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Drought Strategy Tolerance of Four Barley Cultivars and Combined Effect with Salicylic Acid Application

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Abstract

This investigation was conducting to explain that four barley genotypes varied in their drought tolerance according to their genotype and their tested organs. It can be recorded that growth parameters (fresh, dry matter and length, water content, leaf area and pigment contents) were decreased as decreasing M. C. in the soil. This indicated that Giza 123 was the superior in its drought tolerance and Giza 129 was the interior and both cv. Giza 2000 and cv. Giza 124 were the intermediated between them. This concomitant with increase in soluble sugar and soluble protein content of both organs in Giza 123 and shoot Ca^{++}, this related with lower value of OP other than genotypes, in Giza 2000 this was related with a huge accumulation in soluble protein of shoot and root, shoot amino acids and root proline reach 3-folds than control plants as decreasing M. C. Whereas drought stress increased soluble protein only in Giza 124 while in Giza 129 decreasing M. C. increased soluble protein, amino acids and proline contents in shoot and root and shoot Ca^{++}. The values of OP increased as decreasing M. C. in four barley cultivars concomitant with their drought tolerance. Also, SA application was markedly enhanced the production of growth parameters in shoot and root with varied degree according to each tested barley genotypes. SA application was significantly increased OP in shoot, root and spike of barley pants. Spraying vegetative parts with 0.5 mM SA was markedly increased the soluble sugar, soluble protein and amino acids in shoot, root and spike of four barley cultivars. On the other side, SA application lowered the accumulation of proline in shoot and root of barley genotypes. SA treatment induced no significant change in K^{+}, Ca^{++}, and Mg^{++} in shoot, root and spike of Giza 123, it significantly increased K^{+}, Ca^{++}, and Mg^{++} in shoot and root of Giza 2000. SA application enhanced accumulation of K^{+}, Ca^{++} in shoot and root of Giza 124 and K^{+}, Ca^{++} and Mg^{++}in three organs of Giza 129.

Keywords

Drought, Barley Cultivar, Salicylic Acid

1. Introduction

Recent scientific advances make exploration of genotypic selection to drought stress was feasible and could result in large gains in productivity. Drought stress is one of the most adverse factors on plant growth and productivity, it induced morphological, physiological and biochemical changes, reduced CO_2 assimilation, leaf area, pigment content, stem expansion, root proliferations disturbs water use efficiency. The role of chemical constituent's accumulation in drought plants has been researched to understand plant tolerance to dehydration. Also drought increase active oxygen ROS species generation and can be controlled by increased antioxidant enzmes [1] [2] [3] [4]. SA is involved in the regulation of important plant physiological processes such as photosynthesis, nitrogen metabolism, proline (Pro) metabolism, production of glycinebetaine (GB), antioxidant defense system, and plant-water relations under stress conditions and thereby provides protection in plants against abiotic stresses [5]. A common effect of abiotic stresses, including drought, is oxidative damage due to a loss of balance between the production and elimination of reactive oxygen species (ROS) [6]. If not effectively and rapidly removed from plants, excessive ROS may damage a wide range of cellular macromolecules such as lipid, protein and DNA and ultimately cause cell damage. Salicylic acid (SA) is a naturally occurring phenolic compound. SA plays an important role in the regulation of plant growth, development, ripening, and defense [7] [8]. In general, low concentrations of SA may enhance the antioxidant capacity in plants, but high concentrations of SA may cause cell death or susceptibility to abiotic stresses [8] [9]. In addition to being an important component of biotic stress tolerance mechanism, SA also regulates various aspects of plant responses to abiotic stresses through extensive signaling cross-talk with other growth hormones [5] [10] [11] [12]. Many studies have so far been conducting to identify its role in abiotic stress tolerance in different crops. Results of these studies suggest that exogenously applied SA can induce drought resistance in wheat [13] [14] [15]. The present work was to study the mechanisms of drought tolerance strategy of four barley cultivars and their response to the exogenous addition of salicylic acids.

2. Materials and Methods

2.1. Experimental Sites and Drought Stress Treatments

Barley grain cultivars were obtained from the breeding program of seeds station, Beni-Suef, Egypt. Barley grains were surface sterilized by immersion in a mixture of ethanol 96% and H_2O_2 (1:1) for 3 minutes, followed by several washings with sterile distilled water, seeds were grown in 1 kg pots in Botany and Microbiology

Department garden. Barley plant was considered as one of the most important crop plants in Egypt because of its contribution as nutrient foods for people. Barley is a major cereal grain commonly found in bread, beverages and various cuisines of every culture. It remains one of the most widely consumed grains, globally, source of dietary. Fiber, vitamins and minerals are not found in refined and enriched grains. Five seeds were sown in each pot and soil was brought to field capacity. The seedlings were left to grow under the desired soil moisture content levels (90%, 70%, 50% and 30%). Soil moisture content was measured by calculate the soil field capacity, this consider as 100% moisture content and so could be determine the other lower soil moisture content. The clay soil comprise four components minerals and soil organic matter make up the solid fraction, whereas air and water comprise the pore space fraction. A typical agricultural soil is usually around 50% solid particles and 50% pores (Adapted from Brady and Weil, 2002) [16]. Soil particle of clay is <0.002 invisible to naked eye. Considerations of working in controlled environments were followed by Tibbitts & Langhans [17].

2.2. Drought Stress and Treatments with Salicylic Acid

The previous treatments were repeated for treatment with spraying vegetative parts with 0.5 mM SA as second groups. Three replicate was made for each treatment and plants were grown in natural conditions for crop yield production at 120-days.

2.3. Laboratory Analysis for Metabolities

At the end of the experimental period (120 days) plant height, dry matter yield of the different organs (shoot, root and spike) were determined. Plant height was determined by direct measurement from soil surface to the tip of the flag leaf. Determination of the dry matter involved harvesting and careful separation of fresh organs. Fresh organs were then dried in an oven at 80˚C. Successive weighing was carried out until a constant dry weight was recorded. The plants were uprooted, roots carefully separated from the soil, washed and the length of roots were measured. From determining the shoot and root weight, was calculated. Leaf area was determined by measuring leaf length and maximum width and applying the formula; Leaf area = k (leaf length * leaf maximum width) Cm^2 $plant^{-1}$. This formula provided a simple way for determination of leaf area particularly in the field where large leaves had to be measured. The coefficient k was calculated and assigned different values for different grasses [18] [19], and recently reviewed and given a value of 0.75 for maize [20]. Dry matter was determined after drying plants in an aerated oven at 70˚C to constant mass. Pigment content was measured by Metzner [21]. Saccharides were determined by the anthrone-sulfuric acids method [22]. Soluble protein was measured according to Lowry *et al.* (1951) [23]. Amino acids and proline were measured by Moore and Stein (1948) and Bates *et al.* (1973) [24] [25]. Sodium and potassium were de-

termined by flam photometric method [26], and calcium and magnesium by the versene titration method [27].

2.4. Statistical Analysis

The experimental data were subjected to the one way analysis of variances (ANOVA test) using the SPSS version 11.0 to quantify and evaluate the source of variation and the means were separated by the least significant differences, L. S. D. at P level of 0.05% [28]. Experimental data were subjected to one way analysis of variance and the means were separated by the least significant differences, L. S. D. [28]. Correlation coefficients were calculated using statgraphics 5.0 software.

