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Effect of foliar application of salicylic acid on growth, yield, physiological and biochemical characteristics of mung bean (*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 2018 with Mung bean varieties PDM-139 (Samrat) and HUM-12 (Malviya Jagriti). Effect of Salicylic acid under Salt stress condition on Mung bean with seven treatments and three replications were laid out in complete randomized Design. This research was undertaken to asses the impact of 100 ppm and 150 ppm of Salicylic acid (SA) on alleviation of oxidative, ionic and osmotic stress of different concentration levels of salt stress (0, 100, 150 mM Nacl, respectively). Salinity caused, the level of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) and accumulation of Na^+ ions in plant parts to increase and decreased the phosynthetic pigment, relative water content, plant growth and yield. Exogenous foliar spray of SA increased the level of antioxidant enzymes activities [Peroxidase (POX), Proline, Ascorbate Peroxidase (APX)], photosynthetic pigments, plant growth and yield. Salicylic acid (SA) concentration @ 100 ppm shows the best result in all the growth and yield parameters.

Keywords: Salicylic acid (SA), hydrogen peroxide (H_2O_2), ascorbate peroxidase (APX), peroxidase (POX)

Introduction
Mung bean (*Vigna radiate* L.), alternatively known as the Moong bean, Monggo, Green gram, or Mung is a plant species in the legume family. It belongs to the genus vigna. It is said to have originated from India and must have been derived from var. *sublobata* which occurs wild throughout India and Burma (Aykroyd and Doughty, 1964). In India Mung bean is cultivated in area of 3.38 million hectares with an average productivity of 4.7 qt / ha and production of 1.61 million tonnes. In Uttr Pradesh green gram is cultivated in an area of 0.72 lakh hectares with an average productivity of 5.5 q/ ha and production of 0.40 lakh tonnes (IIPR, Annual Report, 2015 – 2016). Mung bean has multipurpose uses as it is an excellent source of high quality protein (25%) having high digestibility. It is a good source of Riboflavin, Thiamine and Vitamin C (Ascorbic acid). It is also used as green manure crop and feed for cattle.
Salinity stress is one of the most atrocious environmental factors restricting the productivity of Mungbean in arid and semiarid regions. Salt stress is a major abiotic stress that causes detrimental effect on plant growth and productivity (Syeed *et al.*, 2011) [13]. Salt toxicity inhibits the plant growth and development and can also lead to physiological water limitation due to severe salt deposition in rhizosphere causing a low osmotic potential and ion imbalance (Zhu, 2002, Munns & Tester, 2008) [16, 10]. Plants can maintain an osmotic equilibrium by the synthesis of compatible solutes such as prolines, glycine betaine, proteins and soluble carbohydrates (Abriz and Torabian, 2017). High level of Na^+ can affect chlorophyll production by preventing chlorophyll synthesis or by accelerating its degradation thus, decreases photosynthesis. Extreme exposure of crops to Na+ toxicity can lead to the production of Reactive Oxygen Species (ROS) such as $O2\bullet^-$ (superoxide radical), H_2O_2, •OH (hydroxyl radical) and ½ O2 (singlet oxygen) which in turn gives toxic responses like lipid peroxidation, DNA mutation and protein degradation. Salt stress induction of ROS accumulation is defused by antioxidant enzyme systems such as Catalase (CAT), Peroxidise (POX), Superoxide Dismutase (SOD), Ascorbate Peroxidise (APX) and Polyphenol Oxidase (PPO) (Mittler *et al.*, 2004) [9].
Salicylic acid (SA) is a hormone-like endogenous growth regulator playing defensive role against biotic and abiotic stress. It is of phenolic nature, which participates in regulation of physiological processes in plant such as growth, photosynthesis, nitrate metabolism, ethylene production, heat production, Flowering and also provides protection against biotic and abiotic

