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Research Article

IMPACT OF FOLIAR APPLICATION OF SALICYLIC ACID ON GROWTH AND LIPID PEROXIDATION IN WATER STRESS TOLERANCE OF *GLYCINE MAX* (L.) MERRILL

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Abstract: Salicylic acid (SA) is an important signal molecule modulating plant response to stress. An earthen pots trial was conducted to study the impact of salicylic acid (SA) @ 100, 200 and 400 ppm on growth and biochemical parameters of *Glycine max* (L.) merrill under waterlogging and drought stress. Shoot, root (length, dry weight), leaf area index, were significantly affected by water stress (waterlogging and drought stress), but SA suppressed these harmful effects. SA also increased RWC which was highly declined due to water stress. Chlorophyll and carotenoids content of stressful plants was destructively affected, but it was reorganized and increased along with increasing SA concentration. Nonetheless, the spray of SA on *Glycine max* seedlings (100, 200 and 400 ppm) resisted waterlogged and drought injuries by way of decrease of lipid peroxidation through reduce of MDA content. Overall, it can be concluded that SA could improve physiological and biochemical properties of *Glycine max* seedlings under water stress.

Keywords: *Glycine max*, lipid peroxidation, Salicylic acid, water stress.

INTRODUCTION

Waterlogging (water stress) is defined as prolonged soil saturation with water at least 20% higher than the field capacity a serious problem which affects crop growth and yield in low-lying, rain fed areas[1]. Most of the rainy season crops, especially legumes, and to lesser extent maize and rice are affected by flooding leading to hypoxic or even anoxic conditions lack of oxygen shifts the energy metabolism from aerobic mode to anaerobic mode, which in turn adversely affects nutrient and water uptake; so the plants show wilting even when surrounded by excess water. Drought is one of the most important manifestations of abiotic stress in plants. It is the major yield limiting factor of crop plants and it actively and continuously determines the natural distribution of plant species. Drought exacerbates the effect of the other stresses to which plants are submitted (abiotic or biotic) and several different abiotic stresses result in water stress (like salt and cold stresses). As sessile organisms, plants have to cope with drought stress at least at some point in their life cycle. Drought being the most important environmental stress severely impairs plant growth and development, limits plant production and the performance of crop plants more than any other environmental factor[2]. Available water resources for successful crop productions have been decreasing in recent years. Furthermore, in view of various climatic change models scientists suggested that in many regions of the world, crop losses due to increasing water shortage will further aggravate its impacts[3]. The susceptibility of plants to drought stress varies in dependence of stress degree, different accompanying stress factors, plant species and their developmental stages[4]. Acclimation of plants to water deficit is the result of different events, which lead to adaptive changes in plant growth and physio-biochemical processes such as changes in plant structure, growth rate, tissue osmotic potential and antioxidant defenses[5]. Salicylic acid (SA) acts as a potential non-enzymatic antioxidant as well as a plant growth regulator, which plays an important role in regulating a number of plant physiological processes including photosynthesis[6]. Some earlier reports show that exogenous salicylic acid could ameliorate the damaging effects of heavy metals in rice[7] waterlogging stress in wheat[8], and salt stress in wheat. These observations suggest that SA being antioxidant could be linked to oxidative stress. Salicylic acid is an important signal element and endogenous growth regulator involved in local and endemic disease resistance in plants. Earlier investigations have shown the role of SA in modulating plant responses to a wide range of oxidative stresses such as heat, drought, chilling, waterlogging, heavy metal and salt stress[9]. SA induces resistance to water

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deficit and ameliorates the damaging effects of heavy metals[10]. Soybean (*Glycine max*) is a major food and oil crop in most countries where salinity and water stress problems exist or might develop. Large areas of formerly arable land are being removed from crop production every year due to increasing soil salinity. Soybean is moderately salt tolerant, and may be cultivated in a light moderate saline soil[11]. The aim of present study was to evaluate the foliar application of Salicylic acid in water stress tolerance of *Glycine max* plants as well as to investigate the relative water content, leaf area index and total protein content.

