



EFFECT OF SALICYLIC ACID ON MORPHOLOGICAL, BIOCHEMICAL AND ANTIOXIDANT PARAMETERS OF MUNGBEAN (*VIGNA RADIATA* L.) UNDER SALT STRESS

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Abstract

The pot experiment was conducted at Field Experimentation Center, Department of Biological sciences, Sam Higginbottom University of Agriculture, Technology & Sciences, Uttar Pradesh during summer season 2016-2017 with Mungbean varieties (NM-98, NM-54). Effect of Salicylic acid under Salt stress condition on Mungbean with five treatment and three replications were laid out in complete randomized Design. This research is based on the Salt stress negatively effect photosynthesis by causing excess accumulation of leaf Na^+ and Cl^- stomatal closure and oxidative stress resulting in the formation of reactive oxygen species (ROS). Exogenously application of Salicylic acid to minimize the Na^+ , Cl^- and ROS contents in Salt stress. Different Concentration of salicylic acid (0.5, 1.0, 1.5 mM) shows different result on Plant growth, Biochemical and Antioxidant under salt stress (75 NaCl mM). Salicylic acid concentration (0.5 mM) show best result in all the growth and yield parameter.

Key words : Salicylic acid, SOD (Super oxide dismutase), MDA (Malondialdehyde), APOX (Ascorbate peroxidase), H_2O_2 (Hydrogen peroxide).

Introduction

Mungbean (*Vigna radiata* L.) commonly called green gram is a crop of leguminosae family. Mungbean takes less time to mature can be cultivated during spring and winter season and fits well in existing cropping pattern of the country. India producing 14.76 million tons of pulses from an area of 23.63 million hectare which is one of the largest pulses producing in the world. In India, mungbean is cultivated in area of 3.38 million hectares with an average productivity of 4.74 qt / ha and production of 1.61 million tonnes. In Uttar Pradesh green gram is cultivated area of 0.72 lakh hectares with an average productivity of 5.5 qt/h. (IIPR Annual Report, 2014-2015). It is also popular for its nutritive value and digestibility containing higher protein contents (28%), fat (1.3%), carbohydrates (60.4%) and reasonable amount of vitamins and essential micronutrients (Akhtar *et al.*, 2013).

Salinity in farms that are in arid and semi-arid areas in almost all the regions of the world is a major problem.

Salt stress is a major abiotic stress that causes detrimental effects on plant growth and productivity (Syed *et al.*, 2011). Salt stress has a triple effect on plant growth. First, it decreases water absorption (osmotic effect). Second, it leads to an imbalance ion and third, it results in plant toxicity ionic effect. Ionic imbalance occurs in the cells due to excessive accumulation of Na^+ and Cl^- ions and reduced uptake of mineral nutrients such as K^+ , Ca^{2+} and Mn^{2+} (Yusuf *et al.*, 2012). Salt stress may negatively affect photosynthesis by causing excess accumulation of leaf Na^+ and Cl^- stomatal closure and oxidative stress resulting in the formation of reactive oxygen species (ROS). Excessive amounts of ROS can enhance membrane lipid peroxidation and electrolyte leakage (Xiaohua *et al.*, 2017).

Salicylic acid a phenolic compound, plays prominent and diversified functions in plant protection under salt stress (Hayat *et al.*, 2012). Salicylic acid is a signal transduction cascades, involved in plant defense mechanisms in response to stress (Wang *et al.*, 2016). Salicylic acid acts as a plant growth regulator and plays an important role in modulating the plant responses to salinity

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(Nazar *et al.*, 2011). Exogenously applied SA was shown to minimize the Na⁺, Cl⁻ and ROS contents, which were increased during salinity stress and application influences a wide variety of plant processes, including stomatal regulation, chlorophyll content, photosynthesis.

Present study is aimed to understand the effectiveness of salicylic acid on growth and yield of Mungbean (*Vigna radiata* L.) under salt stress condition with the objective. To study the effect of salicylic acid on growth and yield parameter in mungbean crop under salt stress condition.

Materials and Methods

The experiment was carried out during summer season 2017, Department of Biological Sciences, SHUATS, Allahabad (U.P.) which is located at 25° 24' 42" N latitude, 81° 50' 56" E longitude and 98 m altitude above the mean sea level. The experiment was laid out in CRD design with five treatments with three replications and out of 2 varieties of mungbean, 3 plants will be tagged randomly for the purpose of Morphological, Biochemical and Antioxidants studies. Treatments will be denoted as T0, T1, T2, T3 and T4 replications as R1, R2 and R3 for variety NM-98 and variety NM-54 treatment will be denoted.

