly. The mean proline content averaged rose from 0.8 µg/ml and 1.8 µg/ml for 0 mM (control) and 0.2 µg/ml

- 1.1 µg/ml (280 mM NaCl) over all varieties. At low salt concentration of 40mM, the variety MAUS-158MAUS162PUSA20&JS-355 shows the average proline content of 1µg/ml-1.9 µg/ml. MAUS57 & MAHABEEJ shows a proline content increase of 2.4 µg/ml - 2.8 µg/ml. At 100 mM concentration of MAUS162, PUSA20&JS-355 shows high proline concentration of.1µg/ml-1.6 µg/ml. The lower proline 0.6 µg/ml - 0.8 µg/ml. MAHABEEJ shows a high proline concentration of 2.2 µg/ml and MAUS-158 & MAUS57. High proline concentration at 160 Mm JS-355show 3.25 µg/ml. At 220 Mm MAHABEEJ the proline concentration was 2.65 µg/ml higher. Additionally, higher proline concentration at 280 mM concen. MAHABEEJ is 2.4µg/ml, respectively.

4. DISCUSSION

4.1 Physiological Parameter: Root Length and Shoot Length

Reducing plant growth under saline conditions may either be due to osmotic reduction in water availability resulting in increased stomata resistance as reported by Gunes et al. [26], it has been reported that salinity stress significantly reduced net photosynthetic rates, increased energy losses for salt exclusion mechanism, substantially reduced nutrient absorption and ultimately reduced nutrient consumption. Our result shows that the root length of all soybean growers has been observed to decrease due to salinity stress. MAUS57 shows higher root length under high salt concentration (280 mM) and MAHABEEJ shows

Table 2. Effect of salt stress on biochemical component in different varieties of soybean (*Glycine max* L.)

	MAUS-158	MAUS-162	MAUS-57	PUSA-20	Mahabeej	JS-355
Protein concentration						
0 mM	3.4	3.3	3.85	2.8	5.1	3
40 mM	3.7	3.7	2.7	3.14	4.3	4.6
100 mM	2.65	3.3	3.4	3.7	2.85	3.3
160 mM	2.7	2.1	3.8	3.95	5.25	4.7
220 mM	4.2	2.65	3.65	3.6	6.05	4.05
280 mM	3.9	3.35	3.14	4.15	4.35	4.3
Proline concentration						
0 mM	1.8	1.8	1.7	1.5	1.45	0.8
40 mM	1.35	1.65	2.4	1	2.8	1.9
100 mM	0.7	1.6	0.8	1.15	2.2	1.6
160 mM	1	0.8	1	1.6	2.6	3.25
220 mM	1	1.15	0.65	0.95	2.65	1.1
280 mM	0.8	1.1	0.25	0.85	2.4	0.8

very low root length at high salt concentration (280 mM) among all varieties. Also, JS-355 shows better root length than all varieties. Reduction was observed in the root length of all soybean cultivators due to salinity stress. From the graphical representation above, we conclude that variety MAHABEEJ shows the reduction in shoot length at high salt concentration (280 mM) and we also analyze that variety JS-355 shows higher shoot length at high salt concentration (280 mM) and MAUS162 also showed better shoot length. MAUS57 showed the mean length of the shoot at all concentrations of salt. MAHABEEJ showed shoot length decrease at all concentrations. Reduction in number of leaves was observed among all the varieties. From the above table and graphical representation, we concluded that variety MAUS162 showed the best outcome for leaf number. And we analyzed that the M5. Also MAUS-158&PUSA20 variety was counted with the similar number of leaves and MAHABEEJ showed the reduction in number of leaves at all salt concentration. Reduction in Fresh weight was observed among all varieties. From the above table and graphical representation we conclude that variety MAUS-158 at all salt concentration showed the high fresh weight among all varieties. Also MAUS162, MAUS57&JS-355 showed better results MAHABEEJ showed fresh weight reduction at all salt concentration. Reduction in dry weight was observed among all the varieties. From the above table and graphic representation, we concluded that MAUS-158 showed the high dry weight of all varieties at all concentrations of salt. After that MAUS162 shows higher Dry Weight at all concentrations of salt. Also JS-355 showed the reduction of dry weight at all concentrations of salt. There is a positive co-relationship between the increase in salt concentration and the decline in morphological parameter [13]. This may be attributed to the decreased Root length, shoot length, No. of leaves content, fresh weight & dry weight of plant.

