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ABSTRACT

Salicylic acid (SA) plays an important role in the regulation of plant growth and development in response to water deficit. The effect of SA (0, 0.4 and 0.8 mM) on some physiological parameters of three soybean genotypes was investigated in three irrigation schedules included (85%, 65% and 45% of field capacity) during 2014–2015. Results showed that water deficit decreased stomatal conductance, leaf area index, relative water content, membrane stability index, yield components and grain yield particularly in L17 genotype. Activities of superoxide dismutase, ascorbate peroxidase and concentration of hydrogen peroxide, proline and total protein were increased in response to water deficit as well as SA applications. SA inhibited catalase activity resulting in increased hydrogen peroxide accumulation in soybean genotypes. Application of 0.4 mM SA decreased the adverse effects of water deficit in soybean genotypes by elevation of antioxidant enzymes activity and reducing malondialdehyde formation especially in Williams genotype.

1. Introduction

Soybean (*Glycine max* L. Merr) is one of the most interesting grain legumes. It is an essential source of protein, edible oil, copper, zinc and manganese for both human and animal nutrition as well as a notable feedstock for biodiesel production (Kuchlan et al. 2017). Imposing plants to water deficit results in the accumulation of osmolytes, stress tolerance proteins and changes in antioxidant enzyme activity (Tripathi et al. 2015). In soybean plant, water deficit stress reduces photosynthetic pigments, stomatal conductance, biomass, growth and finally the grain yield and its components (Hossain et al. 2014; Tripathi et al. 2015; Ruppenthal et al. 2016). Investigation of the morpho-anatomical and physiological basis of changes in drought stress condition can be helped to select varieties with higher productivity (Martinez et al. 2007). Drought tolerant genotypes of soybean could avoid the negative impact of water stress by osmotic adjustment, retain higher relative water content (RWC) and counteract the loss of turgor (Hossain et al. 2014).

Exogenous application of plant growth regulation has been considered as an alternative strategy to minimize negative impacts related to drought stress (Khan et al. 2015). Exogenous application of salicylic acid (SA) at lower concentrations within the range of 0.1–0.5 mM improves photosynthesis, growth and various physiological and biochemical processes, whereas in higher concentrations more than 1 mM, SA may cause stress in plants (Hayat et al. 2010). SA elevates the activities of antioxidant enzymes such as superoxide dismutase (SOD) and ascorbate peroxidase (APX) when sprayed to paraquat stressed barley plants (Ananieva et al. 2004), drought stressed common bean plants (Sadeghipour and Aghaei 2012) and salt stressed soybean (Jaiswal et al. 2014). In contrast, SA reduces catalase (CAT) activity and increases hydrogen peroxide (H_2O_2) concentration, which providing

the systemic acquired resistance and tolerance to oxidative stress (Chen and Klessig 1991). In biotic and abiotic stresses, the elevating effect of SA on cell membrane stability can also be related to the activity of antioxidant enzymes (Pawlowski et al. 2016), accumulation of soluble phenolics and carotenoids that protect plant tissue from oxidative damage (Khan et al. 2015). Increasing proline content by using exogenous SA ameliorates damages caused by water deficit and protects cell membranes against the harmful effects of reactive oxygen species (ROS) (Dučaiová et al. 2013). SA enhances leaf area and dry matter production in lemongrass (Idrees et al. 2010), strawberry (Ghaderi et al. 2015) and soybean (Kuchlan et al. 2017). Saruhan et al. (2012) reported that foliar application of SA increased proline and soluble carbohydrate content in maize genotypes under drought stress. SA application improves RWC and grain yield of common bean in water stress conditions (Sadeghipour and Aghaei 2012). This experiment focused on the effect of foliar application of SA on leaf water content, leaf area index (LAI), grain yield and some physiochemical parameters of soybean genotypes in limited irrigation condition.

2. Materials and methods

2.1. Experimental design and plant growth conditions

The experiment was laid out as split plot factorial based on a randomized complete block design with three replications. Main plots were subjected to irrigation schedules and sub plots included soybean genotypes and SA levels.

This field trial was conducted during summer 2014 and 2015 at Moghan Agricultural Research Center (37° 47′ N, 46° 19′ E and 60 m above sea level) in Ardabil, Iran. The climate of the region is characterized as semi-arid with the mean annual rainfall 250 mm and temperature 23.5°C,

Table 1. Weather data for each month of soybean growth seasons during 2014 and 2015.