3. Results

3.1. Growth Parameters

In cv. Giza 123 decreasing moisture content induced three position the firstly was more or less have the same value of control 100% in shoot fresh matter, secondly a marked increase in shoot dry matter was detected up to 50% M. C., thirdly in root there is a slight decreasing effect in fresh and dry matter up to 70% (**Table 1(a)**). After that level, a significant reduction in fresh and dry matter was induced reach a low level at 30% M. C., the percent of reduction was 84% and 72.4%. Length of both organs showed a decreasing effect reach a 20% at 30% M. C. compared with control plants (**Table 1(a)**). Fresh and dry matter of shoot and root of Giza 2000 generally become more or less unchanged up to 70%, after that a marked reduction was recorded (**Table 1(b)**). The percent of reduction at 30% M. C. was 69.3%, 81.7%, 70.5%, 67.2% in fresh and dry matter of shoot and root respectively. Length in both organs was decreased with decreasing moisture content. The percent of reduction was 58.8% and 75% in shoot and root compared with control plants (**Table 1(b)**). A lowering effect was recorded with decreasing moisture content in fresh and dry matter of shoot, root and length of cv. Giza 124, this effect was more obvious in root than in shoot (**Table 1(c)**). The percent of reduction in fresh, dry matter and length at 30% M. C. was 63.9%, 63.4%, 43.7%, 66.6%, 65.4% and 72%. Also decreasing soil moisture content induced a significant reduction in fresh, dry matter and length of Giza 129 from 70% to 30% M. C. The percent of reduction was 53.2%, 51.4%, 39%, 34.7%, 51.9% and 48.4% in fresh, dry and length of shoot and root at 30% M. C. (**Table 1(d)**). Leaf area smoothly decreased at all M. C. levels in cv. Giza 123 moreover a marked reduction was recorded at 50% and 30% in both Giza 2000 and Giza 124, however a significant reduction was recorded in cv. Giza 129. The percent of reduction at 30% M. C. was 81.8%, 60.7%, 54.9%, 53.3% as compared with control plants (**Figure 1**). Chlorophyll a, b and carotenoids were significantly decreased as decreasing drought stress in four barley genotypes compared with reference control plants (**Figure 2(a)** & **Figure 2(b)**). The percent of reduction in Chl. a, b and c at 30% M. C. level was 80.3%, 98.9%, 67.4%, in Giza 132 plant,

Table 1. The response of Giza 123 (a), Giza 2000 (b), Giza 124 (c) and Giza 129 (d) barley genotypes to drought stress and interactive with SA treatments on fresh, dry matter (g plant^{-1}) and length (Cm) of shoot, root and spike at the final fruiting stage.

(a)

Treat. M. C.	Shoot				Root				Length				Spike			
	M. C.	%	D. m.	%	F. m.	%	D. m.	%	Sh.	%	Ro.	%	D. m.	%	Length	%
90%	7.7	100	2.8	100	4.2	100	0.83	100	90	100	3	100	2.2	100	17	100
70%	8.0	100	3.6	130	3.9	93.3	0.74	89	85**	94	27	90	1.9	89.3	17.5	102.9
50%	7.5	104	3.1	114	3.7	87.8	0.67	80.4	75.9**	84	24	983	1.7	75.6	15**	88.2
30%	7.4	96.9	2.6	96	3.5	84	0.60	72.4	72.3	80	24	80	1.1	52.0	13.5**	79.4
90% + SA	9.7	126	4.6	166	5.2**	124	1.1	132.5	96.3	107	33	110	2.4	109.0	18.5**	108.8
70% + SA	9.4	123	3.9	143	6.5**	156	1.2	144.7	88.2	98	30	99	2.5	114.3	17.5	102.9
50% + SA	7.4	96	3.3	121	5.9**	1421	0.96	115.7	83.7	93	292	95	2.2	100	19.5 * *	1 15
30% + SA	7.2	93	2.7	99	4.1	97.6	0.74	88.9	37.9	87	26	85	1.4	89.3	17.5	100.8
L. S. D. 0.05%	**0.05**		**0.19**		**0.25**		**0.09**		**0.98**		**0.94**		0.16		0.51	

(b)

M. C.	Shoot				Root				Length				Spike			
	M. C.	%	D. m.	%	F. m.	%	D. m.	%	Sh.	%	Ro.	%	D. m.	%	Length	%
90%	4.9	100	1.3	100	3.0	100	2.2	100	0.86	100	85	100	1.8	100	18	100
70%	4.6	93.9	1.4	06.9	2.9	96.7	1.9	89.3	0.76	88.2	79	92.2	1.5**	81.2	17.5	97.2
50%	4.3	87.8	1.2	93.1	2.5	83.3	1.7	75.6	0.66	76.2	66	77.7	1.3**	70.9	17 **	94.4
30%	3.4	69.4	1.0	81.7	2.0	66.7	1.1	52.0	0.52	67.2	58	68.2	0.74**	41.3	11.5**	63.9
90% + SA	9.4	191	2.2	171	3.3	109	2.4	109	0.94	109.3	94	110.6	2.1**	119.1	19**	105.6
70% + SA	6.9	140.2	2.0	155.7	3.9	129	2.5	114.3	1.1	127.6	83	97.7	1.8	100	18	100
50% + SA	6.7	136.4	1.7	129.8	3.8	126	2.2	100	1.0	124.1	59	69.4	1.5 **	83.1	16**	88.9
30% + SA	5.0	102.4	1.2	90.1	2.4	78.7	1.4	89.3	0.85	98.9	55	64.7	0.93	52.1	16**	88.9
L. S. D. 0.05%	0.19		**0.158**		**0.19**		0.16		**0.1**		**1.3**		**0.22**		**0.13**	

(c)

M. C.	Shoot				Root				Length				Spike		Length	
	M. C.	%	D. m.	%	F. m.	%	D. m	%	Sh.	%	Ro.	%	D. m.	%	Spike	%
90%	5.3	100	2.2	100	4.0	1 00	0.77	100	78	100	25	100	1.9	100	15.5	100
70%	5.6	104.4	1.9	98.6	3.6	78.9	0.65	85.4	75	96.1	23.5	94	1.6**	84.3	15	96.8
50%	4.8	90.2	1.8	89.6	3.1	75.6	0.56	72.9	74	94.9	23	92	1.1**	58.9	12**	77.4
30%	3.4	63.9	1.4	63.4	1.3	43.7	0.57	66.6	51	65.4	17	68	0.6**	31.6	9.5**	61.2
90% + SA	5.3	100	2.2	100.9	4.3	105.7	0.80	104.8	81	03.9	28	112	1.9	100.7	16.5	106.5
70% + SA	5.8	109	2.3	105.4	4.1	100.7	0.78	101.6	75	96.2	29	116	1.9	98.7	16.5	106.5
50% + SA	5.7	106.6	2.3	104.1	5.2	129	0.91	119.2	74	94.2	25	100	1.8	97.1	16.5	106.5
30% + SA	4.8	90.8	2.0	90.9	2.9	74	0.57	73.8	65	83.3	23.5	94	0.923**	49	14.5**	93.5
L. S. D. 0.05%	**0.17**		**0.14**		**0.13**		**0.02**		**1.1**		**0.73**		**0.72**		**0.49**	

(d)