stresses such as salinity (Hayat *et al*., 2010) [4]. Salicylic acid application may alleviate the toxic effect of salt stress due to its significant role in stomatal regulation, growth yield and photosynthesis (Arfan *et al*., 2007) [2]. It has been recognised as an endogenous natural signal molecule involved in defense mechanisms by regulating biochemical process (Hadi *et al*., 2014). Salicylic acid enhancing the endogenous antioxidant levels in plants which resulted in better protection against oxidative stress. Several new roles have been reported for SA. These include, mediation of the response to different environmental stresses such as salinity and drought (Golezani and Mahootchi, 2015) [3]. Foliar spray of Salicylic acid enhances photosynthetic activity and plant growth under salinity (Khan *et al*., 2014). Therefore, keeping in view the positive effect of Salicylic acid on plants under salt stress, the present study has aimed to understand the effectiveness of foliar application of Salicylic acid on plants growth, yield, physiological and biochemical characteristics of Mungbean (*Vigna radiata* L.) under salt stress condition.

Materials and Methods

The present investigation entitled-Effect of foliar application of Salicylic acid on growth, yield, physiological and biochemical characteristics of Mung bean (*Vigna radiata* L.) under salt stress‖ was carried out at Department of Biological Sciences, Faculty of Sciences, Sam Higginbottom University of Agriculture, Technology and Science Allahabad (U.P) during the year 2018. The experiment was laid out in Completely Randomized Design comprising of seven treatments with two varieties, PDM-139 (Samrat) and HUM-12 (Malviya Jagriti) of Mung bean each replicated thrice. The seeds of Mung bean varieties were sown separately on 1st March in the pots with soil. The first and second observation was taken at 30 and 60 days after sowing (DAS) that was on 31th March and 30th April 2018 respectively, the observations were recorded on growth, physiological and biochemical parameters. At 90 DAS that was on 30th May 2018, yield parameters were taken. The manures and fertilizers and the recommended dose of fertilizers (RDF- 60kg N + 30 kg P_2O_5 + 30 kg K_2O ha^{-1}) was applied to the pot before sowing in all pots uniformly before sowing. All the fertilizers were applied in a single dose at the time of sowing in the pots as basal dose. Salt with two different concentration were incorporated in the pots before sowing according to the treatments and the foliar application of two different concentration of Salicylic acid were done at 25 and 45 DAS according to the treatments. Seven treatments were included in the trial were *viz*; T_0 (Control), T_1 (NaCl 100mM), T_2 (NaCl 150mM), T_3 (Salicylic acid 100 ppm + NaCl 100 mM), T_4 (Salicylic acid 100 ppm + NaCl 150 mM), T_5 (Salicylic acid 150 ppm + NaCl 100 mM), T_6 (Salicylic acid 150 ppm + NaCl 150 mM).

Result and Discussion

The results of the investigation, regarding the effect of foliar application of Salicylic acid on growth, yield, physiological and biochemical characteristics of Mung bean (*Vigna radiata* L.) under salt stress have been presented in tables wherever required. The result of the experiment has been presented under the following heading.

Growth Parameters

Vegetative growth {*viz*., plant height (cm) and number of leaves} in Mung bean plants were lowered with the increase in the concentration of salt treatment as compared to normal conditions. However, exogenous Salicylic acid applications increased these parameters as compared to plant which treated with only salt. The experimental results are clearly indicating that the variety HUM-12 (Malviya Jagriti) had shown better resistance as compare to the variety PDM-139 (Samrat). Both the varieties had shown good result in T3 (Salicylic acid 100 ppm + NaCl 100 mM) nearly as T_0 (control). As compare to Salicylic acid (SA) @ 150 ppm the application of SA @ 100 ppm on plants under two different salt stresses (100 and 150 mM) gave the higher values for these parameters. 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 (Nirmala *et al*. 2015) [12]. Salicylic acid on salt treated plants induces salt tolerance in plant by reducing ionic and osmotic stress and inducing plant growth. Similar findings have been recorded by Tufail *et al*., (2013) [15] and Akhtar *et al*., (2013) [1].

