



## RESPONSE OF SOYBEAN TO WATER STRESS CONDITIONS AND FOLIAR APPLICATION WITH SALICYLIC AND ASCORBIC ACIDS

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**ABSTRACT:** A field experiment was conducted at Etay El-Baroud Agricultural Research station during the two successive seasons 2012 and 2013 to study the effect of three levels of available soil moisture depletion (ASMD) namely wet (25-30%), medium (45-50%) and dry (65-70%) and foliar spray of salicylic and ascorbic acids singly or in combination at the concentration of 100 and 200 ppm on vegetative growth, yield components and some biochemical constituents of soybean (*Glycine max* L.). Increasing soil moisture stress up to 65-70% ASMD significantly decreased shoot and leaf dry weight, leaf area and leaf area index as well as, chlorophyll a, b and chl. (a+ b) content, relative water content in leaves and seasonal water consumptive. Whereas, peroxidase and polyphenol oxidase activities, proline content and water use efficiency were significantly increased when, the plants were received medium treatment compared with wet or dry treatment. Dry treatment significantly decreased plant height, number of branches/ plant, number of pods/ plant, weight of seeds/plant, 100-seed weight, harvest index and seed yield/faddan as well as total carbohydrates, protein accumulation and oil content in seeds. Foliar application of either salicylic or ascorbic acid and their combination had significant effect on growth parameters and yield components in both studied seasons, except, shoot dry weight at 90 days after sowing in the first season, leaf dry weight and number of branches/ plant in the second season as well as, number of pods/ plant which were insignificant affected in both seasons. Foliar application with salicylic or ascorbic acid and their combination induced significant increase for chlorophyll a, b content, peroxidase and polyphenol oxidase activities, proline, total carbohydrates, protein accumulation and oil content in seeds as well as, seasonal water consumptive use and water use efficiency compared with untreated plants. The interaction effect between water stress and foliar application of salicylic and ascorbic acids was found to be significant on leaf area, leaf area index, peroxidase and polyphenol oxidase activities and proline in leaves at 75 days after sowing in the second season. The maximum values of water use efficiency were obtained when plants were irrigated at 45-50% ASMD and sprayed with 200 ppm of salicylic or ascorbic acid and their combination at 100 ppm for each.

**Key words:** Soybean, water stress, salicylic acid, ascorbic acid, polyphenol oxidase, proline.

### INTRODUCTION

Soybean (*Glycine max* L.) is considered one of the main leguminous crops in the world for its importance in human nutrition as good source for protein and oil since it has the highest protein content in comparison with the other leguminous crops. It is necessary to investigate certain abiotic factors such as drought and salinity that may limit the soybean yield (Shilpi and Narendra, 2005).

Drought is perhaps the major factor limiting crop production world-wide (Shangguan *et al.*, 2000). Soil moisture stress affected growth and yield of soybean (Frederick *et al.*, 2001).

Water stress during growth stages of plants adversely affects on many physiological growth process (photosynthesis, translocation of carbohydrates and growth regulators, ion uptake, transport assimilation, N<sub>2</sub> fixation, turgidity, respiration) and shoot traits (Fageria *et al.*, 2006).

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The induction of peroxidase activity in plants occurs in response to numerous biotic and abiotic stimuli.

The roles of peroxidase can play in cell wall toughening and in production of toxic secondary metabolites and its simultaneous oxidant and antioxidant properties make it an important factor in the defense response of plants to a variety of stresses (Idrees *et al.*, 2011).

Oxidation of phenols by polyphenol oxidase leads to formation of quinones and free radicals that can activate enzymes, which form a part of the metabolic processes acting against different stresses (Bhonwong *et al.*, 2009).

There are different approaches to mitigate the drought hazards, which include the development of stress tolerant plants by selection of stress resistant varieties (Ahloowalia *et al.*, 2004) also, *in vitro* selection, use plant growth hormones (ABA, GA, cytokinin, SA), antioxidants (ascorbic acid, H<sub>2</sub>O<sub>2</sub>) and osmoprotectants as foliar application and seed treatment (Farooq *et al.*, 2009).

The survival of plants under such a stressful condition depends on the plants ability to perceive the stimulus, generate and transmit the signals and to initiate various physiological and biochemical changes (Hossain and Fujita, 2009). The molecules such as salicylic and ascorbic acids have been suggested as signal transducers or messengers. These substances have obtained particular attention because of inducing a protective effect on plants under abiotic stress.

The potent impact of salicylic and ascorbic acids on various organs of plant structure and function prompt many investigators to apply them to several crop plants aiming to control pattern of growth and development coupled with enhancement of systematic resistance against various hurtful agents which may appear in the surrounding environments (Amin *et al.*, 2008).

Additionally, the main function of antioxidants such as SA and vitamins were protective of cell membranes and their binding

transporter proteins (H<sup>+</sup> - ATP-ase membrane pumps), maintained their structure and function against the toxic and destructive effects reactive oxygen species (ROS) during stress, in turn, more absorption and translocation of minerals (Fathy *et al.*, 2000).

Salicylic acid is an endogenous growth regulator from phenol compound group that in different of process in plants is operative such as seed germination, stomata closure, nutrient uptake, chlorophyll synthesis, protein synthesis, transpiration and photosynthesis (Khan *et al.*, 2003). Salicylic acid is a plant phenol and today it is in use as internal regulator hormone, because its role in the defensive mechanism against biotic and abiotic stresses has confirmed. Salicylic acid may affect directly on specific enzymes function or may activate the genes responsible for protective mechanisms (Hayat and Ahmed, 2007; Horvath *et al.*, 2007). Role of SA has been well documented in the activation of defense responses against various biotic and abiotic stresses (Zhao *et al.*, 2009; Idrees *et al.*, 2011). Ascorbic acid affects phytohormone-mediated signaling processes during the transition from the vegetative to the reproductive phase as well as the final stage of development and senescence (Barth *et al.*, 2006). It is also important as a cofactor for a large number of key enzymes in plants (Arrigoni and de Tullio, 2000). Ascorbic acid is major water-soluble antioxidant, protecting biologically important macromolecules from oxidative damage caused by hydroxyl radicals, superoxide and singlet oxygen. In addition to its importance in photoprotection its, regulate of photosynthesis (Smirnoff, 2000). Ascorbic acid plays an important role in the regulation of cell cycle and several fundamental processes of plant growth and development.

In this study it seemed necessary to study the effects of various levels of soil moisture stress on growth, yield and some metabolic processes and the role of salicylic and ascorbic acids in amelioration of the adverse effects of water stress.

## MATERIALS AND METHODS

A field experiment was carried out at Etay El-Baroud Agric. Res. Station during the two successive summer seasons 2012 and 2013 to study the effect of soil moisture stress and foliar spray of salicylic and ascorbic acids on the growth, yield and its components, photosynthetic pigments, proline, peroxidase and polyphenol oxidase in leaves as well as crude protein, total carbohydrates and oil content of soybean seeds and water relations *i.e.* relative water content of leaves (RWC), water consumptive use (WCU) and water use efficiency (WUE).

The experiment was laid out in split plot design with four replicates. The main plots were occupied by soil moisture levels, while sub-plots contained foliar spray of salicylic and ascorbic acids. Plots were separated from each other by 1.5 meters distance to avoid the interference between irrigation treatments. Each sub-plot area was 14 m<sup>2</sup> (3.5×4m) and included 5 ridges, 4m long and 70 cm apart.

Some physical and chemical properties of the experimental site are shown in Table 1.

The treatments were as follows:

### Main Plots (Irrigation Treatments)

A-Irrigation at 25-30% of available soil moisture depletion (ASMD) (wet treatment).

B-Irrigation at 45-50% of ASMD (medium treatment).