Month	Average temp. (°C)		Maximum temp. (°C)		Minimum temp. (°C)		Relative humidity (%)		Day length per month (h)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
May	24.3	24.9	31.7	32.4	16.9	17.4	61.8	60.8	290.9	293.1
June	28.0	29.6	34.3	33.2	21.6	20.5	53.6	57.1	288.2	279.6
July	28.1	28.2	35.6	35.9	20.7	20.7	50.4	58.0	341.6	315.1
August	25.6	23.8	31.7	29.8	19.5	17.8	63.0	68.1	249.2	199.7
September	17.0	17.7	21.6	22.8	12.3	12.5	74.7	75.3	140.7	168.2
October	10.7	10.8	14.8	14.7	6.5	6.8	77.8	80.5	101.8	113.8
Means	22.3	22.5	28.3	28.1	16.25	15.95	63.6	66.6	235.4	228.3

Data from the Pars Abad Meteorological Institute.

respectively. The weather data in 2014 and 2015 are listed in Table 1. The soil of the experimental farm was sandy clay loam having pH 7.8, EC 2 ds m^{-1}, total nitrogen 0.11%, available N 232 mg 100 g^{-1} of soil, available phosphorous 8.1 ppm and available potassium 0.5 mg 100 g^{-1} of soil. This study was conducted in split factorial based on a randomized complete block design with three replications.

Soybean genotypes (Williams, L17 and D42XI9) belong to maturity group III with indeterminate growth habit and have been prepared by the Seed and Plant Improvement Institute, Karaj, Iran. Seeds were sown on 28 May 2014 and 30 May 2015. Before sowing, the seeds were inoculated with the *Bradyrhizobium japonicum* produced by the Research Institute of Soil and Water, Tehran, Iran. Seedlings were arranged in rows with a distance of 0.6 m with a plant density of 50 plants m^{-2}. Phosphorous (60 kg ha^{-1} in super phosphate form) was applied as a basal dose and starter nitrogen was applied as urea form (50 kg ha^{-1}) at the time of planting.

2.2. Irrigation schedules

Irrigation schedules including control [85% field capacity (I_1)] and water deficit conditions [65% of field capacity (I_2) and 45% of field capacity (I_3)]. To prevent water leakage from one plot to another, plots were separated by 1 m unplanted distances.

The volume of water for each irrigation schedules was calculated according to the method of Afshar et al. (2014).

$$V = [(FC - \theta_m) \times Pb \times D_{root} \times A]/E_i,$$

where V = volume of irrigation water (m^3), F_C = moisture content at field capacity (%), θ_m = moisture content before irrigation (%), pb = soil bulk density (g cm^3), A = irrigated area (m^2), D_{root} = root depth (m), E_i = irrigation efficiency

The required water volume in each treatment was calculated based on the water distribution efficiency of 80% by flume and a chronometer in root development depth in each plot (0.4 m). To reduce any rainfall effects on water deficit treatments, each plot was covered by a shelter protector.

2.3. Spraying treatments

SA (hydroxybenzoic acid-2 C$_7$H$_6$O$_3$, MW = 138.12 g mol^{-1}) was purchased from Merck (Germany) and was dissolved in ethanol (1 g 10 ml^{-1}), then, concentrations of 0.4 and 0.8 mM were made up with distilled water. SA was sprayed on the foliage of the plants from each irrigation schedule treatments with an atomizer back pack foliar spraying (Solo 451, Germany). Control group of plants were sprayed with ethanol/water. Plants were treated with SA at the V1 stage (fully developed leaves at unifoliolate node) and at the R1

stage (beginning bloom). Soybean genotypes were sampled at the R4 stage (full pod). Plant productivity analysis was measured at full maturity stage.

2.4. Stomatal conductance

Stomatal conductance was measured on uppermost fully expanded leaves (three leaves per plant) from 11:00 to 14:00 pm at photosynthetic active radiation > 1,000 mM m^{-2} s^{-1}, leaf temperature between 29°C and 32°C with a portable porometer system (AP4, Delta T, Devices Ltd., UK).

2.5. Chlorophyll and carotenoids content

For chlorophyll and carotenoids determination, fresh leaf samples were washed with distilled water and samples (1 g) were ground in 90% acetone using a pestle and mortar. Samples centrifuged at 2500×g for 10 min, then the absorbance of supernatant was measured at $\lambda = 663$, 646 and 470 nm using 90% acetone as a blank by a spectrophotometer. Content of chlorophyll a, chlorophyll b and carotenoids (µg g^{-1} Fw) was calculated according to Lichtenthaler and Buschmann (2001) using the following formulae:

$$\text{Chlorophyll } a = 12.21 \text{ OD}_{663} - 2.81 \text{ OD}_{646},$$

$$\text{Chlorophyll } b = 20.13 \text{ OD}_{646} - 5.03 \text{ OD}_{663},$$

$$\text{Carotenoids} = (1000 \text{ OD}_{470} - 3.27$$
$$\text{Chlorophyll } a - 104 \text{ Chlorophyll } b)/229.$$

2.6. Membrane stability index

Leaf membrane stability index (MSI) was estimated as reported by Premchandra et al. (1990). Leaf discs (200 mg) from the young fully expanded leaves were cleaned with deionized water, placed in tubes with 15 ml of double-deionized water in two sets. One set was incubated for 2 h at 25°C. Subsequently, the electrical conductivity of the solution (EC1) was determined. The second set was heated in water bath at 95°C for 20 min and its conductivity (EC2) was measured. MSI was defined as follows: MSI = [1 − (EC1/EC2)] × 100.