Yield Parameters

Yield parameter {*viz*., number of pods per plant and seed yield per plant (g)} in Mung bean plants were lowered with the increase in the concentration of salt treatment as compared to normal conditions. However, exogenous Salicylic acid applications increased these parameters as compared to plant which treated with only salt. The experimental results are clearly indicating that the variety HUM-12 (Malviya Jagriti) had shown better resistance as compare to the variety PDM-139 (Samrat). Both the varieties had shown good result in T3 (Salicylic acid 100 ppm + NaCl 100 mM) nearly as T_0 (control). As compare to Salicylic acid (SA) @ 150 ppm the application of SA @ 100 ppm on plants under two different salt stresses (100 and 150 mM) gave the higher values for these parameters. 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) [4]. Application of Salicylic acid in salt treated Mung bean plants were beneficial which may be due to its influence on translocation of CO_2 assimilation into the seeds and enhancement of photosynthetic rate. Similar findings were reported by Khan *et al*., (2003) [8] and Karlidag *et al*., (2009) [7].

Physiological Parameters

Physiological parameters {*viz*., relative water content (%) and total chlorophyll content (mg/g FW)} in Mung bean plants were lowered with the increase in the concentration of salt treatment as compared to normal conditions. However, exogenous Salicylic acid applications increased these parameters as compared to plant which treated with only salt. The experimental results are clearly indicating that the variety HUM-12 (Malviya Jagriti) had shown better resistance as compare to the variety PDM-139 (Samrat). Both the varieties had shown good result in T3 (Salicylic acid 100 ppm + NaCl 100 mM) nearly as T_0 (control). As compare to Salicylic acid (SA) @ 150 ppm the application of SA @ 100 ppm on plants under two different salt stresses (100 and 150 mM) gave the higher values for these parameters. The chlorophyll content of soybean leaves was increased due to application of salicylic acid (Khan *et al*., 2003) [8]. Foliar application of SA may increases the leaf diffusive resistance and lower transpiration rates and protects relative water content. Similarly it had been reported by Szepsi *et al*., (2005) [14].

Table 1: Effect of salt stress and Salicylic acid on plant height (cm) and number of leaves in Mung bean varieties.

Treatment S Symbol	Treatment Combinations	Plant Height (Cm)				Number Of Leaves Per Plant			
		PDM-139		HUM-12		PDM-139		HUM-12	
		30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
T_0	Control	22.80	39.47	23.84	40.56	12.27	21.33	12.89	22.28
T_1	NaCl 100 mM	17.52	28.23	18.06	29.54	7.37	14.61	7.42	15.37
T_2	NaCl 150 mM	16.15	27.04	16.59	27.56	7.14	14.20	7.18	14.39
T_3	SA 100ppm + NaCl 100 mM	21.70	37.29	22.26	38.69	10.27	19.63	11.13	20.70
T_4	SA 100ppm + NaCl 150 mM	19.92	33.75	20.03	34.79	8.97	18.10	9.04	19.15
T_5	SA 150ppm + NaCl 100 mM	19.76	33.32	19.90	34.36	8.35	17.99	8.35	18.86
T_6	SA 150ppm + NaCl 150 mM	18.52	29.23	18.80	32.04	7.81	16.96	7.94	17.80
TAB. F 5%		S	S	S	S	S	S	S	S
S.Em		0.049	0.128	0.036	0.135	0.300	0.136	0.203	0.126
C.D at 5%		0.149	0.389	0.109	0.409	0.909	0.413	0.615	0.381

Table 2: Effect of salt stress and Salicylic acid on number of pods per plant and seed yield per plant (g) in Mung bean varieties.

Treatment S Symbol	Treatment Combinations	Number Of Pods Per Plant		Seed Yield Per Plant (G)	
		PDM-139	HUM-12	PDM-139	HUM-12
T_0	Control	17.19	17.22	9.23	9.29
T_1	NaCl 100 mM	9.09	9.18	4.98	5.11
T_2	NaCl 150 mM	8.88	8.98	4.51	4.75
T_3	SA 100ppm + NaCl 100 mM	15.20	15.69	8.29	8.54
T_4	SA 100ppm + NaCl 150 mM	12.71	12.84	6.99	7.09
T_5	SA 150ppm + NaCl 100 mM	12.19	12.24	6.70	6.86
T_6	SA 150ppm + NaCl 150 mM	11.03	11.12	5.39	5.54
TAB. F 5%		S	S	S	S
S.Em		0.063	0.067	0.036	0.062
C.D at 5%		0.192	0.202	0.109	0.189