MATERIALS AND METHODS

Growth conditions and Plant material:

The experiment was carried out in greenhouse ambient of school of Forestry and Environmental Science at Sam Higginbottom Institute of Agriculture, Technology and Sciences (SHIATS) (Deemed-to be-University), Allahabad-211007, India, during the months of July to October of 2011. The plants grown in greenhouse ambient under natural conditions day/night (minimum/maximum air temperature and relative humidity were: 22.4/37.6 °C and 76 to 81%, respectively, as well as the average photoperiod was of 12 h of light and maximum active photosynthetical radiation of 623 $\mu mol^{-2}\ s^{-1}$ (at12:00 h). *Glycine max* seeds were collected from Genetics and Plant Breading department, SHIATS (Deemed-to be-University), Allahabad, India, were surface sterilized with 0.01 % aqueous solution of mercuric chloride followed by repeated washing with double distilled water (DDW). These seeds were sown in earthen pots (10 inches diameter) filled with sandy loam soil and farmyard manure (mixed in the ratio of 6:1) and lined in a green house. At 20 days stage, plants were sprayed with 100, 200 and 400 ppm of salicylic acid (SA). Each seedling was sprinkled thrice. The nozel of the sprayer was adjusted in such a way that it pumped out 1 ml in one sprinkle. Therefore, each plant received 3 ml of SA solution. After completing last treatment of SA, Waterlogging stress and Drought stress with 30-35% moisture retention was maintained for 30 days. The experiments were allocated to eight groups as follows:T_0 (Normal irrigation),T_1 (Waterlogging control), T_2 (Waterlogging + 100 ppm SA), T_3 (Waterlogging + 200 ppm SA), T_4 (Waterlogging + 400 ppm SA),T_5 (Drought control), T_6 (Drought + 100 ppm SA), T_7 (Drought + 200 ppm SA) and T_8 (Drought + 400 ppm SA).The plants were sampled at 10, 20 and 30 days after maintaining water stress to assess the following observations:

Growth parameters:

Shoot and root length were measured from the base of the stem and base of the root respectively. Plants dried at 70°C were weighed for determination of dry matter production. One gram fresh weight of shoot and root from various samples were taken, wrapped in aluminum foil and oven dried at 70°C in hot air oven until a constant weight was recorded. Direct measurement of LAI is labor intensive, involving removal of all leaflets in a given ground area, determining the area of the leaflets, and dividing the total leaf area removed by the ground area.

Relative water content determination:

Relative water content (RWC) was determined using fresh leaf discs with 2 cm^2 diameter. After weighing, they floated on deionized water for saturation until 24 hours. Saturated leaf weight was recorded and the Dry mass was noted after dehydration at 70°C for 48 h. The following formula was used to calculate RWC[12]:

$$RWC = \frac{\text{Fresh weight-dry weight}}{\text{Turgor weight-dry weight}} \times 100$$

Estimation of Photosynthetic pigments

Chlorophyll content estimation:

Chlorophyll content was estimated by Arnon and Stout method[13]. Briefly, one gram of shoot was homogenized in 5 ml of 80% acetone (acetone: water, v/v). Extraction was done then cooled. The suspension was centrifuged at 5000 rpm for five min and supernatant was used for measuring chlorophyll content. The absorbance was recorded at 665 and 663 nm. Blank (only organic solvent used for tissue homogenization) was taken to be 80% acetone.

Carotenoids content estimation:

Total carotenoids in the plant tissues were estimated according to the method by Jensen[14]. One gram of each sample were extracted with 80% methanol and centrifuged. The supernatants were concentrated to dryness. The residues thus obtained were dissolved in 15 ml of diethyl ether and after addition of 15 ml of 10% methanolic KOH, the mixture was washed with 5% ice-cold saline water to remove alkali. The collective saline washings were extracted with ether (3:15 v/v). The ether extract from both were mixed together followed by washing with cold water till alkali free. The alkali free ether extract was dried over anhydrous Na_2SO_4 for two hours in the dark. The ether extracts were filtered and its absorbance was measured at λmax 450 nm by using ether as blank.

