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## Evaluation of selenium and salicylic acid effect on physiological and qualitative characteristics of dry-land wheat cultivars

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## **ARTICLE INFO**

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#### Keywords:

Electrolyte leakage Grain yield Proline content Wet Gluten content ABSTRACT - This experiment was carried out as factorial based on a randomized complete block design with three replications at the research station of the Islamic Azad University-Arak Branch, Iran, in 2011 and 2012. Experimental factors consisted of three levels of salicylic acid (SA); seed priming with distilled water (hydro priming), seed priming with 0.5 mM SA, and 0.5 mM SA seed priming 1 mM SA spraying; two levels of selenium (Se); 0 and 20 g ha<sup>-1</sup>, and three rain-fed wheat cultivars: Azar 2, Sardari, and Rasad. The results showed that SA seed priming combined with foliar application together with spraying of Se increased the relative water content in Azar 2 and Rasad cultivars compared to the hydro priming. The foliar application of Se increased the leaves proline amounts and grain Se concentration in wheat cultivars. Priming with SA combined with a spray of Se decreased the electrolyte leakage by 32% as compared to the control. The application of SA combined with foliar application of Se increased the proline amounts of leaves and grain Se concentration. Seed priming of SA in combination with foliar application of Se increased the grain gluten content in Sardari and Rasad cultivars. The highest productivity index (63.12%) and grain yield (1585.01 kg ha<sup>-1</sup>) were obtained from Azar 2 cultivar. The results suggested that applying SA and Se may help in alleviating damage and improving the tolerance of drought stress and grain quality in wheat.

## **INTRODUCTION**

Wheat is essential nourishment for more than 1/3 of the world's population and crop yield is considerably influenced by global climate changes and limited water resources in the environment (Chaves and Oliveira, 2004). In Iran, more than 5 million hectares are cultivated with wheat, while some 60.75% of which are rain-fed wheat (Ahmadi et al., 2016). Drought stress is an important environmental factor constraining crop productivity. Water deficit stress severely limits crop growth, especially in arid and semiarid regions of the world as it affects all stages of plant growth and development (Shamim et al., 2009).

Searching for suitable ameliorants or stress alleviants is a task for plant biologists. Recently, investigators have identified several beneficial effects of selenium (Se) on plants (Terry et al., 2000). In higher plants, however, the role of Se is still unclear (Hawrylak-Nowak, 2008). Selenium can increase the tolerance of plants to UV-induced oxidative stress, delay senescence, promote the growth of ageing seedlings, and regulate the water status of plants exposed to drought (Germ et al., 2007). Yao et al. (2011) reported 42, 56 and 40% increases in the contents of proline, phenolic compounds and flavonoids, respectively, in the leaves of wheat seedlings after treatment with UV-B and Se. A significant increase in the activity of superoxide dismutase, catalase and glutathione peroxides enzymes and the levels of abscisic acid and proline was observed with an increase in the water stress level in the leaves of all the safflower cultivars genotypes investigated (Sajedi et al., 2012).

Salicylic acid (SA) is a common plant-produced phenolic compound having key roles such as stomatal movement, seed germination, ion absorption, and responses to environmental stresses (Khademi et al., 2012). The exogenous application of SA helped in the activation of a range of plant defense genes, increased resistance to infection, decreased damages caused by exposure to ultraviolet light and ozone, and improved drought tolerance in plants (Rasmussen et al., 1991). Some earlier reports showed that exogenously applied SA could ameliorate the damaging effects of both drought stress (Waseem et al., 2006) and salt stress (Arfan et al., 2007) in wheat. Pirasteh-Anosheh et al. (2012) also showed that exogenous applications of SA alleviated drought stress harmful effects considerably in two wheat cultivars. These observations suggest that SA, being an oxidant, could be linked to oxidative stress. SA has been shown to interfere with the biosynthesis and/or action of ethylene, abscise acid, and cytokines in plants (Hayat et al., 2010). At present, the growth of wheat has been seriously influenced by drought in many regions. The current work studies the influence of Se and SA supplementation on physiological traits of wheat cultivars grown under dry

land conditions in order to promote improved drought stress tolerance and increase the quality in wheat.

## MATERIALS AND METHODS

A 2-year experiment was performed in 2011 and 2012 at the Research Station of Islamic Azad University-Arak Branch, Iran (34° 30' N latitude, 40° 41' E longitude, 1779 m above sea level). This region has a semi–arid climate based on metrological data from Arak, Iran (Table 1). The physical and chemical properties of the soil were determined for depths of0 to 30 cm (Table 2).