M. C.	Shoot				Root				Length				Spike			
	F. m.	%	D. m.	%	F. m.	%	D. m.	%	Sh.	%	Ro.	%	D. m.	%	Length	%
90%	7.9	100	2.8	100	3.6	100	0.80	100	64.8	100	24.8	100	2.0	100	18.5	100
70%	7.7	98.1	2.3	82.9	3.1	87.2	0.64	79.7	58.4	90.2	21.6	87.1	1.1**	53.1	15**	81.0
50%	5.6	70.6	1.9	68.1	2.4	66.9	0.52	64.7	52.4	80.8	22.8	91.9	0.38**	18.4	13**	70.2
30%	4.2	53.2	1.5	51.4	1.4	39	0.28	34.7	33.6	51.9	12	48.4	0.24**	11.7	9**	48.7
90% + SA	7.7	103.5	2.8	100	3.8	105.3	0.82	101.5	84	129.3	30	120.9	2.1	102.8	19	102.7
70% + SA	6.0	106.8	2.9	105.3	4.3	119.5	0.69	85.7	81	125	28	112.9	1.6**	77.6	16**	86.5
50% + SA	6.1	101	2.1	74.5	3.7	103.9	0.56	69.4	82	126.5	29	116.9	1.1**	53.1	15**	81.1
30% + SA	5.9	85.3	2.0	71.6	2.9	80.2	0.53	65.3	67	103.4	27	108.9	0.75**	36.7	12.5**	67.6
L. S. D. 0.05%	**0.17**		**0.15**		**0.15**		**0.02**		**1.0**		**0.87**		**0.13**		**0.75**	

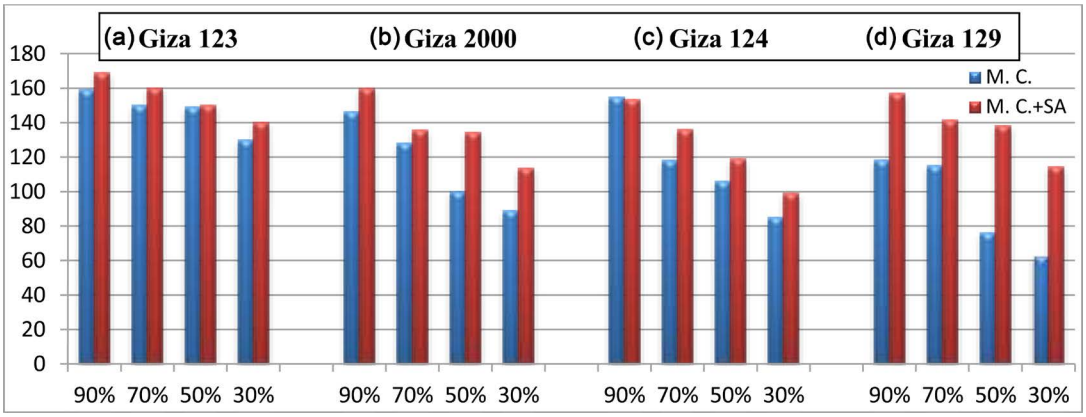
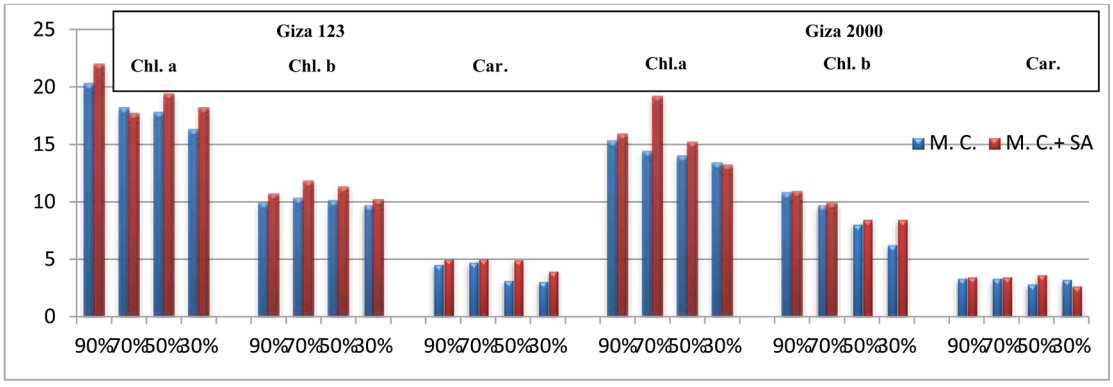


Figure 1. The response of Giza 124 (a), Giza 2000 (b), Giza 124 (c) and Giza 129 (d) barley genotypes to drought stress and interactive with SA treatments on leaf area at the final fruiting stage.

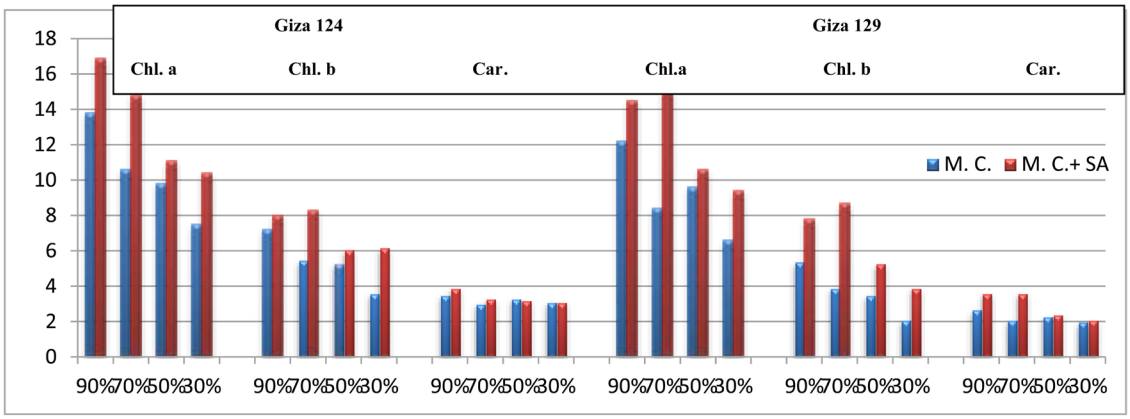
87.6%, 58.3%, 98.1 in Giza 2000 plant, 54.3%, 50%, 88.6%, in Giza 124 plant, 54.1%, 38.9%, 74.1% in Giza 129 plant. Water content was reduced in shoot and root as decreasing M. C. levels in four tested genotypes, and the low values were recorded at 30% M. C. level (Table 2). The highest value in water content was recorded in Giza 123 while the lower one was observed in Giza 129. The percent of reduction at 30% M. C. level was 97.9%, 85.3%, 66.7%, 71.4%, 85.8%, 75%, 39.2% and 39.3% in Giza 124, Giza 2000, Giza 124 and Giza 129 respectively (Table 2).

3.2. Metabolities in Shoot and Root

Soluble sugar and soluble protein significantly accumulated as decreasing moisture content in both shoot and root of Giza 123 (Table 3(a)). This more obvious in case of soluble sugar in root than in shoot and in case of soluble protein in shoot than in root. The high value was recorded at 30% M. C. in both contents, the percent of increase at that level was 114%, 160%, 150%, and 200% compared with control pants. However amino acids become around the value of control in



(a)



(b)

Figure 2. The response of Giza 123 (a), Giza 2000 (a), Giza 124 (b) and Giza 129 (b) barley genotypes to drought stress and interactive with SA treatments on pigment contents (mg g^{-1} d. m.).

Table 2. The response of Giza 123, Giiza 2000, Giza 124 and Giza 129 barley genotypes to drought stress and interactive with SA treatments on water content in shoot and root at the final fruiting stage.