Protein estimation:

Protein content in the plant extracts was determined according to Lowry *et al.,*[15]. One gram fresh leaves were homogenized with 10 ml phosphate buffer (1mM, pH 7.0). The homogenate was centrifuged at 8000 rpm for 30 minutes. The supernatant was used for protein estimation. Its 100 µl, and 200 µl of the aliquots were taken in triplicate for test and maintained to 500 µl by water, followed by the addition of 5 ml of

reagent-C, (reagent-C: 95 ml of reagent-A mixed with 5 ml of reagent-B, Reagent-A: 2% sodium carbonate in 0.1 M NaOH, Reagent-B: 1% copper sulphate ($CuSO_4.5H_2O$), 2% potassium-sodium tartarate in ratio of 1:1 was also mixed properly and incubated for 10 min at room temperature. 500µl of 1N Folin-Ciocalteu's phenol reagent was mixed and vertexed quickly. This reaction mixture was incubated for 30 minutes at 37°Cand its absorbance was recorded at λmax 660nm. The amount of protein was calculated by comparison with standard curve of BSA drawn under identical experimental conditions.

Determination of lipid peroxidation:

Lipid peroxidation was measured as described by Hodges *et al.,*[16]. Approximately 0.5 g plant tissues was homogenized in 80% ethanol and centrifuged at 3000 rpm. Afterwards, the extract obtained was analyzed in two steps. At the first step, 1 volume of 20% (w/v) (TCA) and 1 volume of 0.01% BHT (an antioxidant used to block lipid peroxidation during the assay) were added to 1 volume of supernatant. At the second step, 1 volume of 20% TCA that contained 1 volume of 0.65% TBA and 1 volume 0.01% BHT were added to 1 volume extract taken from the supernatant. After vortexing the sample for 10 sec, they were incubated in a hot water bath adjusted to 95°C for 25 min followed immediately by a shock treatment in an ice bath. The cooled samples were centrifuged at 3000 rpm and absorbance values of supernatants were measured in spectrophotometer. First step samples were measured at 532 and 600 nm, whereas second samples at 440, 532 and 600 nm.

Statistical Analysis:

All the experiments were performed in triplicate. Values in the tables indicate mean values ± SD. Differences among treatments were analyzed by Two Way ANOVA with multiple observations, taking p <0.05 as significant according to Fisher's multiple range test.

RESULTS

In the present study water stress adversely affected growth parameters (shoot and root length, shoot and root dry weight, number of leaves, leaf area index (LAI))and biochemical parameters (total chlorophyll content, carotenoids, total protein content and lipid peroxidation).On the other hand, applications of SA gradually lightened the negative effects of water stress on growth and biochemical parameters. Moderate levels of SA application (200 ppm) showed highest performance under waterlogging and drought stress.

Plant growth parameters

Effect of water stress and protective action of salicylic acid on the shoot and root length of *Glycine max* cultivar is shown in table 1 and 2. Water stress significantly reduced the shoot and root length whereas the foliar application of salicylic acid (SA) stimulated shoot and root length. Amongst SA treatments, 200 ppm concentration showed the highest stimulation of shoots and root length as compared to water stress (Drought and Waterlogging) control. Normal irrigation (T_0) had shown the maximum shoot length (58.33±4.16 cm) at 10 days after treatment (DAT) as compared to other treatment except T_3 treatment (Waterlogging + 200 ppm SA). As increasing days till 30 DAT, shoot length increase was observed in the same pattern. The salicylic acid treatment increased root length as compared to drought and waterlogging control. Waterlogging and Drought stress reduced the root length (11.78±0.280 cm and 10.33±0.152 cm) as compared to control group (13.80±0.655 cm) at 10 DAT. Root length was significantly enhanced by salicylic acid treatment as compared to water stress control. The maximum increment of root length at 200 ppm concentration of SA showed 13.06±0.152, 18.13±0.305, 21.93±0.250 in waterlogging; 12.20±0.100, 15.73±0.152, 18.75±0.152 in drought stress as compared to their respective controls at 10, 20 and 30 DAT respectively **(Table 2)**.