C-Irrigation at 65- 70% of ASMD (dry treatment).

### Sub-plots (Foliar Spray of Salicylic and Ascorbic Acids)

1-Spraying water (control)

2-Spraying 100 ppm salicylic acid (SA)

3-Spraying 200 ppm salicylic acid (SA)

4-Spraying 100 ppm ascorbic acid (ASC)

5-Spraying 200 ppm ascorbic acid (ASC)

6-Spraying 100 ppm salicylic+100 ppm ascorbic acids

Soybean seeds of Giza 111 variety was used in this study, which obtained from Legumes

Research Section, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Soybean seeds were planted on 5/6/2012 and 6/6/2013 in the first and second seasons, respectively in two sides /ridge with 2 seeds in hill spaced 20 cm.

All agricultural practices were carried out according to the recommendations of Ministry of Agriculture, Egypt. Phosphorus fertilizer at the rate of 150 Kg P<sub>2</sub>O<sub>5</sub>/faddan was applied to the soil just before sowing in the form of calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>). Nitrogen fertilizer at the rate of 22.5 Kg N/faddan was applied in two equal doses (before sowing and after thinning).

Salicylic and ascorbic acids were foliar sprayed three times at 33, 47 and 61 days after sowing (spraying every two weeks).

Irrigation treatments were applied after 30 days from sowing.

### Growth Characters

Five plants were randomly taken from each sub-plot at 75 and 90 days after sowing to estimate dry weight of shoot system and leaves, leaf area and leaf area index.

To determine leaf area / plant, 10 disks area equal  $(10 \times 3.14 \times (1.5)^2 = 70.65 \text{ cm}^2)$  according to Hunt (1990) by the following formula:

#### Leaf area / plant, in cm<sup>2</sup>

$$L A = 70.65 \times \frac{\text{dry weight of leaves per plant}}{\text{dry weight of leaves disks}}$$

#### Leaf area index

$$L A I = \frac{\text{leaf area per plant}}{\text{ground area occupied by plant}}$$

Plants samples were dried in an electric oven with drift fan at 70°C for 48 hr., till constant dry weight.

### Yield and Yield Components

Harvesting took place on 3/10/2012 and 4/10/2013 in the first and second seasons, respectively. At harvesting time, five plants from the central row in each sub-plot were randomly taken to determine plant height, number of branches /plant, weight of seeds/plant and 100 – seed weight.

**Table 1. Physical and chemical properties of the experimental site at the two growing seasons (2012 and 2013)**

Soil property	Soil texture	Clay (%)	Silt (%)	Sand (%)	pH	Organic matter (%)	Available N (ppm)	Available P (ppm)	Available K (ppm)
Season 2012	Clay	53.37	34.80	11.83	8.10	1.79	53.0	31.0	339
Season 2013	Clay	54.23	33.62	12.15	8.20	1.85	40.0	29.0	290

Plants in a central area (4 m<sup>2</sup>) in each sub-plot were harvested and weighed then converted to seed yield Kg/faddan.

### Physiological Traits

#### Chlorophyll content of leaves

Chlorophyll a and b content in fresh leaves (as mg/g fresh weight) at 75 days after sowing were determined and calculated according to Moran and Porath (1980).

#### Antioxidant enzymes of leaves

##### Peroxidase activity

The activity of peroxidase enzyme was determined by employing the method of Thimmaiah (1999), after 75 days from sowing, 3 g fresh leaves were ground in a precooled mortar and pestle containing 9 ml of 0.1 M phosphate buffer (pH 7.1). The extract was centrifuged at 3000 rpm at 60c for 20 min. Peroxidase activity was expressed as changes in absorbance min<sup>-1</sup> at 425 nm.

##### Polyphenol oxidase activity

Poyphenol oxidase activity was estimated as described by Mayer and Harel (1979) with some modifications.

##### Proline content of leaves

Proline in leaves was determined according to Bates *et al.* (1973). The results were calculated in mg / g dry weight.

At harvest time, samples of mature seeds were prepared to chemical analysis.

##### Total carbohydrates content

Total carbohydrates were determined in the dried seeds, using phenol sulphuric method (Dubois *et al.*, 1956).

#### Protein content

Total nitrogen was determined using the modified Micro-Kjeldahl method (AOAC, 1988). The total protein was calculated by multiplying the values of total nitrogen by 6.25.

#### Oil content

Oil content was determined using Soxhlet extraction apparatus using petroleum ether as solvent and as percentage calculated on dry weight basis (AOAC, 1990).

#### Water relations

##### Relative water content of leaves, RWC (%)

At 75 days after sowing, leaf samples, were immediately weighed (fresh weight, FW) and transferred into sealed flasks, then rehydrated in water for 5 hr. until fully turgid at 4°C, surface swabbed and reweighed (turgid weight, TW). Leaf samples were oven dried at 70°C for 48 hr., and reweighed (dry weight, DW).

RWC (%) was calculated according to Lazcano-Ferrat and Lovatt (1999) as follows:

$$RWC(\%) = \frac{(FW - DW)}{(TW - DW)}$$

##### Water consumptive use (WCU)

Soil samples were taken, using a regular auger, at planting time, just before and 48 hours after, each irrigation and at harvest for soil moisture determination. Irrigation was applied when the moisture content reached the desired available soil moisture for each treatment. At each sampling date, duplicate of soil samples were taken from 0-15, 15-30, 30-45 and 45-60 cm depths and their moisture content were gravimetrically determined and presented in Table 2.

**Table 2. Some soil moisture parameters at different depths of the experimental site**

Depth (cm)	Field capacity (%)	Wilting point (%)	Available moisture (%)	Bulk density (g/cm <sup>3</sup> )
0 - 15	37.8	18.6	19.2	1.03
15 -30	34.2	16.2	18.0	1.07
30 -45	33.1	15.5	17.6	1.08
45-60	30.6	14.7	15.9	1.1

The depleted soil moisture was detected after each irrigation and the following equation was used for calculating water consumptive use according to (Israelsen and Hansen, 1962):

$$Cu = D \times Bd \times (e_2 - e_1) / 100$$

Where:

Cu = Water consumptive use (ET) in mm.

D = Soil depth (cm)

Bd = Bulk density in g/cm<sup>3</sup>

e<sub>1</sub>, e<sub>2</sub> = Soil moisture content before and after each irrigation.

#### Water use efficiency (WUE)

Water use efficiency in Kg/m<sup>3</sup>/faddan was calculated for each treatment according to the equation described by Pierre *et al.* (1965) as follows:

WUE = seed yield (Kg /fad.) /seasonal water consumption in m<sup>3</sup>/fad.

#### Statistical Analysis

Data were statistically analyzed according to (Snedecor and Cochran, 1980) and means were compared using LSD values at 5% level of probability.

## RESULTS AND DISCUSSION

### Growth Characters

Data presented in Tables 3 and 4 show that increasing soil moisture depletion level from 25-30% up to 65-70%, significantly decreased shoot and leaf dry weight, leaf area and leaf area index in both seasons at 75 and 90 days after sowing.

The minimum values of such traits were obtained from dry treatment (irrigation at 65-70% ASMD). These findings explain that, decreasing available soil moisture level decreased plant growth. The obtained results are in agreement with those reported by (Abdul-Wasea and Elhindi, 2011; Pirzad and Shokrani, 2012).

The decrease in total dry weight may be due to considerable decrease in plant growth, photosynthesis and canopy structure, as indicated by leaf senescence during drought stress (Nautiyal *et al.*, 2009). In this respect Hamayun *et al.* (2010) reported that shoot fresh and dry weights in maize and soybean plants were significantly reduced, when exposed to drought due to reduced shoot growth, increased senescence and switching over the plant growth from shoot growth towards root growth.