Treat. M. C.	Giza 123				Giza 2000				Giza 124				Giza 129			
	Sh.	%	Ro.	%	Sh.	%	Ro.	%	Sh.	%	Ro.	%	Sh.	%	Ro.	%
90%	4.9	100	3.4	100	3.6	100	2.1	100	3.4	100	3.2	100	5.1	100	2.8	100
70%	4.5	91	3.2	94.2	3.5	97.2	2.2	104	3.0	88.2	2.9	90.6	3.2	62.7	2.5	89.3
50%	4.8	97.9	3.0	88.2	3.1	86.1	1.8	85.7	3.0	88.2	2.5	78.1	3.7	72.5	1.9	67.9
30%	4.8	97.9	2.9	85.3	2.4	66.7	1.5	71.4	2.0	85.8	2.4	75	2.0	39.2	1.1	39.3
90% + SA	5.1	104.8	4.4	120.9	7.2	200	2.4	114.3	3.1	91.2	3.8	118.8	4.9	96.1	2.9	103.6
70% + SA	5.5	112.2	5.3	155.9	4.7	130.6	2.8	133.3	2.5	73.5	2.0	62.5	3.1	60.8	3.6	128.6
50% + SA	4.4	89.8	4.9	144.1	5.0	138.9	2.8	133.3	3.4	100	4.3	134.4	4.0	78.4	2.5	89.3
30% + SA	4.5	89.8	3.4	100	3.0	83.3	1.6	76.2	2.8	82.4	2.3	71.9	3.9	76.5	2.7	96.4
L. S. D. 0.05%	2.1		1.1		1.0		1.9		1.5		1.8		1.6		1.1	

shoot at all moisture content levels, in root this effect was observed at 70% M. C. level, afterthat a smooth reduction was recorded in Giza 123 (Table 3(a)). There is a significant reduction in proline content in shoot, whereas a huge accumulation

Table 3. The response of Giza 123 (a), Giza 2000 (b), Giza 124 (c) and Giza 129 (d) barley genotypes drought stress and interactive with SA treatments on soluble sugar (mg g^{-1} d. m.), soluble protein (mg g^{-1} d. m.), amino acids (mg g^{-1} d. m.) and proline content (mg g^{-1} d. m.) of shoot and root at the final fruiting stage.

(a)

Treat. M. C.	Soluble sugar				Soluble protein				Amino acids				Proline			
	Sh.	%	Ro.	%	Sh.	%	Ro.	%	Sh.	%	Ro.	%	Sh.	%	Ro.	%
90%	14.7	100	4.1	100	80	100	16.4	100	48.9	100	50.2	100	3.7	100	0.2	100
70%	15.8	107	4.8	118	100	125	16.8	102	52.2	106	54.1	108	3.6	98	0.8	100
50%	17.5	119	5.4	132	119	149	18.4	112	58.2	119	47.2	94	3.1	85	1.3	650
30%	16.7	114	6.5	160	120	150	32.8	200	53.3	109	44.7	89	6.3	173	1.1	550
90% + SA	16.1	109	5.9	144	100	125	208	127	54.1	111	52.6	105	3.4	93	0.7	350
70% + SA	19.3	131	7.1	173	113	141	24.4	149	58.7	120	56	111	2.1	75	0.3	150
50% + SA	22.1	150	6.6	161	129	161	28.3	172	63.8	131	57.9	115	2.8	76	0.4	200
30% + SA	28.8	196	6.9	171	127	159	29.5	180	67.3	138	65.2	130	3.4	93	0.3	150
L. S. D. 0.05%	0.91		0.77		0.79		0.64		0.18		0.18		0.15		0.05	

(b)

Treat. M. C.	Soluble sugar				Soluble protein				Amino acid				Proline			
	Sh.	%	Ro.	%	Sh.	%	Ro.	%	Sh.	%	Ro.	%	Sh.	%	Ro.	%
90%	18.5	100	7.6	100	83.2	100	18.8	100	47.2	100	51.4	100	3.9	100	0.19	100
70%	19.1	103	7.6	102	92	110	23.6	126	42.8	90.5	48	93	1.9	49	0.35	148
50%	20	107	7.8	103	118	142	34.4	183	52.0	110	50	97	2.2	56	0.58	284
30%	22	117	8.4	111	125	150	38.8	206	66	140	49	95	2.9	74	0.54	305
90% + SA	20	107	8.0	102	84	101	21.6	115	53.5	113	55	107	5.5	141	0.36	189
70% + SA	23	123	9.0	114	99	119	24.4	130	57	120	58	112	5.0	129	0.43	226
50% + SA	27	147	10	128	119	144	31.6	168	60	127	58.3	114	1.0	26	0.28	147
30% + SA	31.4	170	8.4	112	128	154	30	158	67	142	61	118	1.4	37	0.45	237
L. S. D. 0.05%	**0.78**		**0.78**		**1.1**		**0.79**		**0.43**		**0.29**		**0.17**		**0.075**	

(c)

M. C.	Soluble sugar				Soluble Protein				Amino acids				Proline			
	Sh.	%	Ro.	%	Sh.	%	Ro.	%	Sh.	%	Ro.	%	Sh.	%	Ro.	%
90%	23.1	100	9.9	100	64	100	13.6	100	40	100	36	100	1.9	100	2.5	100
70%	16.1	70	8.3	84	94	147	15.2	112	40	100	39	108	1.0	53	2.2	87
50%	16.1	70	7.7	78	98	153	19.6	144	40	100	36	100	1.3	68	2.0	81
30%	14	60	8.5	86	119	186	36.0	265	46.3	117	29	80	1.6	84	2.3	92
90% + SA	25	107	12	116	121	189	18	133	41	103	39	109	1.4	72	2.5	100
70% + SA	22	94	11.4	115	148	232	18	129	43	109	39	108	0.82	43	2.2	88
50% + SA	19	81	13.4	136	139	217	26	192	46	119	43	120	1.4	72	2.1	83
30% + SA	18	78	13	130	137	214	34	253	49	124	43	121	1.5	77	3.7	151
L. S. D. 0.05%	**0.67**		**0.67**		**1.3**		**0.69**		**0.52**		**084**		**0.14**		**0.13**	

(d)

M. C.	Soluble sugar				Soluble Protein				Amino acid				Proline			
	Sh.	%	Ro.	%	Sh.	%	Ro.	%	Sh.	%	Ro.	%	Sh.	%	Ro.	%
90%	18	100	9	100	43	100	16	100	32	100	37	100	2.2	100	1.4	100
70%	16	89	8.1	91	60	141	19	122	49	153	36	98	2.5	115	2.6	186
50%	14	81	3.7	42	64	150	19	118	51	140	46	125	3.6	165	2.8	199
30%	13	71	2.7	31	69	162	22	139	45	159	46	125	3.6	166	2.8	199
90% + SA	17	99	9.2	102	62	146	18	114	37	117	45	121	2.7	123	0.47	33
70% + SA	23	131	9.0	98	79	184	28	173	44	139	45	123	1.8	84	0.93	66
50% + SA	20	111	4.3	48	81	190	24	150	48	150	46	125	4.0	175	0.62	44
30% + SA	21	120	3.7	42	104	244	27	170	53	167	52	140	3.1	144	0.63	45
L. S. D. 0.05%	0.49		0.45		0.73		0.60		0.51		0.57		0.13		0.11	