2.7. Malondialdehyde content

Lipid peroxidation was expressed as equivalents of malondialdehyde (MDA) content, using the thiobarbituric acid method following the method of Valentovic et al. (2006).

2.8. Relative water content

Leaf RWC was determined gravimetrically following the method of Galmes et al. (2007). After measurement of fresh weight, leaves were floated in distilled water in Petri dishes for 24 h at 4°C and weighed again to determine turgid weight, dry weight was determined by drying for 48 h at 90°C. RWC (%) was calculated from the ratio of differences between fresh weight and dry weight, turgid weight and dry weight.

2.9. Leaf area index and specific leaf area

LAI was determined by using Handheld Laser (LAI Meter CI-203, CID, Bio-Science, USA). Specific leaf area (SLA) was determined by drying each sample in electric oven at 70°C to a constant weight.

2.10. Soluble sugars, proline and protein concentration

Total soluble sugars (mg g^{-1} fresh weight of leaves) were determined calorimetrically by the anthrone method (Dubois et al. 1956). Samples (0.5 g) were homogenized with deionized water, extracts were filtered and treated with 5% phenol and 98% sulfuric acid and absorbance at 485 nm was determined. The proline content of the leaves was estimated according to Bates et al. (1973) based on proline's reaction with ninhydrin. Samples were homogenized in sulphosalicylic acid and the supernatant was collected and centrifuged. An equal volume of ninhydrin acid and glacial acetic acid were added to the extract and incubated at 100°C for 1 h and then 5 ml of toluene were added. Proline present in the upper toluene layer was read at 528 nm. Protein concentration was determined using bovine serum albumin as standard, described by Bradford (1976).

2.11. Assay of antioxidant enzymes activity

Catalase (CAT, EC 1.11.1.6) activity was assayed according to Aebi (1984). The 60 μl enzyme extract was added to tris buffer (50 mM, pH = 7) and 0.3 ml H_2O_2 5 mM in the ice bath, the absorbance curve was considered at 240 nm. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by measuring the ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of Becana et al. (1986). One unit of SOD activity was determined as the amount of enzyme required to produce 50% inhibition of NBT reduction at 560 nm. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured by the rate of ascorbate oxidation at 290 nm (ε = 2.8 mM cm^{-1}) according to Ramel et al. (2009).

2.12. H_2O_2 concentration

The content of H_2O_2 was measured according to the modified method of Patterson (1984). Fresh tissue were homogenized with 6 ml ice-cold acetone and centrifuged at 8000×g for 30 min. 0.5 ml of supernatant was mixed with 1.5 ml of a mixture of $CHCl_3$, CCl_4 (1:3 v:v) and 2.5 ml of distilled water. The mixture was centrifuged at 1000×g for 1 min. The water phase was collected and incubated at 37°C for 10 min. Then 1 ml of phosphate buffer (0.2 M pH 7.8) and 1 ml of 4-(2-pyridylazo) resorcinol (200 mM) were added to the samples. The reaction mixtures were incubated at 45°C for 20 min and the absorbance was measured at 508 nm.

2.13. Statistical analysis

Statistical analyses were carried out by analysis of variance using SAS ver.9.1 software. Statistics values were presented as means ± SE of three replicates. Differences between treatments were separated by the least significant difference test at 0.05 probability level.

3. Results

Our results showed that there was no statistically significant difference among two growing seasons for yield components, grain yield and other traits in this study (data not shown). The two growing seasons were somewhat similar in weather conditions. During growing periods, the average of temperature were 22.3°C and 22.5°C, relative humidity were 63.6% and 66.6% and day length per month were 235.4 h and 228.3 h in 2014 and 2015, respectively (Table 1).

Table 2. Effect of different irrigation schedules and salicylic acid on stomatal conductance, content of chlorophyll *a*, *b*, *a/b* ratio, carotenoids, proline, soluble sugars and SLA of three soybean genotypes.

Irrigation schedules	Stomatal conductance (mM m^{-2} s^{-1})	Chlorophyll *a* (μg g^{-1} Fw)	Chlorophyll *b* (μg g^{-1} Fw)	Chlorophyll *a/b*	Carotenoid (μg g^{-1} Fw)	Proline (μg g^{-1} Fw)	Soluble sugars (mg g^{-1} Fw)	SLA (mg cm^{-2})
I_1 (85%Fc)	29.75	1289.26	347.04	3.80	708.56	21.37	1.00	1.06
I_2 (65%Fc)	19.78	985.19	252.97	3.97	654.56	27.56	1.23	1.43
I_3 (45%Fc)	14.89	794.44	192.56	4.26	473.26	36.60	1.65	1.87
LSD$_{0.05}$	3.28	71.03	35.94	0.423	72.89	3.49	0.20	0.31
LSD$_{0.01}$	5.45	117.8	59.6	0.702	120.90	5.78	0.33	0.51
Genotype								
Williams	23.21	1041.11	255.19	4.12	566.44	37.30	1.00	1.31
L17	20.27	980.37	264.04	3.92	633.85	18.52	1.23	1.63
D42X19	20.94	1047.41	273.33	4.00	636.07	29.70	1.65	1.42
LSD$_{0.05}$	1.03	85.20	28.77	0.36	57.83	2.65	0.21	0.10
LSD$_{0.01}$	1.44	119.40	40.34	0.51	81.07	3.71	0.29	0.14
SA								
S1 (Control)	19.86	978.15	244.07	4.18	549.67	18.59	1.32	1.42
S1(0.4 mM)	23.30	1124.82	290.70	3.98	625.60	30.93	1.40	1.38
S2 (0.8 mM)	21.26	965.93	257.78	3.87	661.11	36.00	1.16	1.56
LSD$_{0.05}$	0.51	55.42	24.85	0.352	52.49	1.87	0.17	0.23
LSD$_{0.01}$	0.69	73.31	33.32	0.473	70.39	2.51	0.23	0.31