Treatments detail

Treat-ment	Chemical	Concent-ration (mM)	Mode of application	Duration
T0	Control	-	-	-
T1	NaCl	75	Soil	Before sowing
T2	Salicylic acid + NaCl	0.5+75	Foliar	25 day after sowing
T3	Salicylic acid + NaCl	1.00+75	Foliar	25 day after sowing
T4	Salicylic acid + NaCl	1.5+75	Foliar	25 day after sowing

Observations

Growth parameters

Plant height (cm) : The plant height from ground surface to the tip of the main stem was measured. Average height of the randomly selected three plants was recorded and expressed as plant height in centimeters.

Number of primary branches per plant

The total number of branches arising directly from the main stem was counted at the time of harvest.

Relative water content (%)

The relative content was estimated by the method of

Barrs and Weatherly (1962). Ten leaf discs were collected randomly in each treatment and weighed accurately up to third decimal on a single pan analytical balance. This was considered as fresh weight. The weighed leaf discs were allowed to float on distilled water in a petridish and allowed to absorb water for four hours. After four hours, the leaf discs were taken out and their surface was blotted gently and weighed. This was referred to as turgid weight. After drying in hot air oven at 72°C for 48 hours, the dry weight was recorded and RWC was calculated by using the following formula.

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}}$$

Yield Parameter

Number of pods per plant

The pods of individual plants were counted and average of five plants was recorded as number of pods per plant.

Seed yield per plants (gm)

The matured pods harvest from three randomly selected and tagged plants in each Treatment were sun dried and the seed separated. The average was worked out and expressed as seed yield per plant in gram.

Biochemical Parameter

Chlorophyll content

Chlorophyll was determined according to Wellborn (1983). 1 gram leaves sample was weighed and crushed with 80% acetone made the volume to 10 ml with 80% acetone, centrifuged at 800 ppm for 5 minutes. The supernatant was read under 663, 645 nanometre. The readings were fed in the following formula and results were determined under spectrophotometer.

Chlorophyll content was calculated by using the following formula and expressed in mg g fresh weight⁻¹:

$$\text{Chlorophyll 'a'} = 12.7 \times (A_{663}) - 2.69 \times (A_{645}) \frac{V}{1000 \times w \times a}$$

(mg g⁻¹ fr. wt.)

$$\text{Chlorophyll 'b'} = 22.9 \times (A_{645}) - 4.68 \times (A_{663}) \frac{V}{1000 \times w \times a}$$

(mg g⁻¹ fr. wt.)

where,

A645 = Absorbance of the extract at 645 nm

A663 = Absorbance of the extract at 663 nm

a = Path length of cuvette (1 cm)

V = final volume of the chlorophyll extract (10 ml)

W = Fresh weight of the sample (0.10 g)

Carotenoid content

Carotenoid was determined according to Wellborn (1983). 0.5 gm and homogenized in 10 ml of acetone (80% acetone). Next to the centrifuged at 3000 rpm at 10 min. The absorbance was recorded at 470 nm.

It is calculated by the formula –

Total carotenoids = $[1000A_{470} - (3.27 \text{ Chl-a} + 104 \text{ Chl-b})] / 229$

Protein standard

Estimation of protein was done by pipetting out 50 μ l supernatant containing proteins into test tubes in replicates of three and the total volume was made up to 1 ml. A tube with 1 ml distilled water served as a blank. 3 ml reagent C was added to each tube including the blank and after proper mixing the solution were allowed to stand for 30 min then 0.5 ml reagent D was added and after mixing, the tubes were left at room temperature in the dark for 60 min. Blue colour was developed in the solution. The absorbance was taken at 660 nm in UV-visible spectrophotometer.

A standard graph was drawn and the amount of protein in the sample was calculated and expressed as mg/gm or percentage.

Determination of proline

Proline content was determined by the method adopted by Bates *et al.* (1973). 0.5g of flesh leaf sample was homogenized in 10ml of 3% aqueous sulphosalicylic acid and the homogenate was filtered using what man's No.1 filter paper. Two ml filtrate was taken in a test tube and 2 ml of acid ninhydrin and 2ml of glacial acetic acid was added. This was allowed to react for 1 hour at 100°C in a boiling water bath. The reaction was terminated by placing the tube in an ice box. 4 ml of was added to the reaction mixture. The chromophore containing toluene was separated and absorbance was recorded at 520 nm wavelength using toluene as blank.