Fertilizer amounts were determined based on soil testing analysis; 50% of nitrogen (55 kg ha<sup>-1</sup>) and all of phosphorus (100 kg ha<sup>-1</sup>) were applied at seeding and 50% of nitrogen (55 kg ha<sup>-1</sup>) was applied at tillering stage. Potassium fertilizer was not applied at this experiment. The experimental design was factorial based on a randomized complete block design with three replicates. Experimental treatments comprised three SA levels including seed priming with distilled water (control), seed priming with 0.5 mM SA, and seed priming with 0.5 mM SA in combination with a foliar application of 1 mM SA. The seeds were soaked in aqueous solution for 6 h. SA was foliarly applied at the start of stem elongation or Zadoks Growth Stage (ZGS) 33. Selenium was applied as sodium selenite at rates of 0 and 20 g/ha at the start of stem extension (ZGS 33) and one week before heading (ZGS 53). The dry-land wheat cultivars were Azar 2, Sardari, and Rasad. Wheat seeds were spaced 15 cm apart in 6-m rows. To prevent any likely side effects, 1 m between the plots was not planted.

For measuring relative water content, five flags in the flowering stage were separated at 12 pm and taken to the laboratory at 25°C. In the laboratory, 15 disks of the leaves were immediately prepared and weighed to measure fresh leaf weight. Then, the disks were placed in distilled water for about 24 h until they were completely saturated. At the end of this stage, the leaf disks were dried with towels of dry paper and were weighed again. The samples were placed in an oven for about 48 h at 72°C until they were dried, and then, the weight of the dried leaves was recorded. Relative water content was calculated using the following relation (Dhopte and Manuel, 2002):

$$RWC = \frac{Wf - Wd}{Ws - Wd} \times 100$$

where RWC is the relative water content, Wf is the fresh leaf weight, Wd = is the dry leaf weight, and Ws is the saturated leaf weight.

To calculate cell membrane stability in the flowering stage, five fully developed flag leaves were harvested and 15 discs measuring approximately 3 cm in diameter were prepared from lamina. Then, the drive test tube which contained 10cc manitol solution of -2 bar osmotic potential was moved; after 24 h, the electrical conductivity of each tube at a temperature of 25°C was measured by an electrical conduction device (Aman et al., 2005). The manitol solution with -2 bar osmotic potential was calculated from the Van's Hoff equation (Martinez et al., 2004).

## $\psi_S = -Cm I R T$

 $\psi_S$  is the osmotic potential solution, Cm is the molar (g/L), I = is the ionization coefficient (1 for manitol), R is the gas constant equal 0.083 (Mpa/ mol. K), and T is the temperature (K). Productivity index (%) was calculated as: Spike weight / Total biomass × 100 (Daneshyan et al., 2002).

Months	Mean of temperature (°c)		precipi (mr	precipitation (mm)		Relatively humidity (%)		Evaporation (mm)	
	2011	2012	2011	2012	2011	2012	2011	2012	
November	12.35	8.1	4.0	91.6	34	69	75	43.6	
December	6.6	2.3	20.3	7.2	41	65	0	0	
January	-0.7	3.2	40.5	2.4	76	56	0	0	
February	-0.1	2.3	12.1	26.2	71	59	0	0	
March	6	3.8	71.4	12	64	47	0	0	
April	11.7	10.9	34.5	116.7	46	52	154.2	108	
May	15.7	17	66.6	5.7	57	44	195.7	193.6	
June	19.25	23	14	0	26	27	325.9	322.1	

Table 1. Metrological data in Arak, Iran

Table 2. Physical and chemical soil properties of the experimental site

Soil Depth	Silt	Sand	Clay	Se	N	K	P	pН	EC	OC
(cm)	(%)	(%)	(%)	(ppm)	(%)	(ppm)	(ppm)		(dS m <sup>-1</sup> )	(%)
0-30	48	26	26	0.29	0.15	169	10.1	7.7	4.6	1.6

The free proline amounts were extracted from 0.5 g leaf samples in 3% (w/v) aqueous sulphosalicylic acid and estimated using ninhydrin reagent according to the method cited in Bates et al. (1973). The absorbance of the toluene fraction aspired from the liquid phase was read at 520 nm. The proline amounts were determined using a calibration curve and expressed as  $\mu$ mol proline/g fresh weight.

For measuring Se content in wheat grain, 1 g fine powder samples with particles amount of 0.5 mm were digested with 15 ml of a 7:3 mixture of HNO3 65% and HClO4 70% at 180 to185 °C for 45 min. After cooling, 5 ml of concentrated HCl was added to the sample for reduction of Se<sup>+6</sup> to Se<sup>+4</sup> and continued for 30 min at 100 °C until the sample was completely mineralized. The Se content of test solution was analyzed by atomic absorption (Emami, 1996).