Note: Every column represents the mean of three replicates (mean of two growth seasons, 2014–2015).

Water deficit reduced stomatal conductance compared to well-watered conditions (Table 2). Soybean genotypes responded differently to water deficit, Williams and L17 had the highest ($23.21\ mM\ m^{-2}\ s^{-1}$) and the lowest ($20.27\ mM\ m^{-2}\ s^{-1}$) stomatal conductance, respectively. Exogenous SA application reverses drought induced stomatal closure. Stomatal conductance increased significantly with foliar spray of 0.4 mM SA (Table 2).

Chlorophyll and carotenoid content was affected by water deficit. There was a significant decrease in both chlorophyll a and b and carotenoid content under water deficit in soybean leaves (Table 2). Foliar spray with 0.4 mM SA significantly increased the chlorophyll content (15% chlorophyll a and 19% chlorophyll b) in S2 compared with control. The change in the chlorophyll a/b ratio was not significant in this study (Table 2). Total carotenoids content increased significantly with an increase in SA concentration in soybean genotypes. The highest and the lowest amount of carotenoid were observed at S3 and S1 (550 and 661 $\mu g\ g^{-1}$ Fw), respectively (Table 2).

Water deficit increased membrane lipid peroxidation via increase of MDA concentration and decreased leaf membrane stability index (MSI %). Application of 0.4 mM SA as compared to no application decreased MDA content in Williams genotype by 16.2%, 35.3 and 36% in I_1, I_2 and I_3 treatment, respectively (Figure 1(A,B)).

With increasing water deficit H_2O_2 content increased continuously in all genotypes. Under severe stress condition (I_3), maximum H_2O_2 (37 $\mu M\ g^{-1}$ Fw) was recorded in D42XI9 genotype (Figure 2(D)). Foliar application of SA also increased significantly concentration of H_2O_2 in soybean genotypes leaves and H_2O_2 content was the greatest in 0.8 mM treated plants than in 0.4 mM treated plants and the controls (Figure 2(H)).

In the water deficit conditions, RWC of the leaves reduced, more remarkably in the L17 genotype (a decrease of about 17% in I_2 and 23% in I_3) as compared with I_1 (Figure 2 (A)). SA-treated plants exhibited a slower increase in RWC during water deficit (Figure 2(E)).

Water deficit reduced LAI, while application of 0.4 mM SA increased LAI in three genotypes. LAI in L17 was significantly higher than the Williams and D42XI9 in I_1 and decreased more than the other in I_2 and I_3 treatments (Figure 3(C)). SLA was considerably elevated by water deficit. The highest (1.87 mg cm^{-2}) and the lowest amount of SLA (1.06 mg cm^{-2}) were observed in I_3 and I_1 treatments respectively. SLA in L17 was significantly higher than the Williams

and D42XI9 genotypes (Table 2). It seems that significant increase in SLA by L17 due to more reduction of LAI in this genotype under water deficit conditions.

Proline and soluble sugar content had significantly changed during water deficient stress and SA application. By increasing the stress intensity, proline and soluble sugar content increased likewise, the amount of proline increased up to 28% in I_2 and up to 71% in I_3 and soluble sugar content increased 23% in I_2 and 64% in I_3 compared to well-watered plants in I_1 (Table 2). Proline and soluble sugar content significantly increased with increasing SA levels. The highest content of proline (36 $\mu g\ g^{-1}$ Fw) was observed at S3 (0.8 mM SA) (Table 2).

The total protein content also was affected by irrigation schedules and SA application. Water deficit increased total protein in all genotypes. The highest and lowest amount of total protein was observed in I_3 treatment by Williams and in I_1 by L17, respectively (Figure 2(B)). Application of 0.4 mM SA led to the highest value from this trait. Increasing in SA concentration from 0.4 to 0.8 mM decreased significantly total protein content (Figure 2(F)).