Also, water deficit decreased leaf area index, which induced a reduction in leaf area and number of leaves/ plant. These results are in harmony with those obtained by Abdo and Anton (2009) on sesame and Ali *et al.* (2011) on maize.

Foliar application of salicylic and ascorbic acids alone at 100 and 200 ppm or in combination at 100 ppm promoted growth criteria (shoot and leaf dry weight, leaf area and leaf area index) at two physiological stages (at 75 and 90 days after sowing) in the two seasons compared to the control.

Generally, foliar application of the tested compound led to significant differences between these treatments in both seasons, except shoot dry weight at 90 days in the first season and leaf dry weight in second season which were not significantly affected.

**Table 3. Dry weight of shoot system and leaves, leaf area and leaf area index of soybean plants as affected by soil moisture stress and foliar application of salicylic and ascorbic acids (first season 2012)**

Treatment		Dry weight of shoots (g)		Dry weight of leaves (g)		Leaf area (cm <sup>2</sup> )		Leaf area index	
Irrigation level	Foliar application (ppm)	75 DAS	90 DAS	75 DAS	90 DAS	75 DAS	90 DAS	75 DAS	90 DAS
25-30% ASMD	Water (0)	69.68	206.2	25.21	38.71	3363	5210	2.40	3.72
	100 SA	70.74	207.8	28.69	41.07	3595	5546	2.57	3.96
	200 SA	73.53	214.2	30.39	45.63	4201	6359	3.00	4.54
	100 ASC	70.52	211.0	27.09	39.38	4332	5555	3.09	3.97
	200 ASC	74.67	224.2	29.94	44.28	4538	6067	3.24	4.33
	100 SA+100 ASC	74.29	213.6	31.07	44.66	4629	6304	3.31	4.50
Mean		72.24	212.8	28.74	42.29	4110	5840	2.94	4.17
45-50% ASMD	Water (0)	60.78	198.4	21.98	31.99	3135	4616	2.24	3.30
	100 SA	68.88	212.6	25.08	37.57	3417	4999	2.44	3.57
	200 SA	70.05	208.0	26.78	37.85	3363	5168	2.40	3.69
	100 ASC	65.66	207.6	25.20	38.14	3542	5279	2.53	3.77
	200 ASC	68.30	202.2	25.54	36.80	3338	5386	2.38	3.85
	100 SA+100 ASC	66.24	203.8	27.51	39.82	3305	5583	2.36	3.99
Mean		66.65	205.4	25.35	37.03	3350	5172	2.39	3.70
65-70% ASMD	Water (0)	45.85	188.8	19.83	27.54	2212	3913	1.58	2.80
	100 SA	54.72	201.0	21.17	30.47	2874	4473	2.05	3.19
	200 SA	56.07	204.0	22.75	33.88	3130	4381	2.24	3.13
	100 ASC	48.55	198.4	20.65	28.18	2898	4665	2.07	3.34
	200 ASC	60.07	199.4	21.83	28.43	3197	4779	2.29	3.42
	100 SA+100 ASC	52.81	209.2	23.37	32.91	3232	4843	2.31	3.46
Mean		53.01	200.1	21.60	30.24	2924	4509	2.09	3.22
General mean of foliar application	Water (0)	58.77	197.8	22.32	32.75	2903	4580	2.07	3.27
	100 SA	64.78	207.1	24.98	36.37	3295	5006	2.35	3.58
	200 SA	66.55	208.7	26.64	39.12	3565	5303	2.55	3.79
	100 ASC	61.58	205.7	24.32	35.23	3591	5167	2.57	3.69
	200 ASC	67.68	208.6	25.77	36.51	3691	5411	2.64	3.87
	100 SA+100 ASC	64.45	208.9	27.32	39.13	3722	5576	2.66	3.98
LSD 0.05	Irrigation	3.90	6.0	2.62	3.93	241.3	421.9	0.20	0.30
	Foliar application	4.13	N S	3.02	4.40	438.0	467.2	0.32	0.33
	Irri. × Foliar appli.	N S	N S	N S	N S	N S	N S	N S	N S

SA = salicylic acid

ASC= ascorbic acid

ASMD = available soil moisture depletion

**Table 4. Dry weight of shoot system and leaves, leaf area and leaf area index of soybean plants as affected by soil moisture stress and foliar application of salicylic and ascorbic acids (second season 2013)**

Treatment		Dry weight of shoots (g)		Dry weight of leaves (g)		Leaf area (cm <sup>2</sup> )		Leaf area index	
Irrigation level	Foliar application (ppm)	75 DAS	90 DAS	75 DAS	90 DAS	75 DAS	90 DAS	75 DAS	90 DAS
	<b>Water (0)</b>	63.79	198.2	23.41	34.88	3313	5199	2.37	3.71
<b>25-30%</b>	<b>100 SA</b>	68.31	202.0	23.96	35.95	3349	5426	2.39	3.88
<b>ASMD</b>	<b>200 SA</b>	72.28	207.0	24.50	36.46	3872	5836	2.77	4.17
	<b>100 ASC</b>	65.69	211.4	23.30	35.28	4053	5480	2.90	3.91
	<b>200 ASC</b>	68.57	212.8	25.28	37.15	3975	5841	2.83	4.17
	<b>100 SA+100 ASC</b>	70.97	217.4	25.82	36.37	3938	6081	2.81	4.34
<b>Mean</b>		68.27	208.1	24.38	36.02	3750	5644	2.68	4.03
	<b>Water (0)</b>	57.20	190.2	19.32	29.65	3038	4663	2.15	3.33
<b>45-50%</b>	<b>100 SA</b>	62.37	202.0	18.31	32.06	3150	4720	2.25	3.37
<b>ASMD</b>	<b>200 SA</b>	64.08	206.4	22.14	34.84	3311	4956	2.36	3.54
	<b>100 ASC</b>	61.91	204.2	21.28	31.99	3136	4918	2.24	3.51
	<b>200 ASC</b>	63.31	207.6	20.60	33.18	3302	5117	2.36	3.66
	<b>100 SA+100 ASC</b>	63.99	209.4	23.06	35.41	3256	5223	2.33	3.73
<b>Mean</b>		62.14	203.3	20.79	32.86	3199	4933	2.28	3.53
	<b>Water (0)</b>	46.73	180.8	16.87	24.99	2129	4082	1.52	2.89
<b>65-70%</b>	<b>100 SA</b>	50.22	186.2	17.76	28.33	2801	4249	2.00	2.84
<b>ASMD</b>	<b>200 SA</b>	56.46	189.6	18.73	29.28	3194	4311	2.28	3.08
	<b>100 ASC</b>	52.87	187.6	17.99	29.00	2809	4290	2.01	3.07
	<b>200 ASC</b>	56.98	182.8	19.13	29.53	2831	4438	2.02	3.17
	<b>100 SA+100 ASC</b>	59.28	190.6	20.10	31.43	2993	4512	2.14	3.22
<b>Mean</b>		53.76	186.3	18.43	28.76	2793	4313	2.00	3.05
	<b>Water (0)</b>	55.91	189.7	19.87	29.84	2827	4648	2.01	3.31
<b>General mean of foliar application</b>	<b>100 SA</b>	60.30	196.7	20.01	32.11	3100	4798	2.22	3.36
	<b>200 SA</b>	64.27	201.0	21.79	33.53	3459	5034	2.47	3.60
	<b>100 ASC</b>	60.16	201.1	20.86	32.09	3333	4896	2.38	3.50
	<b>200 ASC</b>	62.95	201.1	21.67	33.29	3370	5132	2.41	3.67
	<b>100 SA+100 ASC</b>	64.74	205.8	22.99	34.40	3396	5272	2.43	3.77
<b>LSD 0.05</b>	<b>Irrigation</b>	2.99	2.88	2.45	2.62	220.1	230.2	0.16	0.21
	<b>Foliar application</b>	4.45	8.43	N S	N S	200.4	249.9	0.14	0.20
	<b>Irri. × Foliar appli.</b>	N S	N S	N S	N S	347.0	N S	0.25	N S

SA = salicylic acid

ASC= ascorbic acid

ASMD = available soil moisture depletion

The most effective treatment on growth parameters was obtained when plants were sprayed with 100 ppm SA+100 ppm ASC which caused increasing in the leaf dry weight, leaf area and leaf area index in the first season, as well caused increasing in shoot dry weight in the second season. Whereas, 200 ppm increased leaf dry weight, leaf area and leaf area index at 90 days in first season. Ascorbic acid at 200 ppm increased shoot dry weight in first season at 75 days after sowing. Also, 100 ppm SA + 100 ppm ASC increased shoot dry weight, leaf area and leaf area index at 90 days in the second season.