Table 1: Effect of Salicylic acid (SA) on shoot length of *Glycine max* under water stress

Treatment	10 DAT	20 DAT	30 DAT
T_0	58.33±4.16	69.33±2.51	83.66±3.21
T_1	46.00±1.00	57.00±1.00	67.33±1.52
T_2	54.00±2.00	63.00±1.00	74.66±1.52
T_3	62.00±3.00	74.33±2.52	86.00±3.00
T_4	58.00±1.00	69.00±1.00	79.66±2.08
T_5	40.00±2.00	50.33±1.52	61.00±2.00
T_6	46.33±1.52	57.33±1.52	67.00±1.00
T_7	55.00±1.00	65.66±1.52	75.66±1.52
T_8	48.66±0.57	59.00±1.00	69.00±1.00

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

SE due to Irrigation = 0.471 **CD due to Irrigation = 0.936**
SE due to Days = 0.577 **CD due to Days = 1.146**
SE due to SA levels = 0.744 **CD due to SA levels = 1.480**

Table 2: Effect of Salicylic acid (SA) on root length of *Glycine max* under water stress

Treatment	10 DAT	20 DAT	30 DAT
T_0	13.80±0.655	18.70±0.556	23.30±0.458
T_1	11.78±0.280	15.63±0.450	20.50±0.360
T_2	12.50±0.264	16.55±0.377	21.35±0.150
T_3	13.06±0.152	18.13±0.305	21.93±0.250
T_4	12.80±0.200	17.53±0.152	21.60±0.100
T_5	10.33±0.152	13.66±0.251	17.05±0.229
T_6	11.06±0.152	14.93±0.152	17.73±0.152
T_7	12.20±0.100	15.73±0.152	18.75±0.152
T_8	11.46±0.076	15.11±0.125	18.21±0.125

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

SE due to Irrigation = 0.259 **CD due to Irrigation = 0.515**
SE due to Days = 0.317 **CD due to Days = 0.631**
SE due to SA levels = 0.409 **CD due to SA levels = 0.814**

Shoot and root dry weights were significantly affected by different drought and waterlogging levels (Table 3 and 4). Shoot and root dry weights were highly decreased on waterlogging (0.192±0.005, 0.066±0.002 gm) and drought control (0.164±0.0085, 0.059±0.002 gm) as compared to normal control (0.374±0.007, 0.075±0.002 gm). Along with increasing SA concentration, shoot and root dry weights were improved on both drought and waterlogging stress levels. At 200 ppm concentration of SA had more ameliorative effects and the higher amounts of shoot and root dry weights, 0.217±0.0075 gm, 0.238±0.007 and 0.086±0.001 gm, 0.080±0.001gm in waterlogging and drought stress respectively as compared to respective controls at 10 DAT, shoot and root weights were also increased in the same mode as 20 DAT and 30 DAT (Table 3 & 4). These results show that although shoot and root dry weights were significantly affected by waterlogging and drought stress, but SA could improve these parameters and this effect enhanced with increasing SA concentration. Numbers of leaves were decreased at waterlogging control (3.66±0.577) and drought control (3.00±1.00) as compared to normal control (5.66±0.577). Along with increasing SA concentration, numbers of leaves were increased on both drought and waterlogging stress. Numbers of leaves were increased when waterlogging and drought stress plants treated with 100 (5.00±1.00, 4.00±1.00), 200 (5.33±0.577, 4.66±0.577) and 400 ppm (4.66±0.577, 4.33±1.52) concentration of SA at 10 DAT. Numbers of leaves were increased as the identical manner with increasing days at 20 DAT and 30 DAT (Table 5).The leaf area index was nearly the same at the beginning of the study but changed significantly by 10 days after treatment (DAT). The leaf area index was 3.36±0.076, 4.40±0.100 and 3.71±0.125 under waterlogging at 100, 200 and 400 ppm SA concentration respectively as compared to waterlogged control (3.01±0.104), whereas under drought stress leaf area index increased at 100 ppm (3.15±0.060), 200 ppm (3.94±0.040) and 400 ppm (3.42±0.070) concentration of SA as compared to drought control (2.70±0.050) at 10 DAT. Later, it increased in the similar manner with increasing days at 20 DAT and 30 DAT (Table 6).