Irrigation schedules induced different changes in the antioxidative enzyme activities, CAT activity increased significantly in the leaves of all the genotypes under water limitation. The highest activity of CAT (32.4 OD mg^{-1} protein min^{-1}) was determined under severe stress treatment (I_3) by L17 genotype and the lowest activity 19.89 (OD mg^{-1} protein min^{-1}) was observed in I_1 by Williams genotype (Figure 2(C)). The significant decrease of CAT activity was observed in three genotypes by SA foliar spraying (Figure 2 (G)). Water-stressed plants showed higher SOD and APX activity in three genotypes as compared to well-watered ones (Figure 3(A,B)). Stressed plants treated with SA showed a greater increase in SOD and APX activities and application of 0.4 mM SA the most effective treatment. SOD activity in D42XI9 leaves and APX activity in Williams under water deficit at 45% of field capacity by foliar application of 0.4 mM SA enhanced considerably when compared with untreated plants.

Water limitation decreased yield components such as the number of grains m^{-2} and pods plant^{-1} and grain yield in three genotypes compared to the control. Despite of in the I_1 treatment, grain yield in three genotypes were close together and in the same statistical group, but with increasing stress severity in I_3 grain yield of L17 decreased significantly compared to other genotypes (Figure 3(D–F)). SA increased number of grains m^{-2}, pods plant^{-1} and grain yield in well-
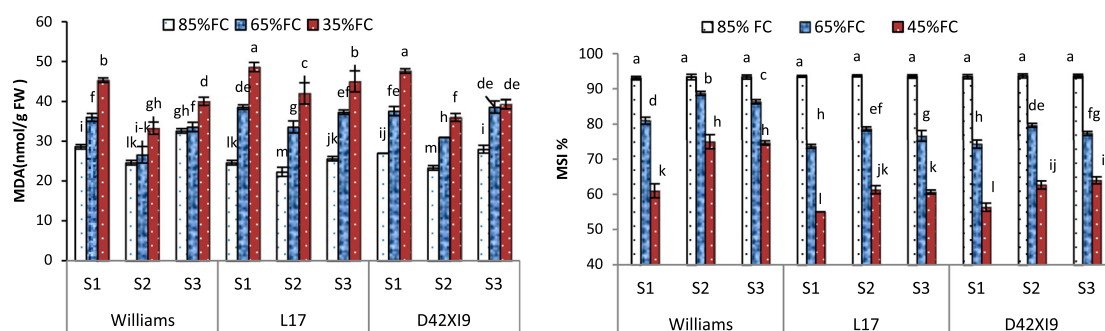
Figure 1. Changes in the concentration of malondialdehyde (MDA) and leaf membrane stability index (MSI %) of three soybean genotypes (Williams, L17 and D42XI9) under different irrigation schedules [85% (I_1), 65% (I_2) and 45% (I_3) of field capacity], were subjected for different concentrations of SA [control (S1), 0.4 mM (S2) and 0.8 mM (S3)]. Every column in each graph represents the mean (±SE) of three replicates of two growth seasons in 2014–2015 and different letters indicate significant differences by LSD $p < .05$.
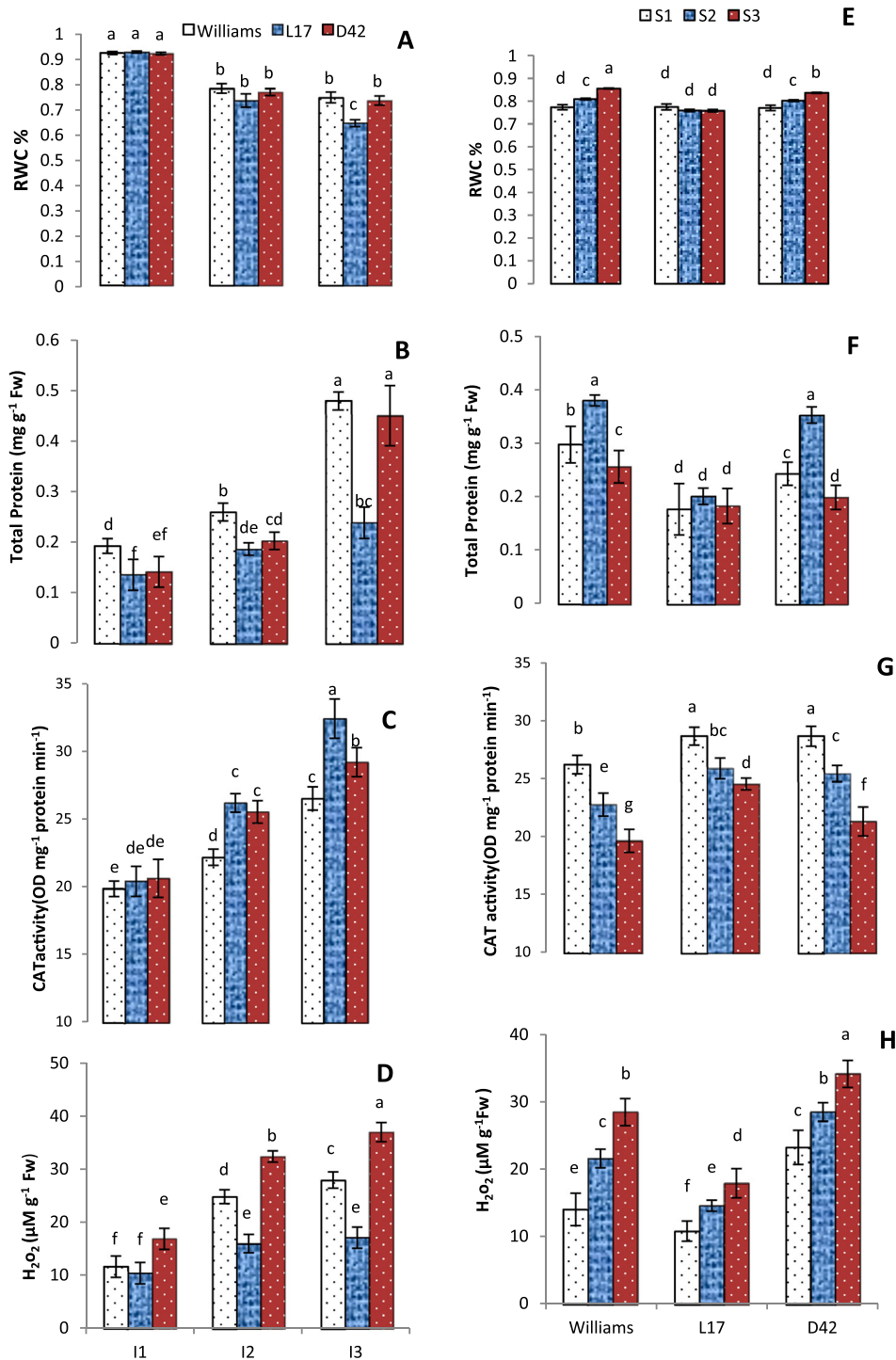
Figure 2. Changes in relative water content (RWC), total protein, catalase activity(CAT), Hydrogen peroxide (H$_2$O$_2$) of three soybean genotypes (Williams, L17 and D42XI9) under different irrigation schedules [85% (I_1), 65% (I_2) and 45% (I_3) of field capacity) (A–D) and different concentrations of SA [control (S1) 0.4 mM (S2) and 0.8 mM(S3)]. Every column in each graph represents the mean (±SE) of three replicates of two growth seasons in 2014–2015 and different letters indicate significant differences by LSD $p < .05$.