For measuring wet gluten, 10g twice-ground-wheat flour was weighed. Flour sample was sieved with a 220 $\mu$ m sieve and transferred to washing container. This container has metal sieves with diameter of 80  $\mu$ m. Twice-ground-wheat flour was mixed with 5cc NaCl<sub>2</sub>% solution for preparation of dough. In order to segregate wet gluten, the dough was washed by distillated water for 5 min. The dough was sieved through sieve 800  $\mu$ m. The glutens retained by the sieves were collected. The sample was centrifuged for 120 s to remove additional solution from gluten. Additional wet was removed from gluten. The gluten was collected and weighed  $(m_1)$ . Wet gluten content was calculated using the following relation (Wheat national standard, number 2087, 1997 and number 2705, 1995).

 $G_{wet} = m_1 \times 10\%$  Where m1 is the weight of wet gluten (g).

At the final harvest, 2  $m^2$  was harvested from the middle of each plot, and grain yield was evaluated. The data were subjected to analysis of variance using SAS V 9.1 software. Means were compared using Duncan's Multiple Range test at P=0.05.

#### **RESULTS AND DISCUSSION**

According to the analysis of variance, year, salicylic acid, the interaction effect of cultivars and salicylic acid and three-way interaction effect of treatments (P=0.01) and the effect of selenium and two-way interaction effect of cultivars and selenium (P=0.05) were significant on electrolyte leakage (Table 3).

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					Mean Squar	e		
SOV	Df	Electrolyte leakage	Relative water content	Proline content	Selenium content	Wet Gluten content	Productivity index	Grain yield
Year (Y)	1	774.52 <sup>ns</sup>	$0.80^{ns}$	746.28**	0.093*	182.13 <sup>ns</sup>	986.62**	333455.43 <sup>ns</sup>
Year×replication (R)	4	10292.97	68.72	24.75	0.010	32.76	46.65	27688.04
Cultivars (C)	2	45038.16**	35.89 <sup>ns</sup>	1144.75**	0.571**	70.13**	726.43**	493884.21**
$Y \times C$	2	263.68 <sup>ns</sup>	1.75 <sup>ns</sup>	1.80 <sup>ns</sup>	0.226**	36.74**	701.62**	77215.92 <sup>ns</sup>
Salicylic acid (SA)	2	49485.6**	189.6*	1233.50**	0.060**	13.85 <sup>ns</sup>	133.01 <sup>ns</sup>	61702.31 <sup>ns</sup>
Y×SA	2	21.74 <sup>ns</sup>	16.65 <sup>ns</sup>	136.40**	0.141**	33.34**	128.86 <sup>ns</sup>	180403.03**
C×SA	4	25975.83**	479.04**	6.95 <sup>ns</sup>	0.624**	38.87**	167.78 <sup>ns</sup>	100832.02*
$Y \times C {\times} SA$	4	130.08 <sup>ns</sup>	4.91 <sup>ns</sup>	51.07**	0.984**	7.46 <sup>ns</sup>	291.06 <sup>ns</sup>	33898.29 <sup>ns</sup>
Selenium(Se)	1	16039.11*	29.97 <sup>ns</sup>	921.78**	7.373**	5.67 <sup>ns</sup>	331.73 <sup>ns</sup>	6908 <sup>ns</sup>
Y×Se	1	1818.27	90.45	101.15**	4.95**	66.03**	164.18	296987.65**
C×Se	2	15059.95*	214.75*	4.18 <sup>ns</sup>	0.68**	37.99**	1263.97**	95010.19
Y×C×Se	2	39.26 <sup>ns</sup>	16.84 <sup>ns</sup>	21.88*	1.58**	55.46**	159.71 <sup>ns</sup>	153112.04*
SA×Se	2	7706.97 <sup>ns</sup>	45.01 <sup>ns</sup>	5.72 <sup>ns</sup>	0.186**	37.57**	1.48 <sup>ns</sup>	40368.55 <sup>ns</sup>
Y×SA×Se	2	265.80 <sup>ns</sup>	20.82 <sup>ns</sup>	16.35 <sup>ns</sup>	0.35**	29.40*	63.49 <sup>ns</sup>	25623.23 <sup>ns</sup>
$C \times \ SA{\times}SE$	4	27981.96**	92.66	23.06*	0.254**	9.72	217.16	8560.76 <sup>ns</sup>
$Y{\times} C \times \ SA{\times}SE$	4	449.70 <sup>ns</sup>	22.70 <sup>ns</sup>	9.14 <sup>ns</sup>	0.527**	34.16**	313.41*	39469.66 <sup>ns</sup>
Error	68	3551.04	52.62	6.45	0.007	6.48	111.92	32777.93
CV (%)	18.04	12.33	17.53	10.16	10.11	5.58	17.53	12.45