was induced in root Giza 123 plant reach 3-folds at 30% M. C. levels (Table 3(a)). Decreasing moisture content induced no significant change in soluble sugar in Giza 2000 till 50% M. C. level and then a slight increase was recorded in both shoot and root (Table 3(b)). However a significant accumulation in soluble protein was detected in shoot and root. The maximum percent value was recorded at 30% M. C. level reach a 150% and 206% compared with control plants. While decreasing moisture content induced an increasing effect in amino acids content in shoot while a slight decreasing effect was induced in root (Table 3(b)). There is a two situation in case of proline content, a decreasing effect in shoot was induced and in root an increasing effect was exhibited (Table 3(b)). While a marked decreasing effect was occurred in soluble sugar in shoot and root of cv. Giza 124, an increasing effect was induced in soluble protein of both organs reach a high percent value at 30% M. C. level 186% and 265% (Table 3(c)). Amino acids give the value of control up to 50% M. C. level, then a smooth increasing effect in shoot and a reduction in root was recorded (Table 3(c)). A surprise position was detected in proline content decreasing effect was observed in shoot and root of Giza 124 plant (Table 3(c)). The data in Table 3(d) revealed that soluble sugar in shoot and root of Giza 129 was significantly decreased as decreasing moisture content reach a low value at 30% M. C. level. This reduction was more pronounced in root than shoot organ. Soluble protein and amino acids were markedly elevated with decreasing moisture content. The percent of increase at 30% M. C. level was 162%, 139%, 159% and 125% of both content in shoot and root compared with control plants control. Proline content was markedly accumulated as decreasing soil moisture content in shoot and root of Giza 129, reach a high value at 30% M. C. level 2-folds compared with control plants (Table 3(d)). K^+, Ca^{++}, and Mg^{++} were generally remain unchanged in shoot and root of both barley Giza 123 and Giza 2000 (Figure 3(a)). Except of this trend root K^{++} and Ca^{++} in root of Giza 2000 and Ca^{++} in both organs of Giza 123 tended to increase as decreasing M. C. level. K^+ content tended to

decrease in both shoot and root of Giza 124 (**Figure 3(b)**). While Ca^{++} content decreased in shoot, increase in root of Giza 124, moreover Mg^{++} do not change with increasing drought stress in shoot while tended to increase in root (**Figure 3(b)**). In Giza 129 K^{+} content was lowered as decreasing M. C. level while Ca^{++} was increased in shoot, in root this content remain unchanged (**Figure 3(b)**). Mg^{++} content tended to decreased in shoot while tended to increase in root.

3.3. Metabolities in Spikes

Soluble sugar, soluble protein, amino acids and proline tended to smoothly increase especially at 50% and 70% M. C. in spikes of Giza 123 (**Table 4(a)**). In spikes of Giza 2000 drought stress increased soluble sugar and soluble protein while amino acids tended to exhibit an irregular pattern and a reduction in proline content was recorded (**Table 4(a)**). A slight accumulation in soluble sugar, soluble protein and amino acids in spikes of Giza 124 (**Table 4(b)**) was induced. Moreover a hug increases in soluble sugar and proline content was recorded in spikes of Giza 129 (**Table 4(b)**). The activation was reached 2.5-folds in soluble sugar and 8-folds in proline content in spikes of Giza 129 (**Table 4(b)**). On the other side a small increase in soluble protein and amino acids in spikes of Giza 124 was induced. In spikes of Giza 123 both K^{+} and Mg^{++} were markedly increased as increasing drought stress, reached 2-folds at 50% M. C. level in case of K^{+} and at 30% M. C. level in case of Mg^{++} (**Table 5(a)**). While Ca^{++} increased at 70% M. C., decreased at 50% and 30% M. C. levels compared with control plants (**Table 5(a)**). Ca^{++} and Mg^{++} were significantly increased in Giza 2000 especially at 70% and 50% M. C. and gave the same values, however K^{+} was smoothly decreased at 70% and 50% M. C. levels and dramatically reduced at 30% M. C. level (**Table 4(a)**). Drought stress induced reduction in K^{+}, Ca^{++} in both Giza 124 and Giza 129 and Mg^{++} in Giza 124 especially at lower moisture content (50% and 30% M. C.) (**Table 5(b)**). Except of this trend Mg^{++} in Giza 129 significantly increased compared with control plant (**Table 5(b)**). Osmotic pressure was increased in shoot, root and spike of four barley tested genotypes with varied degree, moreover root organ was the lowest values than shoot and spike organs (**Figures 4(a)-(d)**). The percent of increase at 30% M. C. was 132.6%, 117.7% and 115.5% in shoot, root and spike of Giza 123, in Giza 2000 it was 136.4%, 126.3%, 127.8%, in Giza 124 it was 134.5%, 105.5%, 106.3% and finally in Giza 129 this percent was 135.2%, 113.0% and 139.9% compared with control plants.

3.4. SA Application

SA application was markedly enhanced the production of fresh, dry mater, length of shoot and root with varied degree according to each tested barley genotype (**Tables 1(a)-(d)**). The medium percent of different moisture content levels in fresh, dry matter and length of shoot and root after spraying with 0.5 mM

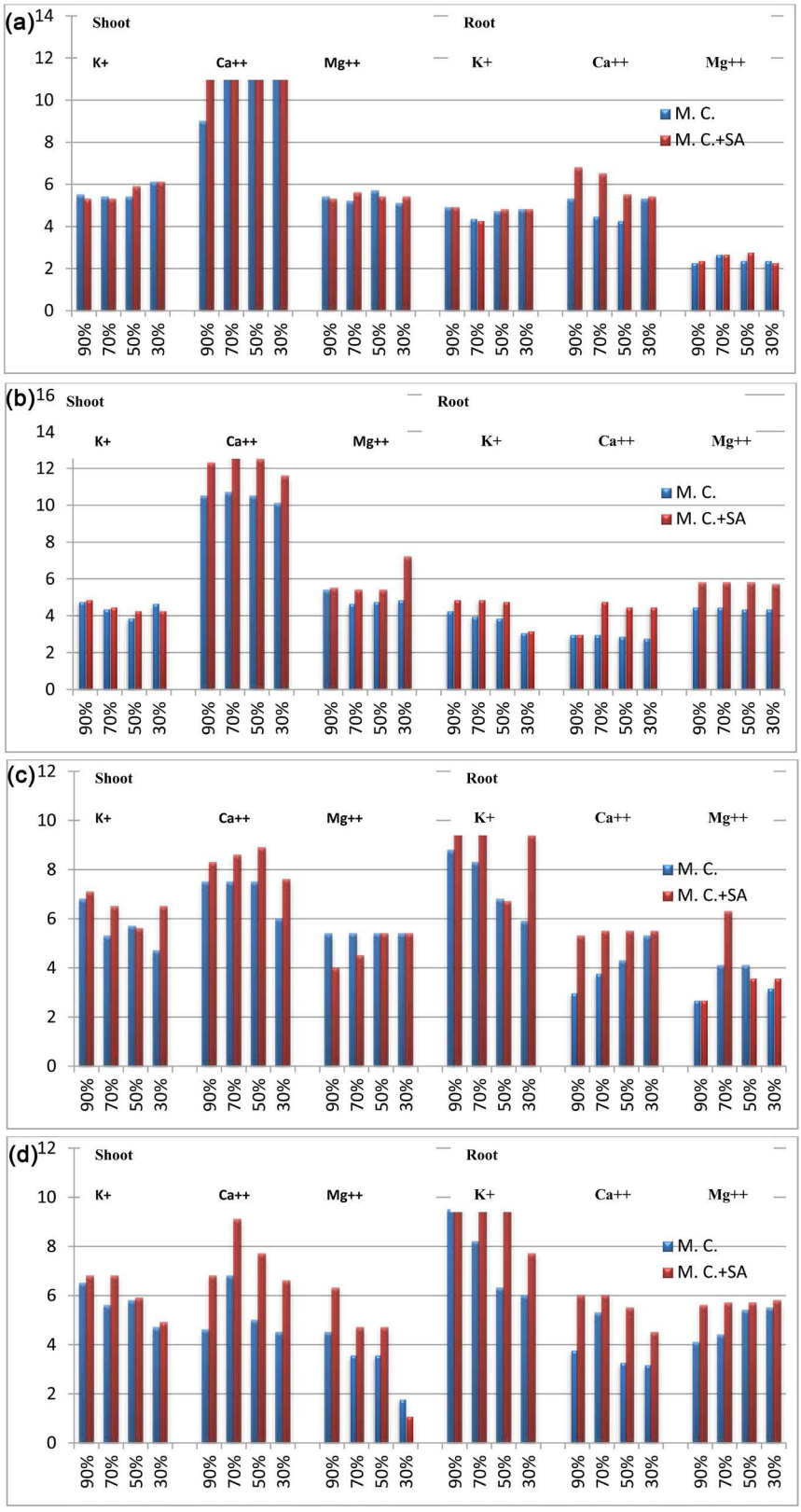


Figure 3. The response of Giza 123 (a), Giza 2000 (b), Giza 124 (c) and Giza 129 (d) barley genotypes to drought stress and interactive with SA treatments on K^{+}, Ca^{++} and Mg^{++} contents (mg g^{-1} d. m.) of shoot and root at the final fruiting stage.