It is calculated by the formula:

$[(\mu\text{g proline/ml X ml toluene}) / 115.5 \mu\text{g}/\mu\text{mole}] / [(g \text{ sample}) / 5] = \mu\text{moles proline/g of fresh weight}$

Determination of Hydrogen Peroxide (H₂O₂)

To estimate the hydrogen peroxide, content the method given by Velikovo *et al.* (2000) was used in this present investigation.

500 mg of leaf tissue was homogenated with 5.0 ml of Trichloro Acetic acid (TCA) and cooled immediately in ice bath. Then it is centrifuged at 12000g for 15 min and the supernatant was collected. 0.5 ml of supernatant was transferred to test tube and 0.5 ml of 10mM

phosphate buffer (pH7.0) AND 1.0 ml of potassium iodide (1M) were added. After the addition, the solution was mixed vigorously and the absorbance was recorded at 390 nm. H₂O₂ content was determined by using an extinction coefficient (E) of 0.28 $\mu\text{m}^{-1}\text{cm}^{-1}$ and expressed as n mol g⁻¹ fw.

Determination of Peroxidase determination

Phosphate buffer 100 mM.

Solution A : contains 6.8 g of KH₂PO₄ in 500 ml of distilled water.

Solution B : contains 8.71g of K₂HPO₄ in 500 ml of distilled Water.

Phosphate buffer was prepared by mixing 87 ml of solution A and 15 ml of solution B. And the pH was set to 6.1.

It was prepared by dissolving 124 μ l of 30% H₂O₂ (30 ml H₂O₂ in 70 ml DW) in distilled water where the final volume was made up to 100ml.

Guaicol (96 mM)

It was prepared by dissolving 1075 μ l of guaicol in distilled water, where the final volume was made up to 100 ml.

Procedure

The reaction mixture was prepared by adding 1.0 ml of phosphate buffer, 0.5 ml of guaicol, and 0.5 ml of H₂O₂, 0.1 ml of crude enzyme and 0.9 ml of distilled water. And the absorbance was measured at 470 nm.

Determination of Ascorbate peroxidase determination (APX)

Ascorbate peroxidase is one of the most widely distributed antioxidant enzyme, which reduce hydrogen peroxide to water using reduced ascorbate as the electron donor. It plays a more important role in scavenging ROS than other antioxidative enzyme since ascorbate, in addition to reacting with H₂O₂, may react with superoxide, singlet oxygen and hydroxyl radical.

Reagents

1. 100 mM potassium phosphate buffer (pH7.0)
2. 15 mL-ascorbate
3. 10 mM H₂O₂

Procedure

APX is assayed by the method of Nakano and Asada (1981).

1. Set up a reaction mixture (3.0 ml) by adding 2.7 ml of 100mM potassium phosphate buffer (pH 7.0). 0.1 ml L-ascorbate and 0.15 ml H₂O₂.

2. Initiate the reaction by adding 50 μ l of enzyme extract and record the decrease in absorbance at 290 nm spectrophotometrically for 2 min against blank which corresponds to the oxidation of ascorbic acid.

3. Calculation the enzyme activity using the molar extinction coefficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ for ascorbate. One enzyme unit is expressed as the amount required to oxidize one nmole of ascorbate min^{-1}

Determination of Leaf Malondialdehyde (MDA)

It is determined by using the method given by CakMak and Horst (1991). 500mg of leaf sample is homogenated in 5 ml of 0.1% (w/v) TCA and centrifuged at 10000 g for 20 min. 0.5 ml supernatant was taken in a test tube and 0.1 ml of 0.5% (w/v) thiobarbituric acid 20% TCA was added and incubated in boiling water bath for 30 mins. The reaction was stopped by placing in ice bath. And the reaction mixture was centrifuged at 10000g for 5 min and the absorbance of the supernatant was recorded at 532 nm.

Determination of Superoxide Dismutase (SOD)

Determination of Superoxide Dismutase SOD by Dhindsa (1981) method.