Table 3: Effect of salt stress and Salicylic acid on relative water content (%) and total chlorophyll content (mg/g FW) in Mung bean varieties

Treatment S Symbol	Treatment Combinations	Relative Water Content (%)		Total Chlorophyll Content (Mg/G Fw)	
		PDM-139	HUM-12	PDM-139	HUM-12
T_0	Control	72.03	71.85	1.23	1.27
T_1	NaCl 100 mM	67.52	67.49	0.88	0.90
T_2	NaCl 150 mM	67.12	67.09	0.80	0.81
T_3	SA 100ppm + NaCl 100 mM	70.85	70.86	1.17	1.21
T_4	SA 100ppm + NaCl 150 mM	69.53	69.60	1.13	1.15
T_5	SA 150ppm + NaCl 100 mM	69.35	69.30	1.09	1.12
T_6	SA 150ppm + NaCl 150 mM	68.87	68.83	0.96	0.95
TAB. F 5%		S	S	S	S
S.Em		0.076	0.094	0.009	0.013
C.D at 5%		0.230	0.286	0.027	0.040

Table 4: Effect of salt stress and Salicylic acid on Hydrogen Peroxide (H_2O_2), Peroxidase (POX), Proline and Ascorbate Peroxidase (APX) in Mung bean varieties.

Treatment Symbol	Treatment Combination	H_20_2 (µmol/mg FW)		POX (Ug^{-1} FW)		Proline (µg/g FW)		APX (µmol/mg FW)	
		PDM- 139	HUM- 12	PDM- 139	HUM- 12	PDM- 139	HUM- 12	PDM- 139	HUM- 12
T_0	Control	0.65	0.65	0.49	0.51	0.64	0.67	0.52	0.54
T_1	NaCl 100 mM	1.19	1.17	0.54	0.56	0.74	0.79	0.84	0.86
T_2	NaCl 150 mM	1.26	1.25	0.52	0.52	0.73	0.76	0.77	0.79
T_3	SA 100ppm + NaCl 100 mM	0.93	0.91	0.81	0.83	0.83	0.86	1.09	1.13
T_4	SA 100ppm + NaCl 150 mM	1.13	1.12	0.74	0.76	0.79	0.83	1.04	1.08
T_5	SA 150ppm + NaCl 100 mM	1.18	1.18	0.70	0.71	0.78	0.82	1.02	1.04
T_6	SA 150ppm + NaCl 150 mM	1.21	1.20	0.65	0.67	0.76	0.79	0.96	0.99
TAB. F 5%		S	S	S	S	S	S	S	S
S.Em		0.003	0.003	0.009	0.009	0.008	0.009	0.004	0.006
C.D at 5%		0.010	0.010	0.027	0.027	0.025	0.028	0.011	0.019

Biochemical Parameters

Biochemical Parameter {*viz.*, Hydrogen Peroxide (H_2O_2), Peroxidase (POX), Proline and Ascorbate Peroxidase (APX)} in Mung bean plants were lowered with the increase in the concentration of salt treatment as compared to normal conditions. However, exogenous Salicylic acid applications increased these parameters as compared to plant which treated with only salt except in H_20_2 as it is a ROS. The experimental results are clearly indicating that both the varieties had shown good result in T3 (Salicylic acid 100 ppm + NaCl 100 mM) nearly as T_0 (control). As compare to Salicylic acid (SA) @ 150 ppm the application of SA @ 100 ppm on plants under two different salt stresses (100 and 150 mM) gave the higher values for these parameters except H_20_2. H_20_2 was found highest in T_2 (NaCl 150mM) and lowest in T_0 (Control). ROS acts as a signal molecule and plants initiate antioxidant mechanism for protection against ROS (Nazar *et al.,* 2011) [13]. The increased activity of antioxidant may be the result of interaction between SA and antioxidant. Similar results were found by Mittler *et al*., (2004) [9], Hussein *et al*., (2007) [5] and Jaiswal *et al*., (2014) [6].

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