watered and water deficit conditions. The effect of 0.4 mM SA was more considerable. Application of 0.4 mM SA increased grains number m^{-2} in Williams by 9.3%, 7.5% and 9.2%, in L17 by 4.6%, 3.3% and 29.1% and in D42XI9 4.6%, 7.3% and 13.7%, pods per plant in Williams by 31%, 20% and 11%, in L17 by 17%, 13% and 8% and in D42XI9 18%, 15% and 15% and grain yield in Williams by 1.7%, 15.9% and 15.1%, in L17 by 1.6%, 2.9% and 0.1% and in D42XI9 4.2%,15.3% and 26.1% compared to the values of the untreated plants under non-stressed (85% of field capacity) and water-stressed (65% and 45% of field capacity) conditions, respectively (Figure 3(D)).

4. Discussion

The drought induces reduction in the chlorophyll content could be attributes to inducing the chloroplast destruction and the instability of chlorophyll protein complex (Khan et al. 2015). Both the chlorophyll *a* and *b* decreases during water stress (Farooq et al. 2009). In contrast, Silvente et al. (2012) observed no effect of water stress on chlorophyll content as well as chlorophyll *a/b* ratio in tolerant and sensitive varieties of soybean. Carotenoids have additional roles in scavenging ROS, stabilize photosynthetic complexes, participate in energy dissipation and can help the plants to reduce
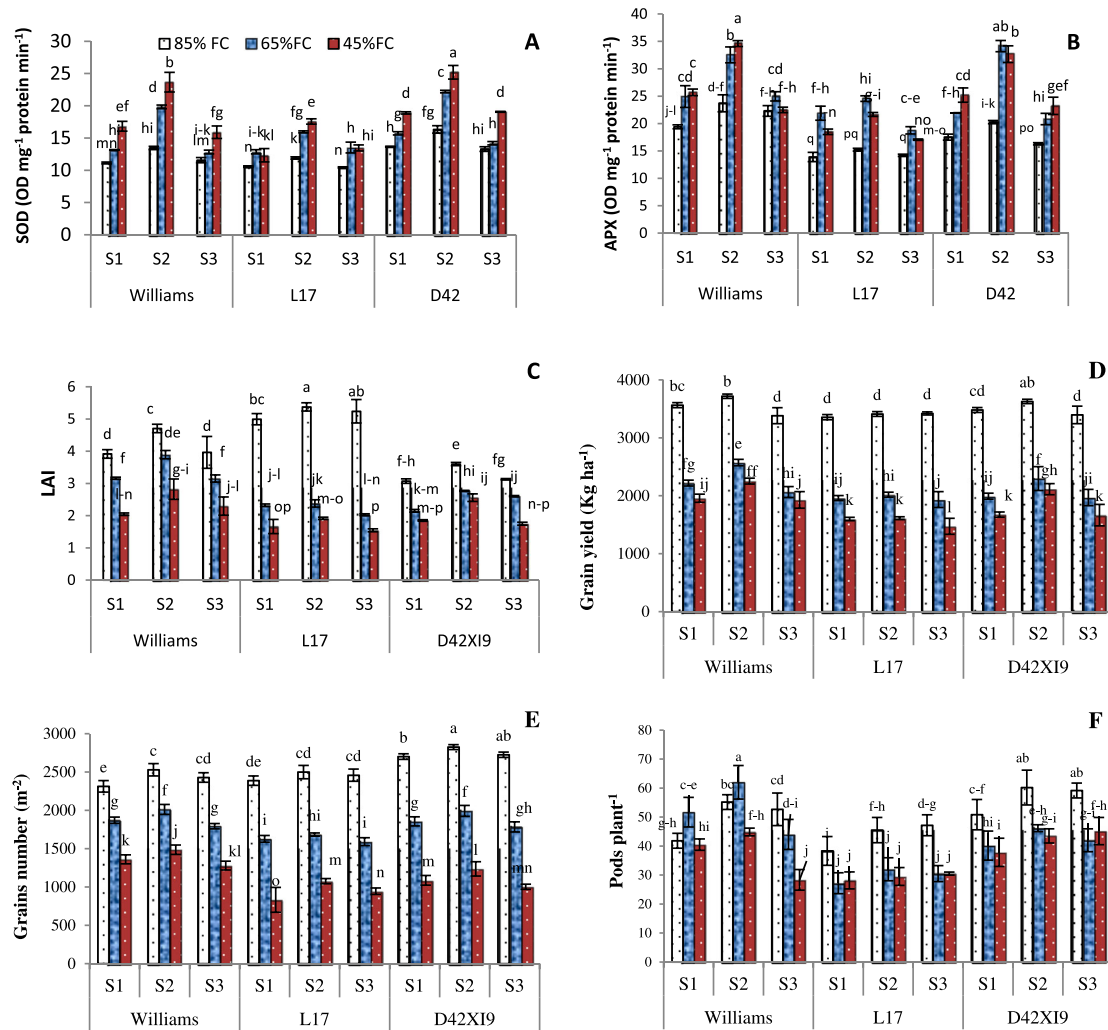
Figure 3. Changes in the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and leaf area index (LAI), grain yield (kg ha^{-1}), grains number m^{-2} and pods per plant of three soybean genotypes (Williams, L17 and D42XI9) under different irrigation schedules [85% (I_1), 65% (I_2) and 45% (I_3) of field capacity) were subjected for different concentrations of salicylic acid [control (S1), 0.4 mM (S2) and 0.8 mM(S3)]. Every column in each graph represents the mean (±SE) of three replicates of two growth seasons in 2014–2015 and different letters indicate significant differences by LSD $p < .05$.

the adverse effects of drought stress (Moharekar et al. 2003). Spraying soybean genotypes with SA caused stimulation of the total photosynthetic pigments (chl. a, chl. b and carotenoids) in leaves compared with control plants (Table 2). These results are concordant with Moharekar et al. (2003), Idrees et al. (2010), Jaiswal et al. (2014) and Jakhar and Sheokand (2015). In contrast to the negative impact of water deficit, SA applications improved stomatal