Reagents

1. Methionine (200 mM)
2. Riboflavin solution (60 μ M)
3. Nitro -blue tetrazolium solution (2.25mM)
4. EDTA disodium solution (3.0 mM)
5. Phosphate buffer (50 Mm; pH 7.0)
6. Phosphate buffer (50Mm; pH7.8)

Sol A: Potassium dihydrogen phosphate 6.80g was dissolved in water and the volume was made up to 500 ml with double distilled water.

Sol B: Di- potassium hydrogen phosphate 8.71 g was dissolved in water and the volume was made up to 500 ml with double distilled water.

Mix 8.5 ml of sol. A and 91.5 ml of sol. B and final PH was adjusted with the help of PH meter.

Grinding media: (0.1 M phosphate buffer, Ph 7.5, containing 0.5 mM EDTA) in case of SOD, EDTA 0.0186 g is dissolved in phosphate buffer 0.1 M, Ph 7.5 (made by mixing 16 ml of sol. A and 84 ml of sol. B and final pH is adjusted with the help of pH meter) and volume is made to 100 ml with the buffer.

Complete reaction mixture plus KCN 3mM (0.1ml of 90mM sol.) was used to inhibit Cu/Zn-SOD

Complete reaction mixture plus 3 mM KCN (0.1 ml

of 90 mM sol.) and 5 mM H₂O₂ (0.1 ml of 150 mM sol.) were used to inhibit both Cu/Zn- SOD and Fe SOD activities. Separate controls (lacking enzyme) were used for total SOD and inhibitor studies. The absorbency was recorded at 560nm, and one unit of enzyme activity was taken as that amount of enzyme, which reduced the absorbency reading to 50% in comparison with tubes lacking enzyme.

$$\text{Unit (of enzyme)} = \frac{\text{Blank} - \text{Sample}}{\text{Blank}}$$

Results

The experimental results clearly indicating that variety NM-98 showed better resistance as compared to NM-54. Both variety showed good result in T2 treatment of 30, 60 and 90 DAS respectively and minimum height was found in T1 at salt stress and salicylic acid concentration level in both the variety similar results clearly indicating that variety NM-54 showed better resistance in number of primary branches/ plant when it was compared with NM-98. In over all the treatment T2 (salicylic acid concentration 0.5mM) showed best response.

The number of pods per plant better in variety NM-54 as compared variety NM-54. Both variety showed good result in T2 treatment and minimum number of pods per plant was found in T1 at salt stress. The results are clearly indicating that the effect of salicylic acid under salt stress on seed yield per plant (g). There was a reduction has been seen in salt level increases but the NM-54 showing resistance when compared to NM-98. With the increased salt level, the relative water content (%) gradually decreased. Both the variety equally affected levels of salt . In overall the treatment best result was found in T2 treatment salicylic acid and minimum relative water content (%) has been observed in T1 of both the variety. These results also indicating that there is some resistance shown by NM-98 when compared to the NM-54.

From table 3, it is confirmed that there is a significant reduction in chlorophyll a 30 and 60 DAS content (mg/g fw) was observed in salt stress in effect of salicylic acid. The performance was good in T2 of both the variety and minimum performance was given by T1 in both variety. Although highest chlorophyll a in 30 and 60 DAS was observed in NM-98 compared to NM-54. Similar in chlorophyll b performance was good in T2 of both the variety and minimum performance was given by T1 in both variety. Highest chlorophyll b in 30 and 60 DAS was observed in NM-98 compared to NM-54.

The experimental results clearly indicating that there

Table 1 : Plant height(cm) at 30, 60 and 90 days and number of primary branches shows as effects of salicylic acid under salt stress condition.

Treatments	Plant height 30DAS		Plant height 60DAS		Plant height 90DAS		No. of primary branches	
	NM-98	NM-54	NM-98	NM-54	NM-98	NM-54	NM-98	NM-54
T0	23.84	22.82	40.53	39.36	47.21	46.95	4.47	4.53
T1	19.61	18.65	30.55	29.21	43.11	42.89	3.34	3.37
T2	22.81	21.82	38.73	37.31	46.17	45.98	4.22	4.39
T3	21.75	20.64	36.69	35.12	45.15	44.96	4.15	4.25
T4	20.54	19.54	34.54	33.81	44.12	43.87	3.99	4.04
mean	21.71	20.54	36.21	34.96	45.15	44.93	4.03	4.12
F5%	S	S	S	S	S	S	S	S
SEm±	0.027	0.017	0.111	0.361	0.020	0.044	0.061	0.012
CD5%	0.084	0.054	0.349	1.139	0.062	0.139	0.192	0.037

T0- control, **T1**- 75mM NaCl, **T2**- 0.5mM Salicylic acid+75mM NaCl, **T3**- 1.0mM Salicylic acid+75mM NaCl, **T4**- 1.5mM Salicylic acid+75mM NaCl, **S**- significant

Table 2 : Number of pods per plant, Seed yield per plant(g) and Relative water content(%) shows as effects of salicylic acid under salt stress condition.