Leaf area was increased by advancing soybean age up to 90 days after sowing; this is mainly due to the production of new leaves and leaves expansion through the growth of soybean plant. Hegazi and El-Shraiy (2007) found that foliar application of salicylic acid generally had a positive effect on vegetative growth parameters of common bean. Also, Hassanein *et al.* (2012) on wheat plants.

It was reported that treatment by salicylic acid increase cell division on apical meristem of corn seedling, and improve plant growth under drought stress condition (Shan *et al.*, 2002).

Farahbakhsh and Saiid (2011) showed that salicylic acid seemed to enhance metabolic activities of the cell, which resulted in stem elongation and caused an increase in leaf area. Also, they found that shoot dry weight of maize was increased with increasing SA concentration. Also, Hamad and Hamada (2001) reported that wheat seeds treated with ascorbic acid decreased the bad effectives of drought stress on fresh and dry weight of radical and plumule. The beneficial effects of ascorbic acid upon growth and productivity have been reported on sunflower plants (El-Gabas, 2006).

Amin *et al.* (2008) found that salicylic or ascorbic acid and their combinations increased dry weights of wheat plant and that might be attributed to an increase in number of tillers and spikes as well as leaf area, leading to increased photosynthetic activity.

Baghizadeh and Hajmohammadrezaei (2011) found that in general, salicylic and ascorbic acids significantly relieved the harsh effects of drought on okra germination and growth parameters and it seems that ascorbic and salicylic acids were able to enhance the tolerant ability of the plant to drought stress. Also, Siamak *et al.* (2015) found that salicylic and ascorbic acids increased plant height and plant biomass of chick pea as compared with control under drought stress.

Data in Tables 3 and 4 show that the interaction effect between soil moisture stress and foliar spray of salicylic and ascorbic acids was found to be significant on leaf area and leaf area index at 75 days after sowing in the second season. The maximum values were obtained when plants irrigated at 25- 30% ASMD in combination with foliar spraying with 200 ppm salicylic acid. However, the other growth parameters did not affected significantly.

### Yield and Yield Components

Data in Tables 5 and 6 reveal that soil moisture stress had a significant effect on plant height, number of branches and pods/ plant, seed weight / plant, 100-seed weight, harvest index and seed yield / faddan in the two seasons. Such characters were significantly decreased when the plants were exposed to severe water deficit (irrigated at 65-70% ASMD). These results revealed that increasing soil moisture stress reduced seed soybean growth, which in turn affected yield components. On the contrary, high moisture level enhanced growth plants thereby improved yield components. These results are in the line with those reported by Masoumi *et al.* (2010) who, found that water deficit stress decreased number of pods per soybean plant, thousand seed weight, seed yield and harvest index.

The decrease in yield and yield components of soybean due to water deficiency had also been reported by other researchers (Dominique *et al.*, 2000; Ohashi *et al.*, 2009). Also, Babaeian *et al.* (2011) found that water stress in seed filing stage decreased the amount of photosynthesis material transfer to barley grains, thereby decreased yield and yield components.

Table 5. Yield and yield components of soybean plants as affected by soil moisture stress and foliar application of salicylic and ascorbic acids (first season 2012)

Treatment		Plant	No. of	No. of	Seed	100- seed	Harvest	Seed
Irrigation level	Foliar application (ppm)	height (cm)	branches/ plant	Pods/ plant	weight/ plant (g)	weight (g)	index	yield/ fad.(Kg)
25-30% ASMD	Water (0)	121.8	3.4	98.4	50.00	18.24	0.35	1680
	100 SA	139.4	3.2	116.4	60.25	18.80	0.38	1708
	200 SA	141.0	4.0	115.0	66.38	19.18	0.42	1882
	100 ASC	129.0	3.4	109.0	67.93	19.80	0.45	1720
	200 ASC	138.2	3.8	114.4	66.91	19.96	0.40	1838
	100 SA+100 ASC	145.2	4.2	118.4	67.99	19.69	0.44	1825
Mean		135.8	3.7	111.9	63.25	19.28	0.41	1775.5
45-50% ASMD	Water (0)	116.8	2.8	81.2	44.12	17.77	0.32	1511
	100 SA	125.0	3.2	85.8	54.94	19.16	0.36	1638
	200 SA	123.4	3.8	93.8	58.24	18.11	0.38	1690
	100 ASC	121.4	3.2	89.0	55.97	18.71	0.39	1640
	200 ASC	124.6	3.4	94.2	59.95	19.33	0.38	1707
	100 SA+100 ASC	125.2	3.6	109.8	56.22	19.49	0.37	1652
Mean		122.7	3.2	92.3	54.91	18.76	0.37	1640
65-70% ASMD	Water (0)	97.6	2.4	63.2	31.85	16.32	0.22	1194
	100 SA	108.2	2.6	70.4	38.74	16.85	0.24	1285
	200 SA	111.4	3.2	74.4	39.50	17.28	0.34	1324
	100 ASC	100.8	2.6	75.6	38.52	17.35	0.26	1265
	200 ASC	109.0	3.0	76.4	41.61	17.99	0.31	1294
	100 SA+100 ASC	107.0	3.2	78.8	44.39	17.73	0.32	1301
Mean		107.3	2.8	73.1	39.11	17.25	0.28	1277
General mean of foliar application	Water (0)	122.1	2.9	80.9	42.00	17.44	0.30	1461
	100 SA	124.2	3.0	90.9	42.00	18.27	0.33	1543
	200 SA	125.3	3.5	94.0	54.71	18.19	0.38	1632
	100 ASC	117.1	3.1	91.2	54.14	18.62	0.37	1542
	200 ASC	123.9	3.4	95.0	56.16	19.1	0.37	1613
	100 SA+100 ASC	129.1	3.7	102.3	56.2	18.97	0.37	1593
LSD 0.05	Irrigation	6.6	0.54	24.39	7.41	0.90	0.037	53.45
	Foliar application	7.09	0.45	NS	8.92	1.1	0.059	72.23
	Irri. × Foliar appli.	NS	NS	NS	NS	NS	NS	NS

SA = salicylic acid

ASC= ascorbic acid

ASMD = available soil moisture depletion

Table 6. Yield and yield components of soybean plants as affected by soil moisture stress and foliar application of salicylic and ascorbic acids (second season 2013)