conductance (Table 2). Sadeghipour and Aghaei (2012) observed that decreases in the stomatal conductance were lower in SA treated plants than control for both cultivars of common bean under both well-watered and water-stressed conditions. Restriction of reduction in stomatal conductance by the application of SA plays an important role on maintaining photosynthetic activity and damage is reduced (Idrees et al. 2010). High stomatal conductance and chlorophyll content coupled with higher photosynthetic capacity were responsible for SA-induced improvement of yield components and grain yield under water deficit (Figure 3(D–F)). Proline and soluble sugar concentrations were increased under water deficit conditions and SA foliar application (Table 2). Proline accumulation is proposed to be associated with tolerance to osmotic stress (Farooq et al. 2009). Tripathi et al. (2015) reported that water stress affected proline contents in the

leaves of the sensitive variety of soybean, but not in the tolerant genotype. SA application increases drought resistance by the accumulation of different osmotic compounds including soluble sugar and proline, which is essential for osmotic adjustment mechanism (Idrees et al. 2010; Aldesuquy et al. 2013; Jaiswal et al. 2014). SA also improved RWC (Figure 2 (E)) and LAI (Figure 3(C)) of soybean genotypes under water deficit conditions. Silvente et al. (2012) reported that under water limitation, tolerant variety of soybean showed only a marginal reduction in RWC as compared to the sensitive genotype. In water deficit condition, drought tolerant soybean cultivar shows more LAI compared with less tolerant cultivars that associate with the lowest rate of reduction in stomatal conductance in the tolerant cultivar (Hossain et al. 2014). Increasing LAI in water-stressed plants by application of SA might be due an increased cell turgor pressure because of the accumulation of soluble sugars and other osamatically active substance such as proline (Table 2) and soluble proteins (Figure 2(B,F)). MDA concentration and cell membrane damage increases in water deficit conditions due to increasing the production of ROS (Ruppenthal et al. 2016). The SA application alleviated the damaging effects of water deficit on cell membranes of soybean genotypes and concomitantly increased the SOD and APX activity (Figure 3). MDA triggers