Treatment	No.of pods per plant		Seed yield per plant(g)		Relative water content(%)	
	NM-98	NM-54	NM-98	NM-54	NM-98	NM-54
T0	13	14	4.16	4.30	72.13	71.78
T1	8	9	3.07	3.15	68.14	67.75
T2	12	12.67	4.13	4.23	71.23	70.86
T3	10	10.33	3.96	4.13	70.27	69.83
T4	9	9.33	3.60	3.79	69.32	68.87
Mean	10.47	11.07	3.78	3.92	70.22	69.82
F5%	S	S	S	S	S	S
SEm±	0.869	0.775	0.063	0.044	0.025	0.020
CD5%	2.739	2.441	0.200	0.137	0.079	0.062

T0- control, **T1**- 75mM NaCl, **T2**- 0.5mM Salicylic acid+75mM NaCl, **T3**- 1.0mM Salicylic acid+75mM NaCl, **T4**- 1.5mM Salicylic acid+75mM NaCl, **S**- significant.

is a significant reduction in carotenoid content (mg/g FW) 30 and 60 DAS was observed in salt stress in effect of salicylic acid. From the overall results, the performance was good in T2 of both the variety and minimum performance was given by T1 in both variety and confirmed that there is a significant reduction in protein content (%) was observed in salt stress in effect of salicylic acid. From the overall results, the performance was good in T2 of both the variety and minimum performance was given by T1 in both variety. Although, highest protein content (%) was observed in NM-98 compared to NM-54. From table 4, it is confirmed that there is a significant reduction in Proline ($\mu\text{g/g}$ of fw) was observed in salt stress in effect of salicylic acid. From the overall results, the performance was good in T2 of both the variety and minimum performance was

given by T0 in both variety. Although, highest Proline ($\mu\text{g/g}$ of fw) was observed in NM-98 compared to NM-54.

From table 5, it is confirmed that there is a significant reduction in Ascorbate peroxidase ($\mu\text{mol/mg}$ fw) was observed in salt stress in effect of salicylic acid. From the overall results, the performance was good in T2 of both the variety and minimum performance was given by T0 in both variety. Although highest Ascorbate peroxidase ($\mu\text{mol/mg}$ fw) was observed in NM-98 compared to NM-54. In present investigation, the exogenous application of 0.5 mM SA to salt stress plants resulted in a significant increase in activity of antioxidant in the leaves and there is a significant reduction in Hydrogen peroxide H_2O_2 was observed in salt stress in effect of salicylic acid. From the overall results, the

Table 3 : Chlorophyll a and Chlorophyll b at 30 and 60 DAS shows as effects of salicylic acid under salt stress condition.

Treatments	Chlorophyll a (mg/g FW) 30 DAS		Chlorophyll a (mg/g FW) 60 DAS		Chlorophyll b (mg/g FW) 30 DAS		Chlorophyll b (mg/g FW) 60 DAS	
	NM-98	NM-54	NM-98	NM-54	NM-98	NM-54	NM-98	NM-54
T0	1.42	1.36	1.28	1.24	0.98	0.97	0.96	0.95
T1	1.09	1.02	0.98	0.94	0.90	0.89	0.88	0.87
T2	1.37	1.31	1.22	1.18	0.96	0.95	0.94	0.93
T3	1.28	1.22	1.20	1.16	0.94	0.93	0.92	0.91
T4	1.17	1.11	1.15	1.12	0.92	0.91	0.90	0.89
Mean	1.27	1.20	1.17	1.13	0.94	0.93	0.92	0.91
F5%	S	S	S	S	S	S	S	S
SEm±	0.014	0.016	0.017	0.016	0.002	0.002	0.002	0.003
CD5%	0.045	0.052	0.054	0.052	0.006	0.005	0.007	0.009

T0- control, **T1**- 75mM NaCl, **T2**- 0.5mM Salicylic acid+75mM NaCl, **T3**- 1.0mM Salicylic acid+75mM NaCl, **T4**- 1.5mM Salicylic acid+75mM NaCl, **S**- significant

Table 4 : Carotenoid(mg/g FW), Protein(%) and Proline($\mu\text{g/g}$ of fw) shows as effects of salicylic acid under salt stress condition.