Table 4. The response of Giza 123 (a), Giza 2000 (a), Giza 124 (b) and Giza 129 (b) barley genotypes to drought stress and interactive with SA treatments on soluble sugar (mg g^{-1} d. m.), soluble protein (mg g^{-1} d. m.), amino acids (mg g^{-1} d. m.) and proline content (mg g^{-1} d. m.), in spikes at the final fruiting stage.

(a)

		Giza 123								Giza. 2000							
Treat.	M. C.	Sol. sug.	%	Sol. prot.	%	Am. acid	%	Prol.	%	Sol. sug.	%	Sol. prot.	%	Am. acid	%	Prol.	%
Control	90%	52.5	100	119	100	42.9	100	0.7	100	48	100	107	100	48.2	100	0.69	100
	70%	63*	119.1	125**	104	40.2**	93.2	0.65	93	99.5**	207	114**	107	46.9**	97	0.25**	36
	50%	80**	152	134**	112	50**	115	1.4**	200	61**	127	127**	119	41.4**	86	0.25	36
	30%	60**	115	139**	116	50**	115	0.9**	129	85.5**	178	127**	119	49.2**	102	0.57**	83
	90% + SA	62.5**	119	103**	86.2	46.9**	109	0.3**	43	55	115	108	101	50.1**	104	0.53**	77
	70% + SA	82**	156	128**	107	47.1**	110	0.4**	57	62**	129	102**	95	52**	108	0.34**	49
	50% + SA	46.5	89	130**	109	51.1**	119	0.7	100	85**	177	102**	95	50.3**	104	0.2**	29
	30% + SA	47.5	91	157**	132	42.3**	99	0.5**	71	105**	219	117**	109	75**	155	0.54**	78
L. S. D.	**0.05**	1.0		1.1		**0.24**		**0.08**		**0.76**		**1.6**		**0.58**		**0.08**	

(c)

		Giza 124								Giza 129							
Treat.	M. C.	Sol. sug.	%	Sol. prot.	%	Am. acid	%	Prol.	%	Sol. sug.	%	Sol. prot.	%	Am. acid	%	Prol.	%
Control	90%	35	100	119	100	33.9	100	1.25	100	22.5	100	101	100	43	100	0.62	100
	70%	57**	163	121	102	31**	91	0.56**	45	28.5**	127	107**	106	43	100	0.99**	160
	50%	40**	114	134**	112	36**	106	0.91**	73	56.5**	251	115**	114	46**	108	3.5**	564
	30%	31	89	135**	113	39**	115	0.83**	66	63.5**	282	117**	116	51**	120	5.4**	871
	90% + SA	38	109	124**	104	38**	111	0.44**	35	49.5**	220	109**	108	49**	115	0.68**	110
	70% + SA	59**	167	101**	85	42**	124	0.84**	67	67.5**	330	137**	137	45**	106	1.9**	306
	50% + SA	66**	168	116**	97	39**	114	0.57**	46	68.5**	304	112**	111	36**	84	1.7**	274
	30% + SA	77**	220	106**	89	37**	108	0.84**	67	71**	315	140**	140	58**	131	2.4**	435
L. S. D. 0.05%		**0.35**		1.5		**0.71**		**0.09**		**0.36**		1.5		**0.47**		**0.08**	

SA was 109.5%, 132.3%, 129.9%, 120.3%, 96.3%, 97.3%, in Giza 123. In Giza 2000 this percent of increase was 142.5, 136.7, 112.9%, 103.9%, 90.3%, 85.6%. However in Giza 124 it was 101.6%, 100.7%, 102.4%, 99.9%, 94.4%, 105.5%, and in Giza 129 this increasing percent was 99.2%, 87.9%, 102.2%, 80.5%, 121.1%,

Table 5. The response of cv. Giza 123 (a), cv. Giza 124 (b) and cv. Giza 129 (b) barley genotypes to drought stress and interactive with SA treatments on K^+, Ca^{++} and Mg^{++} (mg g^{-1} d. m.), in spikes at the final fruiting stage.

(a)

M. C.	Giza 123						Giza 2000					
	$K^{+.}$	%	Ca^{++}	%	Mg^{++}	%	$K^{++.}$	%	Ca^{++}	%	Mg^{++}	%
90%	0.8	100	3.8	100	1.4	100	1.33	100	1.5	100	2.7	100
70%	1.1**	141	6**	160	1.8	133	1.3	98	3**	200	3.6**	133
50%	1.8**	221	3**	80	1.8	133	1.3	96	3**	200	3.6**	133
30%	0.73	91	3**	80	2.7**	200	1**	75	1.5	100	2.7	100
90% + SA	1.8**	229	6**	160	4.5**	333	1.7**	127	2.3**	150	2.7	100
70% + SA	1.9**	246	4.5**	120	2.7**	290	1.4	108	1.5	100	2.7	100
50% + SA	2.7**	334	4.5**	120	1.8	133	1.3	98	3**	200	2.7	100
30% + SA	0.8	100	2.3**	60	1.3	132	1.1	85	4.5**	300	2.7	100
L.S.D. 0.05%	**0.22**		0.41		**0.41**		**0.24**		**0.38**		**0.55**	

(b)

M. C.	Giza 124						Giza 129					
	$K^{+.}$	%	Ca^{++}	%	Mg^{++}	%	K^{++}	%	Ca^{++}	%	Mg^{++}	%
90%	1.6	100	4.5	100	2.7	100	2.0	100	3.8	100	1.4	100
70%	2**	125	4..5	100	2.7	100	1.2**	74	3**	80	1.8**	133
50%	1.3	83	3**	67	1.8**	67	1.1**	56	3**	80	3.6**	267
30%	1.2**	72	1.5**	33	0.9**	33	0.53**	26	2.3**	60	3.2**	233
90% + SA	1.7	104	1.5**	33	1.8**	67	0.67**	33	1.5**	40	2.7**	200
70% + SA	2**	125	1.5**	17	0.9**	33	0.6**	29	4.5**	120	2.7**	200
50% + SA	1.3	79	0.75**	17	1..8**	67	0.57**	28	3**	80	1.8	133
30% + SA	1.1**	67	0.75**	17	0.9**	33	0.47**	23	1.5**	40	0.9**	67
L. S. D. 0.05%	**0.26**		0.29		**0.27**		**0.32**		**0.29**		**0.27**	

114.9% compared with unsprayed barley plants. SA increased water content and leaf area at all tested barley cultivars, this effect was more pronounced in Giza 129, Giza 2000, and Giza 124 than Giza 123 especially in leaf area parameter (Table 2 and Figure 1). SA application enhanced the synthesis of photosynthetic pigments in four barley genotypes (Figure 2(a) & Figure 2(b)). This activation effect was more obvious in case of Chl. a in Giza 123 and Giza 2000 (Figure 2(a)) and in case of production Chl. a and Chl.b in both Giza 124 and Giza 129 (Figure 2(b)). Spraying vegetative parts with SA was markedly increased the soluble sugar, soluble protein and amino acids of both shoot and root of four barley cultivars. The medium percent of increase of the previous parameters in plants spraying with SA in Giza 123 was 146.5%, 162.3%, 146.5%, 157%, 125%, 115.3%, was in shoot and root (Table 3(a)). This value in Giza 2000 was 136.8%, 114%, 129.5%, 142.8%, 125.5%, 112.8%, (Table 3(b)). In Giza124 it was 90%,