Table 3: Effect of Salicylic acid (SA) on shoot dry weight of *Glycine max* under water stress

Treatment	10 DAT	20 DAT	30 DAT
T_0	0.374±0.007	0.418±0.007	0.492±0.005
T_1	0.192±0.005	0.222±0.009	0.275±0.0087
T_2	0.217±0.0075	0.250±0.0097	0.310±0.0140
T_3	0.280±0.0170	0.314±0.0155	0.359±0.0150
T_4	0.244±0.0096	0.279±0.0086	0.331±0.010
T_5	0.164±0.0085	0.188±0.006	0.227±0.0075
T_6	0.187±0.005	0.216±0.006	0.254±0.007
T_7	0.238±0.007	0.276±0.0076	0.308±0.010
T_8	0.216±0.006	0.244±0.0075	0.277±0.005

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

SE due to Irrigation = 0.0031 **CD due to Irrigation = 0.0063**
SE due to Days = 0.0039 **CD due to Days = 0.0077**
SE due to SA levels = 0.0050 **CD due to SA levels = 0.0100**

Table 4: Effect of Salicylic acid (SA) on root dry weight (gm) of *Glycine max* under water stress.

Treatment	10 DAT	20 DAT	30 DAT
T_0	0.075±0.002	0.077±0.002	0.080±0.002
T_1	0.066±0.002	0.068±0.002	0.071±0.002
T_2	0.073±0.001	0.076±0.001	0.079±0.001
T_3	0.086±0.001	0.092±0.002	0.096±0.001
T_4	0.079±0.002	0.083±0.001	0.086±0.005
T_5	0.059±0.002	0.063±0.001	0.069±0.001
T_6	0.069±0.001	0.072±0.005	0.075±0.001
T_7	0.080±0.001	0.084±0.001	0.090±0.002
T_8	0.076±0.001	0.079±0.005	0.084±0.001

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

SE due to Irrigation = 0.0007 **CD due to Irrigation = 0.0016**
SE due to Days = 0.0010 **CD due to Days = 0.0019**
SE due to SA levels = 0.0012 **CD due to SA levels = 0.0024**

Table 5: Effect of Salicylic acid (SA) on number of leaves of *Glycine max* under water stress.

Treatment	10 DAT	20 DAT	30 DAT
T_0	5.66±0.577	8.33±0.577	11.0±1.00
T_1	3.66±0.577	6.33±0.577	8.33±0.577
T_2	5.00±1.00	7.33±0.577	9.00±0.00
T_3	5.33±0.577	9.00±1.00	11.33±1.527
T_4	4.66±0.577	7.66±1.154	10.33±1.527
T_5	3.00±1.00	4.33±0.577	7.33±1.527
T_6	4.00±1.00	5.33±0.577	8.66±1.154
T_7	4.66±0.577	6.66±0.577	10.0±1.00
T_8	4.33±1.52	5.66±0.577	9.00±1.00

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

SE due to Irrigation = 0.153 **CD due to Irrigation = 0.305**
SE due to Days = 0.188 **CD due to Days = 0.373**
SE due to SA levels = 0.242 **CD due to SA levels = 0.482**

Table 6: Effect of Salicylic acid (SA) on leaf area index of *Glycine max* under water stress.