Treatments	Carotenoid (mg/g FW) 30 DAS		Carotenoid (mg/g FW) 60 DAS		Protein content from seed (%)		Proline content ($\mu\text{g/g}$ of fw)	
	NM-98	NM-54	NM-98	NM-54	NM-98	NM-54	NM-98	NM-54
T0	6.78	6.75	5.71	5.67	23.53	22.88	0.67	0.65
T1	6.11	6.09	4.98	4.94	19.55	18.76	0.79	0.76
T2	6.64	6.61	5.50	5.46	22.77	21.93	0.86	0.84
T3	6.44	6.41	5.31	5.27	21.70	20.83	0.83	0.80
T4	6.26	6.23	5.15	5.11	20.63	19.86	0.80	0.77
Mean	6.45	6.42	5.33	5.29	21.64	20.85	0.79	0.77
F5%	S	S	S	S	S	S	S	S
SEm±	0.007	0.014	0.022	0.018	0.060	0.042	0.009	0.005
CD5%	0.023	0.045	0.068	0.056	0.188	0.132	0.028	0.016

T0- control, **T1**- 75mM NaCl, **T2**- 0.5mM Salicylic acid+75mM NaCl, **T3**- 1.0mM Salicylic acid+75mM NaCl, **T4**- 1.5mM Salicylic acid+75mM NaCl, **S**- significant.

performance was good in T2 of both the variety and minimum performance was given by T0 in both variety. Although highest Hydrogen peroxide H_2O_2 was observed in variety NM-98 compared to variety NM-54. Significant reduction in Malondialdehyde (MDA) (nmol/g fw) was observed in salt stress in effect of salicylic acid. From the overall results, the performance was good in T2 of both the variety and minimum performance was given by T0 in both variety. Although, highest Malondialdehyde (MDA) (nmol/g fw) was observed in NM-98 compared to NM-54. The significant reduction in Super Oxide Dismutase (SOD) (unit/g fw) was observed in salt stress in effect of salicylic acid. From the overall results, the performance was good in T2 of both the variety and minimum performance was given by T0 in both variety. Although highest Super Oxide Dismutase (SOD) (unit/g

fw) was observed in NM-54 compared to NM-98. Superoxide dismutase (SOD) is the most efficient intracellular enzymatic antioxidant that is found in all aerobic organisms and in all subcellular compartments subject to ROS-mediated oxidative stress.

Discussion

Growth parameters

Vegetative growth (*viz.*, plant height, number of primary branches, Relative water content) mungbean plants were lower at salt stress treatment as compared to normal conditions. However, exogenous Salicylic acid applications increased these parameters as compared to plant which treated with only salt. The application of 0.5 mM Salicylic acid under salt stress gave the higher values for these parameters than the other treatments except

Table 5 : Ascorbate peroxidase ($\mu\text{mol}/\text{mg fw}$), Hydrogen peroxide ($\text{nmol}/\text{g fw}$), Malondialdehyde (MDA)($\text{nmol}/\text{g fw}$) and Super Oxide Dismutase ($\text{unit}/\text{g fw}$) shows as effects of salicylic acid under salt stress condition.

Treatments	APX ($\mu\text{mol}/\text{mg fw}$)		H_2O_2 ($\text{nmol}/\text{g fw}$)		MDA ($\text{nmol}/\text{g fw}$)		SOD ($\text{unit}/\text{g fw}$)	
	NM-98	NM-54	NM-98	NM-54	NM-98	NM-54	NM-98	NM-54
T0	0.55	0.52	0.65	0.67	0.76	0.72	1.08	1.12
T1	0.89	0.87	1.06	1.08	0.94	0.91	1.12	1.18
T2	1.14	1.09	1.32	1.34	1.37	1.33	1.62	1.68
T3	1.06	1.02	1.23	1.25	1.27	1.24	1.44	1.50
T4	0.97	0.94	1.18	1.20	1.16	1.12	1.34	1.41
Mean	0.92	0.89	1.09	1.11	1.10	1.07	1.32	1.38
F5%	S	S	S	S	S	S	S	S
SEm\pm	0.023	0.006	0.015	0.012	0.019	0.018	0.032	0.052
CD5%	0.074	0.020	0.048	0.037	0.059	0.056	0.101	0.163