\* and \*\* : Significant at 5% and 1% probability. ns : Non-significant

Treatments	Electrolyt e leakage ( µs/cm)	Relative water content (%)	Proline content (µmol/g.f.w)	Selenium content (mg/kg)	Wet gluten content (%)	Productivity index (%)	Grain yield (kg/ha)
Years							
2011	332.91a	58.87a	27.63a	0.84a	44.36a	63.38a	1343.36a
2012	327.56a	58.81a	22.37b	0.78b	46.96a	57.33b	1565.59a
Cultivars							
Sardari	315.03b	59.28a	31.44a	0.69c	47.20a	55.17b	1358.57b
Azar 2	370.67a	57.70a	20.94c	0.82b	45.31b	63.12a	1585.01a
Rasad	305.01b	59.55a	22.62b	0.94a	44.47b	62.77a	1419.84b
Year x Cultiv	vars						
2011Sardari	320.16b	59.43a	34.23a	0.67c	46.05bc	53.59c	1231.84c
2011Azar 2	370.44a	57.47a	23.31d	0.80b	44.94c	65.92ab	1526.00ab
2011Rasad	308.14b	59.71a	25.35c	1.06a	42.10d	70.31a	1272.24c
2012Sardari	309.90b	59.13a	28.65b	0.70c	48.35a	56.45c	1485.30b
2012Azar 2	370.89a	57.93	18.57e	0.84b	45.68bc	60.31bc	1644.03a
2012Rasad	301.88b	59.39a	19.90e	0.82b	46.85ab	55.23c	1567.45ab
Salicylic acid							
Sal	373.05a	58.45ab	19.52c	0.86a	46.23a	58.49a	1465.51a
Sa2	308.69b	56.77b	24.32b	0.78b	45.74a	60.24a	1489.24a
Sa3	308.97b	61.31a	31.16a	0.80b	45.00a	62.33a	1408.68a
Selenium							
Se1	342.42a	58.32a	22.08b	0.55b	45.89a	58.60a	1446.48a
Se2	318.05b	59.37a	27.92a	1.07a	45.43a	62.11a	1462.47a

Table 4. Mean comparisons of simple effects of the measured traits

Means followed by the same letters in each column are not significantly different (Duncan multiple range test 5%)

Sa1: Seed priming with distilled water ,Sa2: Seed priming with 0.5 mM SA, Sa3: Seed priming with 0.5 mM SA combined with spraying of 1 mM SA,Se1: Without selenium, Se2: With selenium

The results showed 15% and 17.7% more electrolyte leakage in Azar 2 cultivar than Sardari and Rasad cultivars, respectively. Seed priming with SA or combined with foliar application of SA also decreased the electrolyte leakage by 17.2% compared to hydro priming (Table 4).

The interaction effects of treatments showed that seed priming with SA in Sardari and Azar 2 decreased electrolyte leakage by 19% and 28.9% compared to hydro priming, respectively. Seed priming with SA in combination with a foliar application in the Rasad cultivar decreased electrolyte leakage by 27.7% when compared with the control. The foliar application of Se in the Rasad cultivar decreased electrolyte leakage by 21% when compared with the control (Table 5). Seed priming with SA in combination with a foliar application of Se could decrease electrolyte leakage by 32% when compared with the control (Table 5). The three-way interaction effects of treatments showed that the minimum electrolyte leakage in Sardari (257.9  $\mu$ S/cm) and Rasad (297.7  $\mu$ S/cm) were obtained from the application of SA both as seed priming and the foliar application of Se. The lowest cell electrolyte leakage values of 225.76 and 229.60  $\mu$ S/cm were obtained from Se spray alone or Se spray in combination with SA both as seed priming and as spray in the Rasad cultivar (Table 6).

Two-way interaction of cultivars and salicylic acid (P=0.01), the effect of salicylic acid and two-way interaction of cultivars and selenium (P=0.05) were significant on relative water content (Table 3).