124.5%, 213%, 176.8%, 113.8%, 114.5% (Table 3(c)). In Giza 129 it was 115.3%, 72.5%, 191%, 151.8%, 143.3%, 127.3% compared with control plants (Table 3(d)). On the other side SA application lowered the accumulation of proline in shoot and root of four barley genotypes (Tables 3(a)-(d)). SA application induced unchanged in soluble sugar, a slight accumulation in soluble protein and amino acids, while a reduction in proline in spikes of Giza 123 was recorded (Table 4(a)). Activation in the accumulation of soluble sugar and amino acids especially at 30% M. C. and a reduction in proline was recorded in spikes of Giza 2000 (Table 4(a)). SA treatment increased soluble sugar, unchanged in amino acids content while a reduction was induced in proline in spikes of Giza 124 (Table 4(b)). SA activated the accumulation of following contents, soluble sugar reached 3-folds, protein and proline this with unchanged effet in amino acids as compared with 90% M. C. (Table 4(b)). While spraying vegetative parts with SA induced no significant change in K^+, Ca^{++}, and Mg^{++} in shoot and root of Giza 123. While significantly increased K^+, Ca^{++} and Mg^{++} in shoot and root of Giza 2000 was reorded (Table 5(a) & Table 5(b)). SA application enhanced accumulation of K^+, Ca^{++} in both shoot and root of Giza 124 and K^+, Ca^{++} and Mg^{++} in Giza 129. SA increased K^+ reached 3-folds at 50% M. C. level. Ca^{++} reached 2-folds at 30% M. C. and Mg^{++} in spikes of Giza 123 (Table 5(a)). SA application resulted unchanged in K^+, Ca^{++} and Mg^{++} in both Giza 124 and Giza 129 (Table 5(b)). Except of this position Mg^{++} increased as decreasing M. C. in spikes of Giza 129, the high value was recorded at 30% M. C. (Table 5(b)). SA treatment was activated the value of OP in four tested barley genotypes, this effect was more pronounced in shoot and spike of Giza 129 than other organs of other genotypes (Figures 4(a)-(d)). The percent of activation at lowest value of moisture content (30% M. C.) was 144.2%, 137.9%, 119.4% in shoot, root and spike of Giza 123. In Giza 2000, it was 144.7%, 170.6%, 146.3%, in Giza 124 it was 151.4%, 131.2%, 113.4 and finally in Giza 129 this percent was 177.9%, 125.3% and 156.9% as compared with control plants.

4. Discussion

Previous data can be demonstrated that four barley genotypes varied in their drought tolerance according to their efficiency and tested organs. It can be observed that fresh, dry matter and length of shoot and root decreased as decreasing M. C., the percent of reduction at 30% M. C. level as follows 96.9%, 96%, 84%, 72.4%, 80%, 80%, in Giza 123. In Giza 2000, it was 69.4%, 81.7%, 66.7%, 52.0%, 67.2%, 68.2%, in Giza 124 it was 63.9%, 63.4%,43.7%, 66.6%, 65.4%, 68% and finally in Giza 129 this percent of reduction was 53.2%, 51.4%, 39%, 34.7, 51.9%, 48.4% compared with control plants. This supported by spike production the net result of cultivation, the percent of dry matter and length of spike was 52%, 79.4%, 41.3%, 63.9%, 31.6%, 61.2%, 11.7%, 48.7% in Giza 123, Giza 2000, Giza 124 and Giza 129 respectively. Also, leaf area and pigment production were markedly decreased according to genotype tolerance, it run parallel with fresh

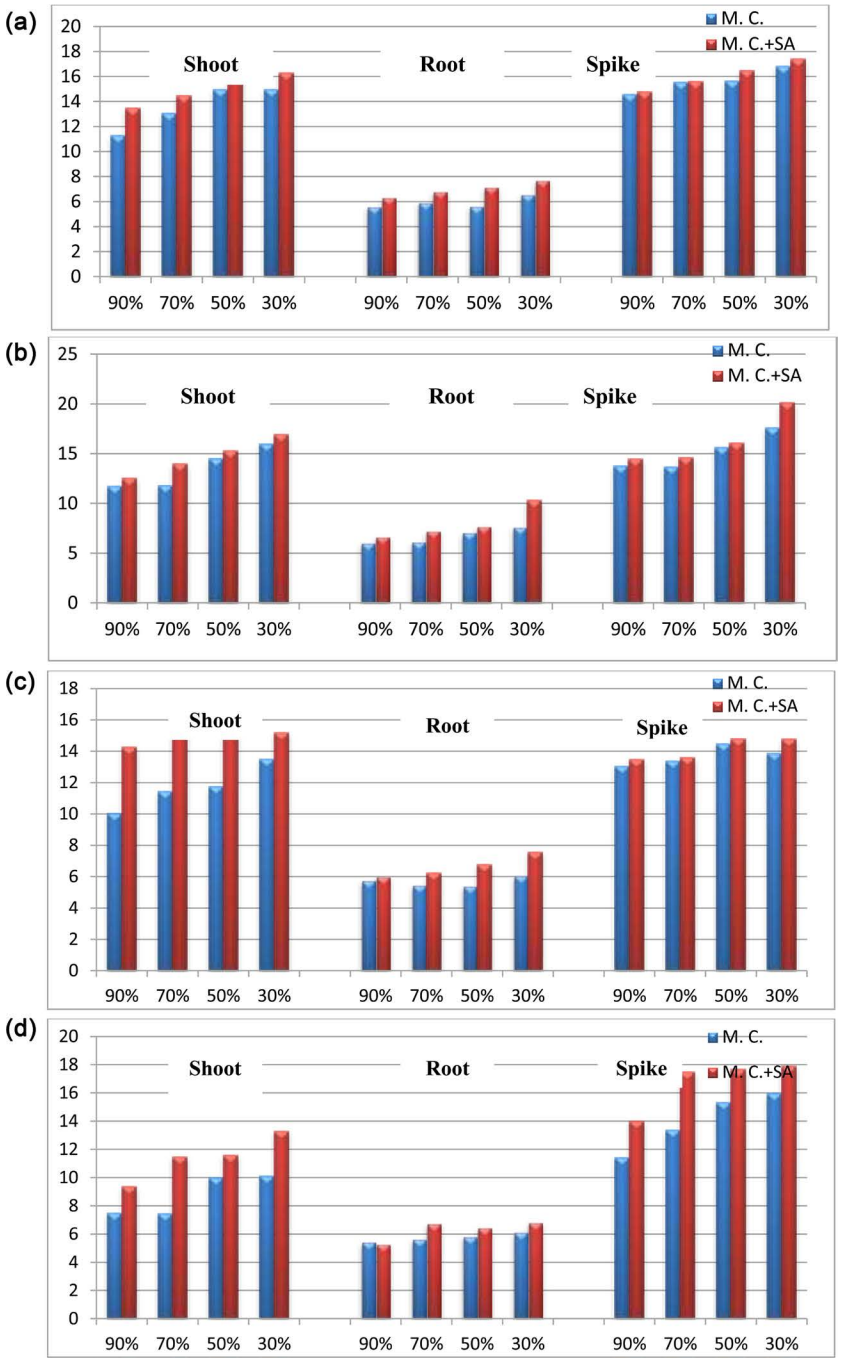


Figure 4. The response of Giza 123 (a), Giza 2000 (b), Giza 123 (c) and Giza 129 (d) barley genotypes to drought stress and interactive with SA treatments on osmotic pressure (mOsmo/H_2O) in shoot, root and spike of at the final fruiting stage.