4.2 Biochemical Parameter: Protein Content

Reduction in protein content under salinity stress also may be due to the disturbance in nitrogen metabolism or inhibition of nitrate absorption as reported by El-Zeiny [27]. It is well known that plants need more energy under saline soil environment. Extra energy could be provided by increased sugar, protein and proline accumulation which is energy rich compounds [28]. Accumulation of protein under salt condition

may play a major role in terms of plants salt tolerance, where the proteins may serve as a reservoir of energy or may be adjuster of osmotic potential in plants subjected to salinity [29-32].

The experimental study indicated that the change in protein concentration due to increase in salt concentration. At 0 mM (control) there is average protein concentration were observed. The variety MAUS-158, MAUS162, MAUS57 show the average protein concentration at all the salt concentration (2.7-3.6 µg/ml). But MAHABEEJ & JS-355 showed the highest protein content (3.0-6.0 µg/ml). But the MAHABEEJ variety show the higher protein content (6.05 µg/ml). Earlier finding suggested there is a positive co-relation between the increase salt concentration and increase in protein content [33].

4.2.1 Proline content

Apart from acting as a cytoplasmic osmotic, proline, an amino acid, can function as a source of carbon and nitrogen for post-stress recovery and growth, as a stabilizer for membrane and protein synthesis machinery, as a scavenger of free radicals, as an energy sink to regulate redox potential and also protect the protein against denaturation. Accumulation of proline has been suggested to be associated with osmotic and saline stress tolerance. Its concentration increases either through foliar spraying of SA or through salt stress [34]. Proline and glycine betaine are known to serve as compatible osmolytes, macromolecular protective agents and also as reactive oxygen scavengers [35,36].

Due to an increase in salt concentration, the experimental study was discussed about the change in proline concentration. Average concentration of proline was observed at 0mM (control). Variety MAUS-158, MAUS162, MAUS57 shows the average concentration of proline at all salt concentrations (0.8-1.8 µg / ml). However, MAHABEEJ&JS-355 show the highest concentration of proline (0.8-3.25 µg/ml). But the variety JS-355 shows the greater proline content (3.25 µg/ml). Earlier findings suggest a positive co-relationship exists between the increase in salt concentration and proline content. This can be attributed to the increased proline expression of stress-responsive proteins within the plants. Salicylic acid is naturally occurring signaling molecule and growth regulator that enhance plant growth especially in growth condition [36,37]. There is no salicylic acid found in the sample because I do not have salicylic acid in

the sample after 20 days because it is signaling hormone produced in the plant after 10-15 hours of stress. In addition, differential expression studies can also be performed by isolating RNA from plants with different responses to salinity to determine the molecular factors involved [8]. These molecular factors (genes) can then be easily and cheaply cloned with the methodology previously described, expressed and characterized, which can in turn be useful in the future for the development of plant science by using recently advanced technologies such as CRISPR / cas and RNAi [6,8,9,38-40].

5. CONCLUSION

From the above study, it is concluded that water stress of different level levels of salinity stress on soyabean varieties. The result also shows that, JS-355 variety demonstrated the better response to all concentrations of salt stress used from 40 mM to 280 mM. As the salt concentration increases, so does the protein and proline concentration. The JS-355 variety showed the better results at all salt concentrations. The highest protein and proline content at high salt concentration was observed in variety JS-355.