Treatment	10 DAT	20 DAT	30 DAT
T_0	5.13±0.305	5.88±0.076	6.36±1.52
T_1	3.01±0.104	3.26±0.065	3.86±0.05
T_2	3.36±0.076	3.70±0.100	4.15±0.05
T_3	4.40±0.100	4.86±0.076	5.46±0.076
T_4	3.71±0.125	4.20±0.100	4.91±0.125
T_5	2.70±0.050	3.10±0.150	3.53±0.076
T_6	3.15±0.060	3.46±0.076	3.98±0.104
T_7	3.94±0.040	4.37±0.092	4.96±0.115
T_8	3.42±0.070	3.75±0.097	4.25±0.093

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

SE due to Irrigation = 0.116 **CD due to Irrigation = 0.231**
SE due to Days = 0.142 **CD due to Days = 0.283**
SE due to SA levels = 0.183 **CD due to SA levels = 0.364**

Relative water content:

Glycine max showed drastic decrement on relative water content in waterlogged control (57.43±1.069) and drought control (49.30±0.721) as compared to control (82.86±0.956) at 10 DAT. SA treatments alleviated reduced RWC to a great extent. The

maximum increment of RWC at 200 ppm concentration of SA showed 76.10±0.900, 71.40±0.916, 66.70±0.800 in waterlogging; 64.30±1.212, 59.00±0.800,54.30±1.014 in drought condition as compared to water stress control at 10 DAT , 20 DAT and 30 DAT respectively **(Table 7)**. RWC was noted significantly decreased with increasing days after treatment.

Table 7: Effect of Salicylic acid (SA) on relative water content of *Glycine max* under water stress.

Treatment	10 DAT	20 DAT	30 DAT
T_0	82.86±0.956	78.38±0.693	73.86±0.702
T_1	57.43±1.069	53.33±0.850	48.96±0.550
T_2	62.46±1.301	58.20±0.916	52.90±0.953
T_3	76.10±0.900	71.40±0.916	66.70±0.800
T_4	69.29±0.862	63.96±0.550	60.26±1.201
T_5	49.30±0.721	44.43±0.862	41.10±0.754
T_6	55.70±0.953	52.60±0.900	47.93±0.702
T_7	64.30±1.212	59.00±0.800	54.30±1.014
T_8	59.00±0.600	56.13±0.802	50.76±0.929

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

SE due to Irrigation = 0.469 **CD due to Irrigation = 0.932**
SE due to Days = 0.574 **CD due to Days = 1.143**
SE due to SA levels = 0.741 **CD due to SA levels = 1.475**

Photosynthetic pigments

Waterlogging and drought stress had a destructive effect on carotenoids of stressful plants **(Table 8).** There was significant decrease in carotenoids content of *Glycine max* leaves under waterlogging control (0.173±0.0240 mg/gm FW) and drought control (0.281±0.0301 mg/gm FW) as compared to normal control (0.488±0.0906 mg/gm FW) at 10 DAT. The SA treatment under water stress (waterlogging and drought) condition resulted higher carotenoid content as compared to that of waterlogging and drought control. At 200 ppm of SA concentration the maximum carotenoids content was recorded in drought conditions with the mean values 0.435±0.0296, 0.655±0.0440, 0.921±0.0265 mg/gm FW and under waterlogging conditions were 0.350±0.030, 0.568±0.050, 0.830±0.0350 mg/gm FW at 10 DAT, 20 DAT and 30 DAT respectively (Table 8).

Table 8: Effect of Salicylic acid (SA) on carotenoids of *Glycine max* under water stress.

Treatment	10 DAT	20 DAT	30 DAT
T_0	0.488±0.0906	0.709±0.0265	0.957±0.0365
T_1	0.173±0.0240	0.338±0.0386	0.555±0.0416
T_2	0.258±0.0240	0.490±0.0366	0.689±0.0250
T_3	0.350±0.030	0.568±0.050	0.830±0.0350
T_4	0.311±0.013	0.524±0.0240	0.741±0.029
T_5	0.281±0.0301	0.542±0.0420	0.822±0.0385
T_6	0.330±0.0140	0.637±0.0356	0.844±0.0329
T_7	0.435±0.0296	0.655±0.0440	0.921±0.0265
T_8	0.381±0.0165	0.613±0.0256	0.873±0.0214