and dry matter of shoot, root and spike. The percent of reduction in leaf area at 30% M. C. level was 81.8%, 60.7%, 54.9% and 53.3% in four tested barley genotypes respectively. The differences in the growth criteria among species and cultivars might be used as a suitable selection criterion for the drought tolerance of these species and genotype. The inhibitory effect of drought on growth parameters could be attributed to the osmotic effect of water stress [29]. Also, the

reduction of yield may be ascribed to the harmful effect of soil moisture stress and nutrient balance disorder in root media [30], or reduced rate of new cell production may be make additional contribution to the inhibition of growth [31]. The reduction in growth criteria due to drought stress might be related to disturbance of water flow from root to shoot [32], decrease in water potential of cell sap [33], or inhibition of cell division [34]. Moreover, the percent of reduction in Chl. a, Chl. b and carotenoids was 80.3%, 98.9%, 67.4%, 87.6%, 58.3%, 97.1%, 54.3%, 50%, 88.6%, 54.1%, 38.9% and 74.1% in four ranking barley genotypes. So lowering in green area of tested genotypes served in reduction in the efficiency of photosynthetic system which directly effected on carbohydrate production [35]. Actually, this indicated that Giza 123 was the superior in its drought tolerance and Giza 129 was the interior and both Giza 2000 and Giza 124 were the intermediated between them. This coincided with increasing in soluble sugar and soluble protein content of shoot, root and spike, shoot Ca^{++}, K^{+} and Mg^{++} in spike of Giza 123. In Giza 2000, this was related with a huge accumulation of soluble sugar in spike, soluble protein of three tested organs, shoot amino acids, Ca^{++} and Mg^{++} in spike. Whereas drought stress increased soluble sugar of spike, soluble protein in shoot, root and spike, Ca^{++} and Mg^{++} in root organ of Giza 124 while in Giza 129 the highest sensitive genotype decreasing M. C. increased soluble sugar of spike, soluble protein and amino acids in three tested organs, shoot Ca^{++}, root $Mg^{++}$was recorded *i.e.* each genotype try to overcome and facing drought stress. The previous observation was induced as a result of increasing OP in shoot, root and spike of four barley genotypes, the percent of increasing in osmotic pressure at 30% moisture content was 132.6%, 117.5%, and 115.4% in Giza 123, in Giza 2000 it was 135.8%, 126.3% and 127.8%, in Giza 124 was 134.4%, 117.6% and 129.3%, finally in Giza 129 it was 135.2%, 113.0% and 135.1% compared with undroughted plants respectively. This mean that the medium percent of increasing in OP value was more or less similar in shoot of four barley genotypes was about 34.5% over the control plants 100% and also in root organ was about 18.6%. However in spike this situation was different the most barley drought tolerant Giza 123 was the lower in percent of increasing OP value was 15.4% over the control value 100% and the most drought sensitive was the higher increasing percent value was 35.1% in Giza 129 genotype. It is interesting to note that the percent of increasing value in OP of Giza 2000 and Giza 124 was respectively intermediated 27.8% and 29.3% over the control plants [36]. Osakabe *et al.* (2017) [37] showed that cell growth caused by cell expansion is regulated primarily by turgor pressure, which is the physical force against the cell wall, and is maintained by osmotic regulation via osmotically active substances, such as potassium ions (K^{+}) as in Giza 123, sugars as in spike of four tested genotypes, protein as in Giza 123, Giza 2000 and Giza 129 and amino acids as in shoot of Giza 2000 and Giza 129 genotype. K^{+} is an essential element in plant growth, and K^{+} uptake and efflux affect plant productivity and control cell water potential and turgor in osmotic regulation. K^{+} affects os-

motic pressure in the root xylem (root pressure), which drives long-distance sap flow from roots to shoots [38]. During water deficit stress, osmotic stress sensing and signaling are pivotal to plant water status and lead to rapid changes in gene expression [39] [40]. Osmotic adjustment helps to maintain cell turgor, which can allow cell enlargement and plant growth during water stress; and it can allow stomata to remain at least partially open and CO_2 assimilation to continue at water potentials that would be otherwise inhibitory [41] (Alves and Setter, 2004). Supported the previous view that drought stress is among the factors most limiting to plant productivity [35] [42] [43] [44] [45] [46]. Plants exposed to drought stress adapt their metabolism in order to cope with the changed environment. Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals and instigate biochemical changes that adjust the metabolism accordingly [47] [48] [49] [50] [51]. One distinctive feature of most plants growing in stress environments is the accumulation of proline [52] [53] and it has been inferred that there may be a relationship between cellular proline level and cell turgidity via osmotic adjustment [54] [55] [56] [57] [58]. From our results it can be confirmed that root is more sensitive than shoot organ in four tested barley genotypes. This related with the higher accumulation of different metabolites as soluble sugar and proline content which the later reached 5-folds than shoot in Giza 123. In root of Giza 2000 soluble protein and proline which later also reached 3-folds compared with control. Whereas in root of Giza 124 a higher accumulation of soluble protein especially at higher drought stress (30% M. C.) was recorded. In Giza 129 a higher accumulation of proline in root than in shoot, these observation was coincided with lower values in water content and OP in root compared with shoot in four tested cultivars. This position throw attention that root sensitivity was induced as a resulted of increase the previous chemical constituents to give chance in increasing OP and gain the requirement of water content and translocation from root to aerial portion for metabolism. Also root sensitivity may be due to, it was considered as the first site of plant in facing water deficit injury than other plant parts [56] [57] [58]. The strategies that plants use to defense drought position involve several mechanisms of stress tolerance, which vary according to the genotype [59] [60] [61]. Many genes involved in stress reactions reveal a complex network of responses required from the perception and recognition of the signals during stress to the final activation of certain genes [62] [63] [64]. Exogenously applications of SA helped to increase plant growth significantly in drought conditions [10] [47] [65] [66] [67] [68] [69]. Exogenously applications of SA strongly inhibited Na^+, K^+ and $Cl^-$and organic solute accumulations (GB and TSC) but stimulated N and RWC [69]. Najafian *et al.* (2009) [70] showed that SA treated plants had a higher shoot and root dry matter, electrolyte leakage, photosynthetic rates, mesophyll efficiency and water use efficiency in compared to control plants when exposed to salt stress. Transpiration rates and stomatal conductance were also significantly iesser in SA treated plants under saline stress

conditions. Foliar application of SA (1.0 μM) strengthened antioxidant defense system in drought-tolerant *Z. mays* cultivar to a great extent [71] [72]. Potential involvement of SA in the 76 identified proteins was reported in drought-exposed *T. aestivum* [14]. Theses identified proteins were advocated to perform major physiological processes such as photosynthesis, carbohydrate and protein metabolism, defense energy production, signal transduction, and toxin metabolism. Some of the recent studies have shown that SA played an important role by regulating many metabolic mechanisms. Marcińska *et al.* (2013) and Nazarli *et al.*, (2014) [73] [74] showed that treatments plants with SA, MeJA and ABA were also effective in enhancing the antioxidant concentrations of proline and soluble sugars. The production of these antioxidants could have been part of a defense system against drought injury, reducing MDA and ELI and maintaining membrane stability. Therefore, this work try to throw attentions on mechanisms of drought tolerance of four barley genotypes and the potential role of SA on this strategy.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

Salicylic acid, SA

Moisture content, M. C.