Treatments		Electrolyte leakage (µs/cm)	Relative water content (%)	Proline content (µmol/g.f.w)	Selenium content (mg/kg)	Wet gluten content (%)	Productivity index (%)	Grain yield (kg/ha)
Cultivars	Salicylic acid							
	Sa1	339.77b	58.34bc	25.66d	0.75d	45.87bc	55.25c	1380.65c
Sardari	Sa2	273.81cd	61.14ab	30.18b	0.79d	47.26ab	56.37bc	1485.22abc
	Sa3	331.50b	58.36bc	38.48a	0.52e	48.46a	53.89c	1209.83d
	Sa1	445.46a	63.58ab	15.93g	0.94b	47.27ab	57.69abc	1550.24ab
Azar 2	Sa2	316.38bc	49.01d	20.15f	0.50e	44.58cd	65.34ab	1593.46a
	Sa3	350.15b	60.51ab	26.74cd	1.01a	44.07cd	66.33a	1611.35a
	Sa1	333.90b	53.43cd	16.96g	0.89bc	45.56bc	62.53abc	1465.63abc
Rasad	Sa2	335.89b	60.16ab	22.63e	1.06a	45.38bc	59.00abc	1389.05bc
	Sa3	245.24d	65.06a	28.27bc	0.85c	42.47d	66.77a	1404.85bc
Cultivars	Selenium							
	Se1	315.64b	61.24a	28.41b	0.51e	47.40a	47.88c	1342.29d
Sardari	Se2	314.41b	57.31ab	34.47a	0.86c	47.00ab	62.46ab	1374.85cd
	Se1	370.81a	54.78b	17.75e	0.64d	46.58ab	67.61a	1632.03a
Azar 2	Se2	370.52a	60.62a	24.13c	1.00b	44.04c	58.63b	1538.00ab
	Se1	340.81ab	58.92ab	20.08c	0.52e	43.69c	60.31ab	1365.12cd
Rasad	Se2	269.21c	60.18a	25.16c	1.36a	45.25bc	65.23ab	1447.57bc
salicylic acid	Selenium							
Sa1	Se1	401.93a	57.34ab	16.99d	0.69c	45.97a	56.97a	1453.87ab
Sal	Se2	344.16b	59.57ab	22.04c	1.04b	46.49a	60.01a	1477.14ab
Sa2	Se1	314.77b	55.54b	21.00c	0.50d	45.29ab	58.34a	1516.40a
Sa2	Se2	302.62b	58.00ab	27.64b	1.07ab	46.20a	62.13a	1462.08ab
Sa3	Se1	310.57b	62.07a	28.24b	0.47d	46.40a	60.49a	1369.16b
Sa3	Se2	307.36b	60.55ab	34.08a	1.12a	43.60b	64.17a	1448.20ab

Table 5. Mean comparisons of two-way interactions between the measured traits

Means followed by the same letters in each column are not significantly significant (Duncan multiple range test 5 %)

Sa1: Seed priming with distilled water, Sa2: Seed priming with 0.5 mM SA, Sa3: Seed priming with 0.5 mM SA combined with spraying of 1 mM SA, Se1: Without selenium, Se2: With selenium

Seed priming with SA combined with a foliar application increased the relative water content in leaves by 4.6% compared to hydro priming. The addition of SA increased the relative water content in the leaves of Sardari and Rasad cultivars, but decreased it in Azar 2 cultivar. In this study, the foliar application of Se in Azar 2 and Rasad cultivars increased the relative water content (Table 5). The interaction effects of cultivars and Se showed that the foliar application of Se in Azar 2 and Rasad cultivars increased the relative water content by 9.6 and 2%, respectively. Seed priming with SA in combination with spray of Se increased the relative water content in Sardari cultivar by 2.5% when compared with the control. Seed priming with SA in combination with a foliar application along with the spray of Se increased the relative water content in Azar2 and Rasad cultivars by 2.2 and 30.7% when compared with the control (Table 6).

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The effect of year, cultivar, salicylic acid and selenium, also interaction effect of year  $\times$  salicylic acid,

year  $\times$  cultivars  $\times$  salicylic acid and year  $\times$  selenium (P=0.01) and three-way interaction effect of year  $\times$ cultivars  $\times$  selenium, also year  $\times$  salicylic acid  $\times$ selenium (P=0.01) were significant on Proline content (Table 3). The results showed that the Proline content in the leaves decreased by 19% in the second year when compared with the first year (Table 4). Seed priming with SA in combination with spray increased the Proline content in the leaves of the studied genotypes. The foliar application of Se increased the Proline content in leaves in three wheat cultivars. Seed priming with SA in combination with spray, along with foliar application of Se increased the Proline content of the leaves (Table 5). The results showed that the addition of Se and SA increased the leaf Proline content in wheat cultivars. When seed priming with SA was combined with the foliar application of Se, there was also a significant increase in the Proline content (Table 6). The simple and interaction effect of treatments on the grain Se concentration were significant (P=0.01) (Table 3).