the action of free radicals and the carotenoids have a key role in protecting against these (Moharekar et al. 2003). Spray with SA decreases the level of MDA induced by water stress with increasing the production of antioxidant enzymes activities like SOD and APX (Sadeghipour and Aghaei 2012). Antioxidant enzymes such as SOD and APX have an important protective role in scavenging ROS and protect plant tissues from oxidative damage (Idrees et al. 2010; Makbul et al. 2011; Jaiswal et al. 2014; Ghaderi et al. 2015). In contrast, the treatment with SA reduced CAT activity and increased H_2O_2 concentration in leaves (Figure 2). CAT belongs to the group of enzymes that regulating the levels of cellular ROS, converts H_2O_2 to H_2O and O_2 in peroxisomes and the mitochondria and protects them from H_2O_2 damages (Ananieva et al. 2004). Our results showed that spraying with SA decreased CAT activity in soybean genotypes (Figure 2(G)). Increased of binding CAT activity by application of SA in extracts from soybean leaves has been reported also by Chen and Klessig (1991). In contrast with these results Ghaderi et al. (2015) showed that exogenous application of 0.1 mM SA improved plant environmental stress tolerance by increasing the activities of antioxidant enzymes such as CAT under drought stress in both cultivars of strawberry. In our study with the increase in concentration of foliar applied SA, the significant increase in H_2O_2 content was detected. While H_2O_2 generation is a continuous process, inhibiting the activity of CAT, one of the primary pathways of H_2O_2 decomposition, could result in H_2O_2 accumulation in plants. Although high levels of H_2O_2 are toxic, but they have significant impact on signal transmission at lower concentrations in many plant species (Ishibashi et al. 2011). These results indicate that SA mode of operation is to bind CAT and prevents its activity by increasing the concentration of H_2O_2. This is related to the fact that SA treatment causes a balanced increase in the production of ROS and activity of antioxidant enzymes and enhanced tolerance of plants to water deficit. Similar mechanism was reported for SA induced multiple water stress tolerance in lemongrass (Idrees et al. 2010), maize (Saruhan et al. 2012) and bean (Sadeghipour and Aghaei 2012).

Grain yield and yield components were improved by application of 0.4 mM SA in stressed and non-stressed plants (Figure 3). The increasing of grain yield due to SA was combined with the improvement of RWC (Figure 2 (E)), reduced stomatal conductance restriction and the stimulant effect on the biosynthesis of photosynthetic pigments in soybean leaves (Table 2). Water stress leads to a reduction of grain yield in soybean due to the reduction of grain number which is mainly determined by pods number (Khatun et al. 2016). Drought stress increases the rate of pod abortion and reduces the seed number (Hossain et al. 2014). SA can increase sink strength via cell division in the immature ovaries and conducts the metabolites stream to the developing grains which leads to reduce the abortion rate (Horvath et al. 2007). Reducing the abortion rate could have significantly increased the number pods plant^{-1} and number of seeds pod^{-1} in soybean as observed by Khatun et al. (2016). Our results suggested that SA enabled the leaf to maintain a high level of RWC, increased the activity of antioxidant enzymes and reduced adverse effect of water deficit in soybean genotypes especially in Williams.

5. Conclusion

The present study indicated that water deficit conditions had several adverse effects in all genotypes, including decreases in stomatal conductance, chlorophyll content, RWC, LAI and grain yield. There was variation among the genotypes in proline, soluble sugars, total protein and H_2O_2 content. Williams was less susceptible to water deficit than D42XI9 and L17 because Williams showed less reduction in stomatal conductance and LAI. Water deficit increased oxidative damage, membrane lipid peroxidation (MDA content) as well as antioxidant enzymes (SOD and APX) activities in soybean leaves, while SA (especially 0.4 mM) induced protection against water deficit via maintenance of membrane stability by decline in MDA content and more increase in antioxidant enzymes activities as well as proline and soluble sugar accumulation. The tested SA doses showed that the foliar spraying of 0.4 mM SA stimulated growth promoting responses, but 0.8 mM SA foliar spraying simultaneously decreased the content of total proteins and CAT activity and led to an increase of H_2O_2 accumulation. Foliar application of 0.4 mM SA improved soybean genotypes tolerance to water stress by limited lipid peroxidation, promotion antioxidant enzymes activity and improvement yield components and grain yield particularly in Williams genotype.

Disclosure statement

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