Treatment		Plant	No. of	No. of	Seed	100- seed	Harvest	Seed
Irrigation level	Foliar application (ppm)	height (cm)	branches/ plant	Pods/ plant	weight/ plant (g)	weight (g)	index	yield/ fad. (Kg)
25-30% ASMD	Water (0)	125.4	3.2	81.6	49.58	18.01	0.36	1665
	100 SA	130.8	3.6	96.2	61.51	19.15	0.41	1681
	200 SA	131.6	4.0	93.6	64.42	19.22	0.40	1796
	100 ASC	125.4	3.4	97.2	63.21	19.18	0.35	1705
	200 ASC	137.2	3.8	93.2	64.06	19.47	0.37	1759
	100 SA+100 ASC	145.8	4.0	108.4	65.69	19.59	0.36	1797
Mean		132.7	3.7	95.8	61.41	19.01	0.38	1734
45-50% ASMD	Water (0)	107.0	2.8	69.6	37.62	17.41	0.25	1483
	100 SA	126.8	3.0	72.8	43.59	17.51	0.33	1575
	200 SA	129.8	3.4	77.2	46.38	18.09	0.36	1674
	100 ASC	109.4	3.2	80.6	41.30	18.05	0.31	1629
	200 ASC	122.0	3.2	82.6	47.47	18.28	0.34	1632
	100 SA+100 ASC	124.2	3.0	81.2	49.60	18.39	0.36	1704
Mean		119.9	3.1	77.3	44.66	17.95	0.33	1616
65-70% ASMD	Water (0)	100.8	2.4	58.2	23.50	16.19	0.17	1227
	100 SA	102.6	2.6	65.0	30.72	16.89	0.21	1283
	200 SA	108.0	2.8	67.8	35.13	16.68	0.22	1292
	100 ASC	101.0	2.4	61.6	28.62	17.42	0.20	1271
	200 ASC	108.8	2.8	61.4	38.35	17.48	0.25	1281
	100 SA+100 ASC	105.0	3.0	68.6	38.27	17.12	0.24	1277
Mean		104.4	2.7	63.8	32.44	16.96	0.22	1272
General mean of foliar application	Water (0)	111.1	2.8	69.8	36.90	17.20	0.26	1458
	100 SA	120.1	3.1	78.0	45.28	17.85	0.32	1513
	200 SA	123.1	3.4	79.5	49.31	18.00	0.33	1587
	100 ASC	111.9	3.0	79.8	44.38	18.22	0.29	1535
	200 ASC	122.7	3.3	79.1	49.97	18.41	0.32	1557
	100 SA+100 ASC	125.0	3.3	86.1	51.19	18.37	0.32	1593
LSD 0.05	Irrigation	5.82	0.52	9.24	6.31	0.34	0.03	68.24
	Foliar application	6.71	NS	NS	8.07	0.80	0.03	57.13
	Irri. × Foliar appli.	NS	NS	NS	NS	NS	NS	NS

SA = salicylic acid

ASC= ascorbic acid

ASMD = available soil moisture depletion

Ali *et al.* (2011) reported that an increase in drought intensity reduced plant height. This is due to the adaptation of maize plants to cope with drought stress; the maize plants started to divert assimilates from stem and utilized them for increased root growth in order to increase the water absorption. Hence the plant height was affected significantly.

Saad El-Deen (2006) reported that the negative effect of water stress on sesame was due to its effect on photosynthesis, cell division and cell elongation during the vegetative growth stage which in turn reduced plant height. In this context, our results showed that foliar spray of salicylic and ascorbic acids singly or in combination, significantly increased all investigated yield components of soybean except number of pods/ plant in both growing seasons where, the maximum values of seed weight/ plant, 100 seed weight and harvest index were recorded when the plants were sprayed with 100 ppm SA + 100 ppm ASC and 200 ppm of ASC or SA, respectively in both growing seasons.

The most effective treatments on seed yield/ faddan in both seasons were the spraying of 200 ppm SA or 100 ppm SA+ 100 ppm ASC.

The increment of the above agronomic characters are in agreement with the findings of Hegazi and El-Shraiy (2007) who found that foliar application of salicylic acid increased all yield parameters of common bean (pod number, pod fresh and dry weight). Also, salicylic acid increased the number of flowers, pods/ plant and seed yield of soybean (Gutierrez-Coronado *et al.*, 1998).

Also, El-Gabas (2006) on sunflower found that spraying ascorbic acid had favorable effect on growth characters and yield particularly with the higher concentration. Amin *et al.* (2008) reported that interaction treatments of salicylic and ascorbic acids increased plant height and yield components of wheat plants compared to their controls. Also, Siamak *et al.* (2015) showed that salicylic and ascorbic acids increased seed yield of chick pea under drought stress.

Data in Tables 5 and 6 show that the interaction effect between soil moisture stress and foliar spray with salicylic and ascorbic acids

was found to be insignificant on yield and yield components.

## Physiological Analysis

### Chlorophyll content

Data presented in Table 7 show that in both seasons chlorophyll a, b and chlorophyll (a+b) contents were significantly increased when soybean plants were watered with wet treatment (25-30% ASMD) as compared with medium or dry treatments. On the other hand, dry treatment scored the lowest values of such pigments. This trend may be due to that water play an important role for pigments formation in leaves. In this respect, Masoumi *et al.* (2010) found that water deficit stress decreased total chlorophyll in soybean plant.

Chlorophyll a, b and total chlorophyll contents were significantly decreased due to leaf senescence acceleration under drought stress for maize plants (Efeoglu *et al.*, 2009). Also, Ali *et al.* (2011) found that drought stress significantly affected chlorophyll a, b and chlorophyll (a+b) concentration of maize plant leaves.

Regarding the effect of foliar spray of salicylic or ascorbic acid, it significantly increased chlorophyll content in both studied seasons. There was a gradual increase in chl. a, b and chl. (a+b) with increasing applied concentration over their corresponding control.

However, the highest recorded values of chl. a, b and chl. (a+b) content were obtained in leaves of soybean plants treated with 200 ppm salicylic acid. Similar results for salicylic acid were obtained by Hegazi and El-Shraiy (2007) on common bean and Farahbakhsh and Saiid, (2011) on maize plants. The stimulative effect of SA might be due to its antioxidantal scavenging effect to the protected chloroplasts and prevented chlorophyll degradation by the toxic reactive oxygen radicals (Aono *et al.*, 1993). El-Gabas (2006) found that ascorbic acid increased chlorophyll a, b, and total chlorophylls in sunflower plant and attributed this to stimulation the biosynthesis of chlorophylls and delay leaf senescence. Amin *et al.* (2008) reported that, ascorbic acid was more effective than salicylic acid in increasing the different photosynthetic pigments in wheat. They also, found that interaction treatments of salicylic and

**Table 7. Chlorophyll content (mg/g/f.wt) and relative water content (RWC) in leaves of soybean plants as affected by soil moisture stress and foliar application of salicylic and ascorbic acids**