Treatments		Electrolyt e leakage (µs/cm)	Relative Proline water content content (%) (µmol/g.f.w)		Selenium content (mg/kg)	Wet gluten content (%) Productivity index (%)		Grain yield (kg/ha)	
Cultivar	Salicylic acid	Seleni um							
	Sal	Se1	348.48bc	60.66abc	22.57hi	0.55hi	46.77a-d	44.41e	1329.65 cde
Sardar i	Sal	Se2	331.06cd	56.03a-d	28.75de	0.95e	44.97b-f	66.09ab	1431.66 a-d
	Sa2	Se1	289.71cde	60.05abc	27.96def	0.60gh	46.27а-е	60.05abc	1527.35 abc
	Sa2	Se2	257.90de	62.23abc	32.39bc	0.98de	48.25ab	62.23abc	1443.10a-d
	Sa3	Se1	308.73cd	63.02abc	34.69b	0.36k	49.15a	63.02abc	1169.87 e
	Sa3	Se2	354.28bc	53.69b-e	42.26a	0.68g	47.77ab	53.69b-e	1249.80de
	Sa1	Sel	415.26ab	61.95abc	14.48kl	0.81f	47.55abc	61.95abc	1611.00 ab
Azar 2	Sa1	Se2	475.66a	65.21a	17.38jk	1.07cd	47.00a-d	65.21a	1489.48 a-d
	Sa2	Se1	335.06cd	44.75e	15.02kl	0.43jk	45.37b-е	44.75e	1674.16a
	Sa2	Se2	297.70cde	53.28cde	25.28fgh	0.57gh	43.80d-g	53.28cde	1512.76 abc
	Sa3	Se1	362.11bc	57.65a-d	23.74gh	0.66g	46.82a-d	57.65a-d	1610.93 ab
	Sa3	Se2	338.20bcd	63.37ab	29.74cd	1.36b	41.32g	63.37ab	1611.76 ab
	Sal	Se1	442.05a	49.39de	13.931	0.67g	43.60d-g	49.39de	1420.98 bcd
	Sal	Se2	225.76e	57.47a-d	19.99ij	1.11c	47.52abc	57.47a-d	1510.28 abc
Rasad	Sa2	Se1	319.52cd	61.83abc	20.01ij	0.47ij	44.22c-g	61.83abc	1347.70 cde
	Sa2	Se2	352.26bc	58.49a-d	25.24fgh	1.65a	46.55a-e	58.49a-d	1430.40 a-d
	Sa3	Se1	260.87de	65.54a	26.30efg	0.41jk	43.25efg	65.54a	1326.68 cde
	Sa3	Se2	229.60e	64.58a	30.24cd	1.30b	41.70fg	64.58a	1483.03 a-d

Table 6. Mean comparisons of three-way interactions between the measured traits

Means followed by the same letters in each column are not significantly different (Duncan multiple range test 5%)

Sa1: Seed priming with distilled water, Sa2: Seed priming with 0.5 mM SA, Sa3: Seed priming with 0.5 mM SA combined with spraying of 1 mM SA, Se1: Without selenium, Se2: With selenium

The results showed that the grain Se concentration decreased by 7% in the second year when compared with the first year. Foliar application of Se significantly enhanced the grain Se concentration in wheat cultivars (Table 4).

Seed priming with SA in combination with a foliar application increased grain Se concentration in Azar cultivar by 6.9% when compared with the control. The maximum grain Se concentration obtained from Rasad and Azar 2 cultivars after applying seed priming with SA and seed priming with SA in combination with a foliar application, respectively. The foliar application of Se in Sardari, Azar 2, and Rasad cultivars increased grain Se concentration by 40.6, 36, and 61.7%, respectively. The foliar application of Se in different levels of SA treatments increased the grain Se concentration (Table 5).

The cultivars effect, year  $\times$  cultivar, year  $\times$  salicylic acid, cultivar  $\times$  salicylic acid, year  $\times$  selenium, cultivar  $\times$  selenium, year  $\times$  cultivar  $\times$  selenium, salicylic acid  $\times$ selenium, year  $\times$  cultivar  $\times$  salicylic acid  $\times$  selenium (P=0.01) and year  $\times$  salicylic acid  $\times$  selenium (P=0.05) were significant on wet gluten content (Table 3).The grain gluten content increased by 5.5% in the second year when compared with the first year (Table 4). The addition of SA increased the grain gluten content in Sardari cultivar, but decreased it in Azar 2 and Rasad cultivars. The highest grain gluten content was obtained from seed priming and seed priming in combination with a foliar application of SA in Sardari cultivar (Table 5). The addition of SA as seed priming in combination with a foliar application of Se increased the grain gluten content in Sardari and Rasad cultivars, but decreased the grain gluten content in Azar 2 cultivar (Table 6).

The simple cultivars effect and two-way effect year× cultivars, year × selenium and interaction effect of year  $\times$  cultivar  $\times$  salicylic acid  $\times$  selenium were significant on the productivity index (Table 3). The results showed that the productivity index decreased by 9.5% in the second year when compared with the first year (Table 4). The highest productivity index was observed in Azar 2 cultivar. Azar 2 produced the maximum grain yield (1585.01 kg/ha), which was 10.4% more than Rasad cultivar and 14.2% more than Sardari cultivar (Table 4). According to the combined mean comparison results, the productivity index of Rasad cultivar in the first year was higher when compared with Sardari and Azar 2 cultivars; however, in the second year, the productivity index of Azar 2 cultivar was higher when compared with Sardari and Rasad cultivars. The highest productivity index and grain yield were observed in Azar 2 cultivar after the application of seed priming and seed priming in combination with a foliar application of SA. The foliar application of Se increased the productivity index of Sardari and Rasad cultivars (Table 5).