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

SE due to Irrigation = 0.012 **CD due to Irrigation = 0.024**
SE due to Days = 0.014 **CD due to Days = 0.029**
SE due to SA levels = 0.019 **CD due to SA levels = 0.038**

Total protein content:

The total protein content was significantly decreased in waterlogging (0.778±0.003mg/gm FW) and drought control (0.635±0.012mg/gm FW) seedlings as compared to normal control (0.819±0.005mg/gm FW) at 10 DAT. The foliar application of salicylic acid (SA) of different concentration (100, 200 and 400 ppm) increased total protein content. At 200 ppm of SA concentration the maximum total protein content was recorded in waterlogging and drought conditions with the mean values 0.955±0.005 and 0.918±0.007 mg/gm FW respectively at 10 DAT (Table 9) and similar trend were also detected with increasing days as recorded on 20 and 30 DAT.

Table 9: Effect of Salicylic acid (SA) on total protein content of *Glycine max* under water stress.

Treatment	10 DAT	20 DAT	30 DAT
T_0	0.819±0.005	0.886±0.007	0.927±0.007
T_1	0.778±0.003	0.831±0.006	0.891±0.012
T_2	0.826±0.005	0.875±0.005	0.945±0.005
T_3	0.955±0.005	1.045±0.044	1.18±0.035
T_4	0.920±0.005	0.967±0.002	1.04±0.035
T_5	0.635±0.012	0.685±0.007	0.734±0.015
T_6	0.757±0.006	0.806±0.005	0.857±0.005
T_7	0.918±0.007	0.971±0.006	1.012±0.016
T_8	0.885±0.006	0.937±0.005	0.970±0.010

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

SE due to Irrigation = 0.0065 **CD due to Irrigation = 0.013**
SE due to Days = 0.0080 **CD due to Days = 0.016**
SE due to SA levels = 0.0103 **CD due to SA levels = 0.021**

Lipid peroxidation:

The damage by waterlogging and drought stress to cellular membranes due to lipid peroxidation as indicated by the accumulation of the malondialdehyde (MDA) levels and the results showed that MDA level was significantly increased in waterlogging (38.63±0.185, 42.91±0.152 and 45.73±0.485nmol/gm FW) and drought control (32.59±0.265, 37.06±0.125 and 41.89±0.226nmol/gm FW) seedlings as compared to normal control (22.66±0.340, 27.65±0.42 and 31.39±0.250 nmol/gm FW) at 10, 20 and 30 DAT respectively (Table 10). SA application reduced lipid peroxidation because MDA content significantly decreased. Application of 200 ppmSA caused reduction of MDA content by 26.81±0.325, 30.92±0.345, 35.70±0.230nmol/gm FW in waterlogging and 25.65±0.305, 29.88±0.252, 33.62±0.191nmol/gm FW under drought conditions, as compared to respective stress control at 10, 20 and 30 DAT respectively.

Table 10: Effect of Salicylic acid (SA) on lipid peroxidation of *Glycine max* under water stress

Treatment	10 DAT	20 DAT	30 DAT
T_0	22.66±0.340	27.65±0.42	31.39±0.250
T_1	38.63±0.185	42.91±0.152	45.73±0.485
T_2	33.59±0.419	38.10±0.267	41.77±0.420
T_3	26.81±0.325	30.92±0.345	35.70±0.230
T_4	28.81±0.225	32.69±0.280	37.01±0.202
T_5	32.59±0.265	37.06±0.125	41.89±0.226
T_6	30.29±0.347	34.85±0.272	38.93±0.202
T_7	25.65±0.305	29.88±0.252	33.62±0.191
T_8	26.85±0.242	32.02±0.166	36.86±0.155

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

SE due to Irrigation = 0.238 **CD due to Irrigation = 0.473**
SE due to Days = 0.292 **CD due to Days = 0.580**
SE due to SA levels = 0.377 **CD due to SA levels = 0.749**