Treatment		2012				2013			
Irrigation level	Foliar application (ppm)	Chl. (a)	Chl. (b)	Chl. (a+b)	RWC (%)	Chl. (a)	Chl. (b)	Chl. (a+b)	RWC (%)
25-30% ASMD	Water (0)	1.96	0.98	2.94	72.19	1.80	0.93	2.73	73.47
	100 SA	2.14	0.99	3.13	80.67	1.93	0.99	2.92	81.03
	200 SA	2.19	1.00	3.19	85.69	2.02	1.03	3.05	86.90
	100 ASC	2.09	1.01	3.10	79.95	1.87	0.90	2.77	80.96
	200 ASC	2.16	1.04	3.20	83.74	1.98	0.98	2.96	83.27
	100 SA+100 ASC	2.24	0.96	3.20	84.38	1.97	0.95	2.92	85.67
Mean		2.13	1.0	3.13	81.10	1.93	0.96	2.89	81.89
45-50% ASMD	Water (0)	1.72	0.79	2.51	67.48	1.67	0.78	2.44	70.58
	100 SA	1.96	0.89	2.85	70.39	1.75	0.88	2.62	71.71
	200 SA	1.99	0.95	2.94	73.66	1.85	0.89	2.74	74.26
	100 ASC	1.84	0.86	2.71	73.57	1.66	0.85	2.51	73.14
	200 ASC	1.95	0.91	2.86	73.84	1.78	0.87	2.65	74.03
	100 SA+100 ASC	1.98	0.97	2.95	74.29	1.81	0.94	2.75	74.45
Mean		1.91	0.90	2.80	72.20	1.75	0.87	2.62	73.03
65-70% ASMD	Water (0)	1.49	0.62	2.11	58.35	1.34	0.66	2.00	60.04
	100 SA	1.73	0.74	2.48	64.48	1.58	0.67	2.25	65.29
	200 SA	1.79	0.79	2.58	69.01	1.67	0.77	2.44	70.39
	100 ASC	1.70	0.70	2.39	66.26	1.43	0.64	2.07	68.80
	200 ASC	1.77	0.79	2.56	66.07	1.66	0.72	2.38	69.31
	100 SA+100 ASC	1.76	0.76	2.53	70.02	1.61	0.74	2.35	70.62
Mean		1.71	0.73	2.44	65.70	1.55	0.70	2.25	67.41
General mean of foliar application	Water (0)	1.72	0.80	2.52	66.00	1.60	0.79	2.39	68.03
	100 SA	1.94	0.88	2.82	71.85	1.75	0.85	2.60	72.68
	200 SA	1.99	0.91	2.90	76.12	1.85	0.89	2.74	77.18
	100 ASC	1.88	0.86	2.73	73.26	1.66	0.80	2.45	74.30
	200 ASC	1.96	0.91	2.87	74.55	1.81	0.86	2.66	75.54
	100 SA+100 ASC	1.99	0.90	2.89	76.23	1.80	0.88	2.67	76.92
LSD 0.05	Irrigation	0.08	0.08	0.15	5.70	0.04	0.05	0.03	3.39
	Foliar application	0.07	0.05	0.09	4.45	0.08	0.07	0.11	3.15
	Irri. × Foliar appli.	N S	N S	N S	N S	N S	N S	N S	N S

SA = salicylic acid

ASC= ascorbic acid

ASMD = available soil moisture depletion

ascorbic acid show a slight increases in chl. a, b and total carotenoids in leaves of wheat plants compared with their controls.

The interaction between soil moisture stress and foliar spray with salicylic or ascorbic acid and their combination had insignificant effect on chl. a, chl. b and chl. (a+b).

#### Antioxidant enzymes

Most of the biotic and abiotic stresses lead to an increase in the production of reactive oxygen species (ROS) such as superoxide radical ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radical (OH) (He *et al.*, 2011; Gorji *et al.*, 2011).

These ROS in high density, hurt cells lipids, proteins and nucleic acids and finally stop the natural metabolism of plant (Badawi *et al.*, 2004; Lai *et al.*, 2007). Plants protect themselves from cytotoxic effects of these ROS with the help of antioxidant enzymes such as peroxidase (POD), polyphenol oxidase (PPO), catalase (CAT) and superoxide dismutase (SOD) induced in plants in response to the stress (Joseph and Jini, 2010; Rani and Jyothsna, 2010; He *et al.*, 2011).

Data presented in Table 8 show that, water deficit stress increased antioxidants content (peroxidase and polyphenol oxidase) significantly, but content of them were more at mild (45-50% ASMD) than high water deficit stress (65-70% ASMD).

In other words, in extreme (high) water deficit stress condition, the antioxidant defensive mechanism of crops will be activated as well and the antioxidants content will increased as compared to wet irrigation (25-30% ASMD). But, due to excessive physiological damages resulted of water deficit stress the antioxidant activities are less than mild water deficit level. Similar results were obtained by Masoumi *et al.* (2010) on maize and soybean as well as Jiang and Zhang (2002) on maize, which might be attributed to inhibitory effects of water stress on protein turnover causing depletion of antioxidants (Bartoli *et al.*, 1999).

Data presented in Table 8 show that foliar spray of salicylic and ascorbic acids and their combination, significantly increased peroxidase and polyphenol oxidase activities in leaves of

soybean plants as compared with untreated plants (control).

The interaction between soil moisture stress and foliar application of SA or ASC had significant effect on peroxidase and polyphenol oxidase activities. The highest values of two enzymes activities were obtained when soybean plants irrigated at medium treatment (50-55% ASMD) and foliar spraying with salicylic acid at 200 ppm and 100 ppm salicylic+100 ppm ascorbic acid. In this concern salicylic-pretreated soybean plants stimulate of antioxidants might be achieved by SA-induced protein synthesis (Kovacik *et al.*, 2009). Also, foliar application of SA increased mineral nutrient content in *Phaseolus vulgaris* seem to involve in stress tolerance mechanism and play an important role to enhance the activity of enzymes responsible for drought resistance (Ghoulam *et al.*, 2002). War *et al.* (2011) found that induction of polyphenol oxidase activity by SA might enable the plants to resist the oxidative damage caused by different stresses. Dehghan *et al.* (2011) show that ascorbic acid may play an important role in salt stress by protecting soybean seedlings from the stress induced oxidative damage through the maintenance and / or increase of the activity of antioxidant enzymes.

#### Proline content

Results presented in Table 8 indicate that increasing water stress from 25-30% up to 65-70%, significantly increased leaf proline concentration. These results are in harmony with those obtained by (Abdo and Anton, 2009; Liu *et al.*, 2011).

Also, Proline accumulation can be met with the stress as temperature, drought and starvation. High levels of proline enabled the plant to maintain low water potentials. By lowering water potentials, the accumulation of compatible osmolytes, involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortages within the organism (Abdo and Anton, 2009). Proline acts as an AOS (activated oxygen species) scavenger (Moslemi *et al.*, 2011). Also, proline is able to scavenge

**Table 8. Peroxidase, polyphenol oxidase, proline of leaves and protein, oil and total carbohydrate content of produced seeds of soybean plants as affected by soil moisture stress and foliar application of salicylic and ascorbic acids (second season 2013)**

Treatment		Peroxidase content	Polyphenol oxidase	Proline content	Protein (%)	Oil (%)	Carbohydrates (%)
Irrigation level	Foliar application (ppm)	(mg/g/f.wt)	(mg/g/f.wt)	(mg/g/d.wt)			
<b>25-30% ASMD</b>	Water (0)	0.397	0.413	0.126	34.54	19.80	31.74
	100 SA	0.508	0.436	0.177	35.32	20.69	32.98
	200 SA	0.584	0.541	0.181	38.06	21.05	33.38
	100 ASC	0.528	0.512	0.163	35.58	20.51	31.85
	200 ASC	0.496	0.556	0.174	37.33	21.06	34.36
	100 SA+100 ASC	0.573	0.574	0.192	38.22	21.50	34.17
<b>Mean</b>		0.514	0.505	0.169	36.51	20.77	33.08
<b>45-50% ASMD</b>	Water (0)	0.561	0.462	0.212	32.06	18.69	29.88
	100 SA	0.679	0.531	0.253	33.68	19.40	30.57
	200 SA	0.723	0.750	0.400	34.75	20.24	31.96
	100 ASC	0.570	0.610	0.225	32.82	19.63	29.55
	200 ASC	0.666	0.736	0.405	34.94	19.27	31.52
	100 SA+100 ASC	0.737	0.778	0.481	34.90	20.25	30.92
<b>Mean</b>		0.656	0.644	0.329	33.86	19.58	30.73
<b>65-70% ASMD</b>	Water (0)	0.410	0.413	0.263	30.18	16.90	27.76
	100 SA	0.513	0.450	0.387	31.83	17.99	28.09
	200 SA	0.723	0.548	0.639	32.61	18.97	29.08
	100 ASC	0.512	0.558	0.395	31.51	17.90	28.55
	200 ASC	0.564	0.632	0.531	31.77	18.65	29.03
	100 SA+100 ASC	0.609	0.626	0.558	32.04	18.98	29.60
<b>Mean</b>		0.555	0.538	0.462	31.65	18.23	28.69
<b>General mean of foliar application</b>	Water (0)	0.456	0.429	0.200	32.26	18.47	29.79
	100 SA	0.567	0.473	0.272	33.61	19.36	30.55
	200 SA	0.677	0.613	0.406	35.14	20.09	31.47
	100 ASC	0.537	0.560	0.261	33.30	19.35	29.99
	200 ASC	0.575	0.641	0.370	34.68	19.66	31.64
	100 SA+100 ASC	0.640	0.659	0.410	35.05	20.24	31.56
<b>LSD 0.05</b>	<b>Irrigation</b>	0.02	0.04	0.02	1.10	0.81	1.45
	<b>Foliar application</b>	0.03	0.03	0.02	1.13	0.98	1.15
	<b>Irri. × Foliar appli.</b>	5.11	0.06	3.33	N S	N S	N S