The cultivars effect, year × salicylic acid, year × selenium (P=0.01) and cultivar  $\times$  salicylic acid and year  $\times$  cultivar  $\times$  selenium (P=0.05) were significant on grain yield (Table 3). The grain yield increased by 14% in the second year when compared with the first year. The grain yield in Sardari, Azar 2, and Rasad increased by 17, 7, and 18.5%, respectively, in the second year when compared with the first year (Table 4). Maximum grain yields of 1632 kg ha<sup>-1</sup> without Se and 1538 kg ha<sup>-1</sup> with Se was observed in Azar 2 cultivar (Table 5). Maximum grain yield of 1516.40 kg ha<sup>-1</sup> was obtained from seed priming with SA and without Se. The maximum grain yield of 1674 kg ha<sup>-1</sup> was obtained from Azar 2 after seed priming with SA and without Se. The highest grain yields for Sardari and Azar 2 cultivars were also obtained from seed priming with SA and without Se treatment. The foliar application of Se together with and without SA increased the grain yield of Sardari cultivar (Table 6). The maximum grain yield for Rasad cultivar was obtained from the foliar application of Se and without SA. Foliar application of selenium alone increased grain yield by 6% when compared with the control. Seed priming in combination with the foliar application of SA together with foliar application of Se increased the grain yield by 4% when compared with the control (Table 6).

According to the combined mean comparison results, the highest decrease of electrolyte leakage with the use of SA was observed in Azar 2 cultivar. Se treatment significantly reduced electrolyte leakage in wheat under drought stress. The regulating effects of Se on reactive oxygen species (ROS) concentrations have been proposed as a key mechanism to counteract symptoms of various a biotic stresses (Feng et al., 2013). The reduction in electrolyte leakage showed the decline of lipid per oxidation. It seems that Se plays an important role in increasing self-protective mechanisms of wheat against drought stress. Khan et al. (2010) reported that the SA treatment alleviated the effects of salinity stress in mango beans via the enhanced lipid per oxidation and electrolyte leakage as well as the induced ant oxidative system.

The addition of SA as seed priming in combination with the foliar application increased the relative water content (Table 3). Singh and Usha (2003) reported that the application of SA (1-3mM) under optimum and stress conditions increased the relative water content in wheat seedlings. Vahdati Mashhadian et al. (2012) reported that SA treatments increased the leaf relative water content and petal water content (%) of cut chrysanthemum flowers by 49 and 73% as compared to the controls, respectively.

Se and/or SA addition significantly increased the Proline content in the leaves of the wheat cultivars. Increase in Proline contents could induce plants in improving stress tolerance. Proline acts multiple roles and may be more crucial in counteracting stress than in playing as a simple compatible osmolyte (Ramachandra Redeve et al., 2004). Proline, soluble sugars contents and antioxidant enzymes activity are part of the defense mechanisms which could induce water deficit stress tolerance to wheat cultivars (Valifard et al., 2012). Treatments with 1.0 and 2.0 mg Se/kg significantly increased Proline content and reduced malondialdehyde content of wheat seedlings (Yao et al., 2009). Tamaoki et al. (2008) reported that, selenium can affect plant growth by influencing the production of jasmonic acid and stress hormone ethylene and proteins. Sajedi and Mashhadi Akbar Boujar (2013) reported that a foliar application treatment with SA caused increases in superoxide dismutase, catalase and glutathione peroxidase enzymes in three wheat cultivars. SA can play an important role in plant tolerance to environment stresses by ethylene biosynthesis, stomatal closure, and respiration. SA could increase the tolerance to salt of wheat seedlings (Dolatabadian et al., 2008). Oraghi Ardebili et al. (2014) reported that the use of Se and/or SA led to the improvement of proline content in soybean when compared to hydro priming.

The results showed that SA seed priming in combination with foliar application, along with spray of Se increased the grain Se concentration in the three studied wheat cultivars. In addition, the rates of 10 and 20 g Se/ha, highly significantly increased Se accumulation in winter wheat grain in three years (Ducsay and Lozek, 2006). Both foliar and soil Se applications significantly improved the Se content in maize grain. Se content in maize grain is found to be linearly correlated with Se application rates (Wang et al., 2013). As a result of pre-seeding treatment of wheat seeds by sodium selenite, the concentration of Se in grain raised from 3 to 6 times, and at foliar treatment of plants from 4 to 8 times when compared with the control options (Kashin and Shubina, 2011).