DISCUSSION

The results of the present study show that waterlogging and drought stress reduced the growth and disturbed the plant metabolism. The foliar application of salicylic acid (SA) was beneficial in overpowering the adverse effects of waterlogging and drought stress by enhancing plant growth parameters, RWC, chlorophyll contents, carotenoids, and total protein content necessary for osmotic adjustment under adverse environmental conditions. The growth parameters (fresh and dry mass of roots and shoots, their lengths and the leaf area index) decreased progressively with the water stress (waterlogging and drought), compared with the control, Moreover, increase in the growth parameters of soybean plants in response to salicylic acid treatment. These results are in agreement with Ghoulam *et al.,*[17] who showed that salinity caused a marked reduction in growth parameters of sugar beet plants. The shoot and root dry weights decreased with water stress (waterlogging and drought). The results were in accordance with Misra and Dwivedi[18]. It was reported that the FW and DW in seedlings were decreased by salinity. Khodary[19] observed that salicylic acid treated maize plants exhibited an increase in tolerance to salinity reflected in growth parameters like length, fresh and dry weight of shoot and root.

The reduction in leaf relative water content was activated by the water deficiency in soil, because during the photosynthesis occur water loss through of the stomatal mechanism and the water assimilation rate is negatively affect during water stress[20]. It may also a consequence of inefficient root system which could not retrieve the water losses because of decreasing its absorbing surface[21]. Reduced RWC due to the salinity stress were also reported by many other researchers[22].

The adverse effect of water stress (waterlogging and drought) on chlorophyll concentration has previously been shown for young peach trees by Steinberg *et al.,*[23] and associated the increased electrolyte leakage to reductions in chlorophyll concentrations. Leaf chlorophyll, an important component of the photosynthetic system governing the dry matter accumulation, was increased significantly with SA application under water stress as compared to the stress control (without SA) and the increase was more with higher level of SA concentration. Similar observations were recorded for photosynthetic rate[24].

Carotenoids acts as accessory pigment and activates defense systems but the effect of SA was not evident under unstressed condition. Carotenoids effectively quench singlet oxygen derived from primary photochemical reactions and hence a close correlation was found between the carotenoid contents of the leaves and the foliar biomass production of tomato genotypes under saltstress[25]. The observed increase in carotenoid content of SA treated leaves of plants under water stress condition may indicate the better defense system induced by SA.

The reduction in the total soluble proteins in the plants under water stress (waterlogging and drought) is due to probable increase of the proteases enzyme activity, in which this proteases enzyme promote the breakdown of the proteins and consequently decrease the protein amount presents in the plant under abiotic stress conditions[26]. In inadequate conditions to the plant active the pathway of proteins breakdown, because the plant use the proteins to the synthesis of nitrogen compounds as amino acids that might auxiliary the plant osmotic adjustment[27]. Similar results on reduction in the proteins were found by Ramos *et al.,*[28] investigating the effects of the water stress in *Phaseolus vulgaris*.

MDA and other aldehyde formations in plants exposed to water stress are reliable indicators of free radical formation in the tissue, and are currently used as indicators of lipid peroxidation[29,24]. Our results supported that the decrease of membrane damage may be related to the induction of antioxidant responses by SA, which protects the cell from oxidative damage. Senaratna *et al.,*[30] suggested that a similar mechanism was responsible for SA induced multiple stress tolerance in bean and tomato plants. Kadioglu *et al.,*,[31] also reported that SA treatment prevented lipid peroxidation in *Ctenanthe setosa* while the peroxidation increased in control plants.

In the present study, the results showed that waterlogging and drought stress decreased the plant growth and biochemical parameters, which were all increased by addition of SA. The protective effect of SA under abiotic stress such as water stress is generally coupled with photosynthetic performance[32]. In general, the water stress directly decline leaf area,

number of leaves, dry weight, photosynthetic pigments, and total protein content. On the other hand, application of SA gradually mitigated the negative effects of water stress especially, on growth parameters. Moderate levels of SA application (200 ppm) showed highest performance under water stress. Our results showed that although *Glycine max* is a sensitive plant to water stress, it was confirmed that exogenous SA application can help waterlogging and drought tolerance of this crop.

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