ACKNOWLEDGEMENT

The authors thank Dr. Manoj Pohare for overall manuscript check and checking the English in the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pohare MB, Akita M. Development of a precursor in a soluble form for protein import into chloroplasts. *International Journal of Bio-Technology and Research*. 2016;6(5):918.
2. Eberhard S, Finazzi G, Wollman FA. The dynamics of photosynthesis. *Annu. Rev. Genet.* 2008;42:463-515.
3. Von Caemmerer S, Quick WP, Furbank RT. The development of C4 rice: Current progress and future challenges. *Science*. 2012;336:1671.
4. Pohare MB, Rathod HP, Shahakar SB, Kelatkar SK, Suryawanshi PP. Effects of UV radiations on morphological characters in *in vitro* regenerated polianthetuberosa. *Research Journal of Agricultural Sciences*. 2012;3:1307-1308.
5. Pohare MB, Batule BS, Bhor SA, Shahakar SB, Kelatkar SK, Varandani SP. Effect of gamma radiations on the morphological characters in *in vitro* regenerated Polianthetuberosa. *Indian Horticulture Journal*. 2013;3: 95-97.
6. Pohare MB, Sharma M, Wagh SG. CRISPR/Cas9 genome editing and its medical potential. In Kumar S (Eds), *Advances in Biotechnology and Biosciences*. Pusa, India. NavNik Publications. 2019;3:69-90. Available: <https://doi.org/10.22271/ed.book.466>
7. Wagh SG, Kobayashi K, Yaeno T, Yamaoka N, Masuta C, Nishiguchi M. *Rice necrosis mosaic virus*, a fungal transmitted *Bymovirus*: Complete nucleotide sequence of the genomic RNAs and subgrouping of bymoviruses. *J Gen Plant Pathol* 2016b; 82:38-43. Available: <https://doi.org/10.1007/s10327-015-0618-7>
8. Wagh SG, Pohare MB, Daspute AA, Wadekar HB, Bhor SA. RNA isolation from purified *Rice necrosis mosaic virus* particles. *Archives of Current Research International*, 2020;19(3):1-6 Available: <https://doi.org/10.9734/acri/2019/MAUS-1589i330157>
9. Pohare MB, Wagh SG, Udayasuriyan V. *Bacillus thuringiensis* as Potential Biocontrol Agent for Sustainable Agriculture. In Yadav AN (Eds) *Current Trends in Microbial Biotechnology for Sustainable Agriculture*. Springer Nature (In press); 2020.
10. Chapman VJ. The salinity problem in general, its importance and distribution with special reference to natural halophytes. In: A. Poljakoff-Mayber, and J. Gale (eds), *Plants in Saline Environments*. 1975;5:7—21.
11. Epstein EJD, Norlyn DW, Rush RW, Kings DB, Kelly DB, Worna AF. Saline culture of crops. A general approach. *Science*. 1980; 210:399—404.
12. Wagh SG, Pohare MB. Current and Future Prospects of Plant Breeding with CRISPR/Cas. *Current Journal of Applied Science and Technology*. 2019;38(3):1-17. DOI:10.9734/CJUST/2019/MAUS578i330360