The results showed that grain yield in the three studied cultivars increased in the second year. It seems that the cause of increase in yield in the second year could be better climate conditions, especially greater rainfall during the stages of stem elongation and double ridge formation (Table 1). Although SA and Se did not significantly affect grain yield, SA and Se treatments enhanced grain yield relative to the control treatment. Lyons et al. (2009) reported that although there was no change in total biomass, Se treatment was associated with a 43% increase in seed production in Brassica plants. Sajedi et al. (2011) reported that the application of Se or micronutrients under stress conditions significantly enhanced the antioxidant activity as well as grain yield in corn.

The highest productivity index and grain yield were observed in Azar 2 cultivar. Therefore, this cultivar had more stability in grain yield during the two years of the study (Table 4).The maximum productivity index and grain yield were obtained in Azar 2 cultivar after seed priming and seed priming in combination with a foliar application of SA. It seems that the increase of grain yield in Azar 2 with the application of SA was due to increases in the productivity index. Jiriaie and Sajedi (2012) reported that the application of SA increased the grain yield by 9.4% when compared with the control in the wheat Shahriar cultivar. Pirasteh-Anosheh et al. (2015) reported that SA foliar application at 2.0mM under non-saline and at 1.41 mM under saline conditions could be considered as the best concentrations for improving barley performance.

### CONCLUSIONS

Conclusively, SA treatment in combination with Se reduced the damage caused by unfavorable conditions in rain-fed wheat cultivars by increasing proline content and reducing electrolyte leakage. The addition of Se or SA decreased electrolyte leakage in the three cultivars and hence enhanced grain yield. It can be concluded that

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the application of SA as seed priming together with the foliar application of Se can help alleviate oxidative damage and thus improve the tolerance of environment stresses. In addition, the rates of 20 g ha<sup>-1</sup> selenium, highly significantly increased Se accumulation in grain in the two experimental years. Foliar Se applications can reliably and effectively increase the grain Se concentration in the three studied wheat cultivars.

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دانتگاه شسراز

تحقیقات کشاورزی ایران (۱۳۹۶) ۳۶(۲) ۹۱–۱۰۰

بررسی تاثیر سلنیوم و سالیسیلیک اسید بر خصوصیات فیزیولوژیکی و کیفیت دانه در ارقام گندم دیم

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> **واژه های کلیدی:** نشت یونی عملکرد دانه محتوی پرولین برگ محتوی گلوتن مرطوب

**چکیدہ** - این آزمایش به صورت فاکتوریل در قالب طرح بلوکھای کامل تصادفی در سه تکرار در سالهای ۱۳۹۰و ۱۳۹۱ در مزرعه تحقیقاتی دانشگاه آزاد اسلامی واحد اراک اجرا شد. عوامل مورد آزمایش شامل سالیسیلیک اسید در سه سطح شامل پیش تیمار بذر با آب مقطر، پیش تیمار بذر با ۰/۵ میلی مولار سالیسیلیک اسید، پیش تیمار بذر با۰/۵ میلی مولار سالیسیلیک اسید توام با محلول پاشی ۱ میلی مولار سالیسیلیک اسید، سلنیوم در دو سطح، بدون مصرف و محلول پاشی ۲۰ گرم در هکتار و سه رقم گندم شامل آذر ۲، سرداری و رصد بودند. نتایج نشان داد که پیش تیمار بذر توام با محلول پاشی با سالیسیلیک اسید محتوای آب نسبی برگ در ارقام آذر ۲ و رصد را نسبت به شاهد افزایش داد. محلول پاشی با سلنیوم مقدار پرولین در برگ و غلظت سلنیوم در دانه را در ارقام گندم افزایش داد. پیش تیمار بذر توام با با سالیسیلیک اسید توام با محلول پاشی با سلنیوم میزان نشت یونی را ۳۲ درصد نسبت به شاهد كاهش داد. محلول پاشی سالیسیلیک اسید توام با سلنیوم مقدار پرولین برگ و غلظت سلنيوم دانه را افزايش داد. پيش تيمار با ساليسيليک اسيد توام با محلول پاشي سلنيوم مقدار گلوتن دانه را در ارقام رصد و سرداری افزایش داد. بیشترین شاخص بازآوری (۶۳/۱۲٪) و عملکرد دانه (۱۵۸۵/۰۱ کیلوگرم در هکتار) از رقم آذر ۲ حاصل شد. نتایج نشان داد که كاربرد سالیسیلیک اسید و سلنیوم می تواند خسارت ناشی از تنش خشكی را تعدیل نماید و تحمل به تنش خشکی و کیفیت دانه در گندم را بهبود دهد.