SA = salicylic acid

ASC= ascorbic acid

ASMD = available soil moisture depletion

hydroxyl radical and stabilize the structure and function of macromolecules such as DNA, protein and membranes interaction with those macromolecules (Simaei *et al.*, 2011).

Foliar application of salicylic and ascorbic acids singly or in combination, significantly increased leaf proline concentration compared with untreated plants (control). The interaction between soil moisture stress and foliar application with salicylic and ascorbic acids on leaf proline content was found to be significantly effect.

The highest values of leaf proline content were obtained from dry treatment (65-70% ASMD) with foliar spraying with salicylic acid at 200 ppm and 100 ppm salicylic+ ascorbic acids. Similar results were obtained by (Hassanein *et al.*, 2012).

#### **Protein, oil and carbohydrates content of seeds**

Table 8 show that total carbohydrates, protein accumulation and oil contents in soybean seeds were significantly increased under wet conditions (irrigation at 25-30% ASMD). While, increasing water deficit up to 65-70% ASMD, significantly decreased those traits. Plants received irrigation at 45-50% ASMD (medium treatment) had intermediate values. Fattahi-Nejsiani *et al.* (2009) and Hassanein *et al.* (2009) reported that protein content in soybean seeds was decreased but proline content was increased under drought stress. They also suggested that drought stress had a stimulatory effect on protein hydrolysis resulting in proline accumulation and /or inhibited protein synthesis are accompanied by inhibition of growth. Mourad and Anton (2007) found that total carbohydrates content of sorghum grains gradually decreased with increasing soil moisture stress. Also, Abdo and Anton (2009) found that increasing soil moisture stress decreased total carbohydrates and oil content in sesame seeds. Bayramov *et al.* (2010) explained the carbohydrates reduction under water stress conditions, that water shortage causes stomatal closure and this in turn prevents CO<sub>2</sub> diffusion into the air inside the tissues of plants and consequently the pigments and photosynthesis becomes low resulted from low

expression of enzymes involved in photosynthesis under drought conditions.

The roles of carbohydrates had markedly regulated the osmotic pressure in plants, and were recorded as important defense substances alleviating protoplasm coagulation under various stress factors (Vassiliev and Vassiliev, 1936). Siamak *et al.* (2015) found that soluble carbohydrates were increased as a result of drought stress in chick pea plants.

Concerning the effect of foliar spraying, Table 8 indicate that soybean plants treated by salicylic and ascorbic acids or their combination, significantly increased total carbohydrates, protein accumulation and oil content of seeds compared with control. The highest values of such traits were obtained by spraying plants with salicylic or ascorbic acid at 200 ppm also, their combination at 100 ppm. Similar results were obtained by Amin *et al.* (2008) who found that foliar application of salicylic and ascorbic acids individually or their combination increased crude protein and total carbohydrates in wheat grains. Hassanein *et al.* (2012) found that foliar application of wheat plant with SA increased total carbohydrates and protein accumulation when compared to the control treatment.

Also, Hegazi and El-Shraiy (2007) reported that the application of SA increased total soluble protein content in leaves of common bean.

Plants produce proteins to reaction biotic and abiotic stresses that were induced by some phytohormones such as salicylic and ascorbic acid (Davis, 2005); these compounds can decrease the drought effects in plants under stress conditions.

Increase in protein content plays an important role in plant defense (Chen *et al.*, 2009). Also, War *et al.* (2011) found that protein content was increased in groundnut plant treated with SA. The increase in protein content was reflected in increased peroxidase and polyphenol oxidase activities which, play an important role in plant defense under drought stress.

The interaction effect between soil moisture stress and foliar application with salicylic and ascorbic acids and their combination recorded no significant effect on total carbohydrates, protein and oil content of soybean seeds.

## Water Relations

### Relative water content

Relative water content in leaves (RWC %) was proposed as a good indicator of plant water status because RWC through its relation to cell volume may be more closely reflects the balance supply to leaf and transpiration rate (Sinclair and Ludlow, 1985).

Table 7 show that increasing water stress from 25 - 30% up to 65-70% ASMD significantly decreased relative water content of leaves at 75 days after sowing in both seasons. Similar results were obtained by Abdo (2007) on maize plants as well as Abdo and Anton (2009) on sesame plants. In other study, leaf relative water content (RWC), water potential, osmotic potential, turgor potentials and osmotic adjustment were significantly decreased under severe drought stress due to the excessive water loss (Machado and Paulsen, 2001). Also, Ali *et al.* (2011) found that under severe drought stress plants failed to maintain the turgor, this might be due to the excessive water loss through transpiration required to reduce the leaf temperature.

Foliar application of salicylic or ascorbic acid significantly increased RWC in soybean leaves compared with the control. The highest values of relative water content were obtained when the plants were sprayed with 100 ppm SA+ 100 ppm ASC in the first season and 200 ppm SA in the second season.

The interaction between soil moisture stress and foliar application of salicylic or ascorbic acid singly or in combination had insignificant effect on RWC of leaves in both seasons.

### Seasonal water consumptive use

Seasonal water consumptive use (WCU) by soybean plant under various treatments is presented in Table 9. Results indicated that the values of WCU ranged from 32.5 to 51.9 cm for the two successive seasons under study.

Results showed that the highest value of WCU was achieved under wet treatment; however the lowest value was obtained from dry treatment. The medium treatment had intermedium value. Similar results on sorghum were obtained by (Mourad and Anton, 2007).

Such results could be explained on the basis that frequent irrigation provides chance for more luxuriant use of water. These findings could be ascribed to the availability of soil water to soybean plants in addition to higher evaporation rate from wet than from dry soil surface. In this connection, Ibrahim (1981) showed that the increase in evapotranspiration rate by maintaining soil moisture at high level can be attributed to excess available water in the root zone to be consumed by the plants. These results are in line with those reported by Abdo and Anton (2009) on sesame plant.

### Water use efficiency

Table 9 show that water use efficiency (WUE) under both studied seasons recorded the maximum value when plants irrigated at 45 - 50% ASMD (medium treatment), whereas it was lower under both wet and dry treatments due to the high seed yield/ faddan which obtained from medium treatment in proportion to the low water consumed. It could be concluded that medium soil moisture level seemed to be more efficient in consuming water compared with either low water deficit (wet treatment) or severe soil moisture stress (dry treatment). In other words, from the stand point of water conservation, medium treatment seemed to be more economic for saving water and gained a suitable seed yield and also, allows soybean plants to use water more efficiency. Similar results on sorghum was obtained by Mourad and Anton (2007) and Abdo and Anton (2009) on sesame plant.