13. Agarwal DK, Billore SD, Sharma AN, Dupare BU, Srivastava SK. Soybean: Introduction, improvement and utilization in India-problems and prospects. *Agric. Res.* 2013;2(4):293–300.
14. U.S. Department of Agriculture. Oil Crops Outlook--July 1998. Economic Research Service, United States Department of Agriculture, Washington, D.C; 1998.
15. Shala, Stacey. United States Department of Agriculture: Economic research service. Food Security Assessment, Regional overview Information Bulletin; 2001.
16. Javaid A, Mahmood N. Growth, nodulation and yield response of soybean to bio fertilizers and organic manures. *Pakistan J. Bot.* 2010;42:863-871.
17. Farhoudi R, Sharifzadeh F, Poustini K, Makkizadeh MT, Kochakpour M, the effects of NaCl priming on salt tolerance in canola (*Brassica napus*) seedlings grown under saline conditions. *Seed Science and Technology.* 2007;35:754-759.
18. Tabbed RA. Physiological responses of the soybean plant to bring and salinity stress. *Asia Life Science.* 1992;1:61-74.
19. Thalloth AT, Kabesh M. Growth and yield of foliar nutrition with molybdenum, boron, zinc and different level of salinity. *J. Agric. Sci.* 1988;13:2105-2112.
20. Zhu JK. Plant salt tolerance. *Trends in Plant Science.* 2001;6:66-71.
Available:[http://dx.doi.org/10.1016/S1360-1385\(00\)01838-0](http://dx.doi.org/10.1016/S1360-1385(00)01838-0)
21. Gupta B, Huang B. Mechanism of salinity tolerance in plants: Physiological, biochemical and molecular characterization. *Int. J Genomics.* 2014;18. DOI: 10.1155/2014/701596
22. Sofy MR. Effect of gibberellic acid, paclobutrazol and zinc on growth, physiological attributes and the antioxidant defense system of soybean (*Glycine max*) under Salinity Stress *International Journal of Plant Research.* 2016;6(3):64-87.
23. Sonneveld C, Welles GWH. Yield and quality of rockwool-grown tomatoes affected by variations in EC-value and climatic conditions. *Plant Soil.* 1988;111: 37-42.
24. Pawar KR, Wagh SG, Sonune PP, Solunke SR, Solanke SB, Rathod SG, Harke SN. Analysis of water stress in different varieties of maize (*Zea mays* L.) at the early seedling stage. *Biotechnology Journal International.* 2020;24(1):15-24.
Available:<https://doi.org/10.9734/bji/2020/MAUS1624i130094>
25. Pawar KR, Wagh SG, Daspute AA, Avhad GB, Choudhary PD, Harke SN. *In vitro* regeneration of potato (*Kufri pukhraj*). *Journal of Pharmacognosy and Phytochemistry.* 2019;8(5):515-518.
26. Gunes A, Inal A and Alpaslan M. Effect of salinity on stomatal resistance, proline and mineral composition of pepper. *J. Plant Nutr.* 1996;19:389–396.
27. El Zeiny HA, Abou LB, Gaballah MS, Khalil S. Antitranspirant application to sesame plant for salinity stress Augmentation. *Journal of Agricultural and Biological Science.* 2007;3:950–959.
28. Banaras HN, Mohamad A, Jelte R. Salt tolerance in fodder beet and sea beet: analysis of biochemical relation, *Bull. J. Plant Physiol.* 2004;30:78-88.
29. Pessaraki M, Tucker T. Uptake of nitrogen-15 by cotton under salt stress. *Soil Science Society of America Journal.* 1985;49:149-152.
30. Pessaraki M, Huber J. Biomass production and protein synthesis by alfalfa under salt stress. *Journal of Plant Nutrition.* 1991;14: 283-293.
31. Dandve MS, Wagh SG, Bhagat PR, Pawar K, Timake SA, Daspute AA, Pohare MB. Bacterial and fungal pathogen Synergetics after co-infection in the Wheat (*Triticum aestivum* L.). *Biotechnology Journal International,* 2019;23(4):1-9.
Available:<https://doi.org/10.9734/bji/2019/MAUS1623i430085>
32. Ingram J, Bartels D. the molecular basis of dehydration tolerance in plants. *Annual Review of Plant Biology.* 1996;47:377-403.
33. Goudarzi M, Pakniyat H. Salinity causes increase in proline and protein content and peroxidase activity in wheat cultivator *Journal of applied science,* 2009;28:348-353.
34. Shakirova FM, Sakhabutdinova AR, Bezrukova MV, Fatkhutdinova RA. Fatkhutdinova DR. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Science.* 2003;164:317-322.
35. Pawar KR, Janavale GB, Wagh SG, Panche AN, Daspute AA, Pohare MB, Harke SN. Phytochemical analysis of *Simarouba glauca* Dc. and Comparison of its Bioactivity. *Asian Journal of Immunology.* 2019;2(1):1-11.

36. Mansour M. Nitrogen containing compounds and adaptation of plants to salinity stress. *Biologia Plantarum*. 2000; 43:491-500.
37. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*. 2007;59:207-216.
38. Wagh SG. Comparative updates on host-induced gene silencing and CRISPR-Cas9 utilization for improved disease resistance in crops. *Research Journal of Biotechnology*; 2020.
39. Rathod HP, Pohare MB, Bhor SA, Jadhav KP, Batule BS, Shahakar SB, Wagh SG, Wadekar HB, Kelatkar SK, Kulkarni MR. In vitro micro propagation of blue passion flower (*Passiflora caerulea* L.). *Trends in Biosciences*. 2014;7(19):3079-3082.
40. Daspute AA, Yunxuan XI, Gu MI, Kobayashi Y, Wagh SG, Panche A, Koyama H. *Agrobacterium rhizogenes* mediated hairy roots transformation as a tool for exploring Aluminum responsive genes function. *Future Science OA*. 2018; 5(3):205-218.

© 2020 Sidhikki et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/55505>