T0- control, **T1**- 75mM NaCl, **T2**- 0.5mM Salicylic acid+75mM NaCl, **T3**- 1.0mM Salicylic acid+75mM NaCl, **T4**- 1.5mM Salicylic acid+75mM NaCl, **S**- significant.

control. This positive effect of SA could be attributed to an increased CO_2 assimilation and photosynthetic rate and increased mineral uptake by the stressed plant under SA treatment (Karlidag *et al.*, 2009). The effects of salt on inhibition of cell division and elongation or due to the accumulation of Na^+ in plant tissues and creates an oxidative stress (Ahmad *et al.*, 2016). foliar SA application can increase the leaf diffusive resistance and lower transpiration rates and protect relative ware content.

Yield parameter

Yield parameter (number of pods per plant, seed yield per plant) mungbean plants were lower at salt stress treatment as compared to normal conditions. However, exogenous Salicylic acid applications increased these parameters as compared to plant which treated with only salt. The application of 0.5 mM Salicylic acid under salt stress gave the higher values for these parameters than the other treatments except control. Exogenous application of salicylic acid prevented the lowering of IAA and cytokinin levels in salinity stressed wheat plants resulting in the better of cell division in root apical meristem, thereby increasing yield and productivity of plants (Hayat *et al.*, 2010). Salicylic acid induce salinity tolerance by increasing yield was already reported in wheat (Tufail *et al.*, 2013). Salicylic acid could be used as a potential growth regulator to improve plant salinity stress tolerance (Hussein *et al.*, 2007).

Biochemical parameters

Biochemical parameters (Chlorophyll a, chlorophyll b, carotenoide, protein) mungbean plants were lower at salt stress treatment as compared to normal conditions. However, exogenous Salicylic acid applications increased these parameters as compared to plant which treated

with only salt. The application of 0.5 mM Salicylic acid under salt stress gave the higher values for these parameters than the other treatments except control. Higher concentrations of sodium chloride (NaCl) decreases plant height, chlorophyll, carotenoide and protein contents (Wani *et al.*, 2016). The chlorophyll content of soybean leaves was increased due to application of salicylic acid (Khan *et al.*, 2003). Salinity caused a marked reduction in photosynthetic pigments including chlorophyll a, chlorophyll b and carotenoide in fenugreek (Jaiswal *et al.*, 2016). Decrease in protein might be due to the higher salt levels that plays a role in osmotic imbalance (Ahmad *et al.*, 2016). Foliar application of salicylic acid where an enhanced level of proline was found under salt stress.

Antioxidant parameters

Antioxidant parameters (Proline, APX, H_2O_2 , MDA, SOD) mungbean plants were lower at salt stress treatment as compared to normal conditions. However, exogenous Salicylic acid applications increased these parameters as compared to plant, which treated with only salt. The application of 0.5 mM Salicylic acid under salt stress gave the higher values for these parameters than the other treatments except control. Reductions of MDA, proline, SOD and H_2O_2 in SA treated seedlings under salt stress were observed in other plant studies (Hayat *et al.*, 2012). ROS acts as a signal molecule and plants initiate antioxidant mechanism for protection against ROS (Nazar *et al.*, 2011). The most suggested role of Salicylic acid is its involved in scavenging ROS and in protecting antioxidant enzymes. Increasing SOD activity under macromolecules which reflected on inactivating enzymes, SA treatment protected membrane stability from salt-

induced oxidative damage. In addition to SOD, increasing the protection level against oxidative require fast removal of H₂O₂ (Osman *et al.*, 2016).

Conclusion

Based on present study, it was concluded that Salt stress deleterious to Mungbean growth and yield. The exogenous application of salicylic acid (0.5 mM) improved the growth, yield, biochemical and antioxidant parameters by decreasing the harmful effect of Salt stress with compared to the other treatments.

The result shows that NM-98 was observed higher ameliorating effect on Growth, yield, Biochemical and antioxidant of mungbean with T2 (salicylic acid concentration 0.5mM) in compare to NM-54 and all other treatments under Salt stress and the T3 (salicylic acid concentration 1.0mM) also showed better response but T1 (NaCl only) and T4 (salicylic acid concentration 1.5mM) found to hamper all above parameters.

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