Foliar application of SA and ASC acids and their combinations increased WUE for soybean plant as compared with untreated plants in both seasons. Such results revealed that the foliar spraying of SA or ASC increased seed yield more than the increase in water consumed, resulting an increase in WUE. Siamak *et al.* (2015) found that salicylic and ascorbic acids increased water use efficiency as compared with the control of chick pea plants under drought stress. Also, Bakry *et al.* (2012) showed that foliar application of ascorbic acid increased water use efficiency of wheat plants under water irrigation requirements of (80% IR). This means that it can be save 20% of irrigation water.

**Table 9. Seasonal water consumptive use and water use efficiency as affected by soil moisture stress and foliar application of salicylic and ascorbic acids**

Treatment		Seasonal water consumptive use (WCU, cm)		Water use efficiency (WUE, Kg/m <sup>3</sup> /fad.)	
Irrigation level	Foliar application (ppm)	2012	2013	2012	2013
25-30% ASMD	Water (0)	51.9	51.8	0.77	0.76
	100 ppm SA	49.8	49.6	0.82	0.81
	200 ppm SA	46.8	47.2	0.96	0.91
	100 ppm ASC	47.5	48.1	0.86	0.84
	200 ppm ASC	45.1	46.3	1.00	0.90
	100 SA+100 ASC	44.8	45.2	0.97	0.95
Mean		47.7	48.0	0.90	0.86
45-50% ASMD	Water (0)	43.6	42.8	0.83	0.82
	100 ppm SA	41.3	41.8	0.94	0.90
	200 ppm SA	38.6	39.5	1.04	1.01
	100 ppm ASC	41.3	40.5	0.95	0.96
	200 ppm ASC	36.2	37.4	1.12	1.04
	100 SA+100 ASC	35.1	36.2	1.12	1.12
Mean		39.4	39.7	1.00	0.98
65-70% ASMD	water (0)	40.2	39.8	0.71	0.73
	100 ppm SA	37.5	37.4	0.82	0.82
	200 ppm SA	35.7	34.8	0.88	0.88
	100 ppm ASC	38.4	38.1	0.78	0.79
	200 ppm ASC	35.3	33.5	0.87	0.91
	100 SA+100 ASC	32.7	32.5	0.95	0.94
Mean		36.6	36.0	0.84	0.85
General mean of foliar application	Water (0)	45.2	44.8	0.77	0.77
	100 ppm SA	42.9	42.9	0.86	0.84
	200 ppm SA	40.4	40.5	0.96	0.93
	100 ppm ASC	42.4	42.2	0.86	0.86
	200 ppm ASC	38.9	39.1	1.00	0.95
	100 SA+100 ASC	37.5	38.0	1.01	1.00

SA = salicylic acid

ASC= ascorbic acid

ASMD = available soil moisture depletion

The interaction between soil moisture stress and foliar application with SA, ASC or their combination in Table 9 show that the maximum values of WUE (1.12 Kg seeds/m<sup>3</sup> water) were scored from plants irrigated at 45- 50% ASMD (medium treatment) in combination with spraying by 100 ppm of SA+ 100 ppm ASC in both studied seasons.

### Conclusion

It can be concluded that foliar application of soybean plants with 200 ppm salicylic or ascorbic acid and their combination at 100 ppm for each and irrigated at 25-30% ASMD (wet treatment) stimulate the growth of soybean plants *via* the enhancement of the biosynthesis of photosynthetic pigments, improved yield as well as carbohydrates, protein and oil content of soybean seeds.

Increasing water stress from 25-30% up to 65-70% ASMD decreased growth, yield components and metabolic processes, salicylic and ascorbic acids treatments lead to regulation plant metabolism and soybean performance under drought stress.

Higher WUE values were obtained when soybean plants were irrigated at 45-50% ASMD (medium treatment) and sprayed by 100 ppm salicylic + 100 ppm ascorbic acid.

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## استجابة فول الصويا لظروف الإجهاد المائي والرش الورقي بحامض الساليسيليك والأسكوربيك

أمينة إبراهيم الشافعي

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أجريت تجربة حقلية بمحطة بحوث إيتاي البارود خلال موسمي صيف ٢٠١٢، ٢٠١٣ لدراسة استجابة فول الصويا صنف جيزة ١١١ لثلاث مستويات من الرطوبة الأرضية وهي الري عند استنفاد ٢٥-٣٠%، ٤٥-٥٠% و٦٥-٧٠% من الماء الميسر (معاملات رطبة ومتوسطة وجافة على التوالي) وكذلك الرش بحامض الساليسيليك والأسكوربيك عند تركيز ١٠٠ و٢٠٠ جزء في المليون ومخلوط لحامض الساليسيليك والأسكوربيك عند تركيز ١٠٠ جزء في المليون لكل منهما على النمو الخضري والمحصول ومكوناته وبعض التغيرات البيوكيميائية وقد أوضحت النتائج الآتية: أدى زيادة الإجهاد الرطوبي الأرضي حتى معاملة الري عند استنفاد ٦٥-٧٠% من الماء الميسر إلى نقص معنوي للوزن الجاف للمجموع الخضري وكذلك الأوراق ومساحة الورقة ودليل مساحة الورقة ونقص في كلورفيل أ و ب والكلوروفيل الكلي أ+ب والمحتوى النسبي للماء في الأوراق والاستهلاك المائي الموسمي، أدى الري بالمعاملة المتوسطة (الري عند استنفاد ٤٥-٥٠% من الماء الميسر) مقارنة بالمعاملة الرطبة والجافة إلى زيادة معنوية في نشاط إنزيمي البيروكسيديز والبولي فينول أوكسيديز وكذلك كمية البرولين في الأوراق وكفاءة استخدام المياه، أدى تعرض نباتات فول الصويا لظروف الجفاف (الري عند استنفاد ٦٥-٧٠% من الماء الميسر) إلى نقص معنوي في ارتفاع النبات وعدد الفروع والقرون في النبات ووزن بذور النبات ووزن ١٠٠ بذرة ودليل الحصاد وإنتاجية الفدان من البذور وكذلك محتوى البذور من الكربوهيدرات الكلية والبروتين والزيت، أدى الرش الورقي بحامض الساليسيليك والأسكوربيك إلى زيادة معنوية لجميع قياسات النمو الخضري ومكونات المحصول للنبات مقارنة بالنباتات غير المعاملة بالرش الورقي ماعدا الوزن الجاف للمجموع الخضري في موسم الزراعة الأول والوزن الجاف للأوراق وعدد الفروع في النبات في موسم الزراعة الثاني وعدد القرون في النبات في موسم الزراعة، هذا وقد أدى رش النباتات بحامض الساليسيليك والأسكوربيك أيضاً إلى زيادة معنوية لمحتوى الكلوروفيل ونشاط إنزيمي البيروكسيديز والبوليفينولوكسيديز والبرولين والمحتوى النسبي للماء بالأوراق وكمية الكربوهيدرات والبروتين والزيت بالبذور والاستهلاك المائي الموسمي وكفاءة استخدام المياه، كان تأثير التفاعل بين معاملات الإجهاد المائي والرش الورقي للنباتات بحامض الساليسيليك والأسكوربيك معنوي على كل من مساحة الورقة ودليل مساحة الورقة ونشاط إنزيمي البيروكسيديز والبولي فينول أوكسيديز والبرولين للأوراق عند مرحلة النمو ٧٥ يوم من الزراعة في موسم الزراعة الثاني، هذا وتشير نتائج التفاعل إلى أن أعلى قيمة لكفاءة استخدام المياه قد سجلت بواسطة معاملة الري عند استنفاد ٤٥-٥٠% من الماء الميسر مع الرش الورقي للنباتات بحامض الساليسيليك أو الأسكوربيك عند تركيز ٢٠٠ جزء في المليون أو مخلوطهما عند تركيز ١٠٠ جزء في المليون لكل منهما.

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