



## Comparison of physiological and biochemical responses of wheat and barley to Selenium by spraying application under rain fed conditions

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### Abstract

This study investigated the effect of two forms of Se at three rates (0, 18, and 36 g/ha) on wheat and barley under rain fed conditions. Results showed that spraying application of 18 g/ha Se increased Chl *a* and Chl *a+b* contents while the selenium rate 36 g/ha decreased plant pigments. Evaluations of Chl *a*, Chl *b*, chlorophyll total *a+b*, carotenoids, chlorophyll total content, relative water content, and grain yield were higher in barley than in wheat. Foliar applications of selenate and selenite increased relative water content and decreased electrolyte leakage and MDA content in both plants but applying only Se increased catalase activity. The effect of sodium selenate on catalase activity was more than that of sodium selenite in both barley and wheat. Treatments had no significant effect on the glutathione content. In the plants treated with foliar application of 18 g/ha Se, grain yield increased by 6.3% compared with the control. It was concluded that foliar application of 18 g/ha sodium selenate or sodium selenite presents a good antioxidant chemical for improved physiological and biochemical characteristics for both wheat and barley grain yield under semi-arid or arid conditions.

**Keywords:** biochemical traits, plant pigments, rain fed, selenium, spraying application

**Abbreviations:** Catalase: CAT; Chlorophyll: Chl; Glutathione: GSH, Malondialdehyde: MDA; Selenium: Se; Thiobarbituric acid: TBA

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### Introduction

Drought is an abiotic factor that affects crop production. Plant response to water stress is dependent on the intensity and duration of the stress and the plant growth stage (Reddy et al., 2004). Drought stress disrupts plant physiological processes and also changes carbohydrate and nitrogen metabolism; it also induces changes in

protein structure and enzyme activity (Hong-Bo et al., 2009). Plants have complex protective mechanisms to prevent damage from free radicals; such mechanisms include enzyme activity of catalase, peroxidase, and super oxide dismutase as well as non-enzyme compounds such as ascorbic acid, tocopherol, glutathione, and carotenoids (Foyer et al., 2000). In some cereals there is a close relationship between antioxidant activity and stress tolerance (Zhang et al., 2004).

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Selenium is a main component of selenoproteins. An important function of selenium includes antioxidant protection, energy metabolism and redox reaction adjustment during gene expression and transcription (Kong et al., 2005). At low concentration it is a beneficial element for plants as an antioxidant and a growth-promoting agent (Garcia-Banuelos et al., 2011). There is evidence that Se has a protective role in exogenous drought stress in plants and it is possible that Se increases plant tolerance to various abiotic stresses (Hasanuzzaman et al., 2012). Protective effect of Se against oxidative stress is higher in plants due to the increased glutathione peroxidase activity and decreased lipid peroxidation (Djanaguiraman et al., 2005). It has been reported that selenite or selenate at low concentrations had no significant effect on concentration of photosynthetic pigments but at higher concentrations, they disrupted accumulations of chlorophyll *a* and chlorophyll *b* (Hawrylak-Nowak et al., 2015). Yao et al. (2011) reports that at Se-treated wheat plants had enhanced chlorophyll *a* and chlorophyll *a+b* contents. Saffaryazdi et al. (2012) reported that application of sodium selenite at low concentration increased Chl *a* and Chl *a+b* and total chlorophyll contents in spinach, but at higher concentrations it decreased chlorophyll *a* and Chl*b* contents compared to the control. Habibi (2013) found that application of Se, due to increasing enzyme activity of catalase and glutathione peroxidase had no effect on MAD and peroxide lipid content in barley plants.

In Iran, more than 6 million hectares are under wheat cultivation some 61% of which being under rain fed condition. The current paper

reports on an investigation into the influence of selenium on physiological and biochemical characteristics of wheat and barley.

## Material and Methods

### Experimental location

This experiment was performed at the Research Station of Islamic Azad University, Arak Branch, Iran during the growing season of 2014–2015. The location was in Markazi Province, central Iran at the geographical location of 34° 30' N, 40° 41' E and altitude of 1779 m above sea level. According to metrological data from Arak, Iran, the region had a semi-arid climate (Table 1).

### Experimental design, plant material, and growth conditions

The experiment was designed as a factorial, based on a randomized complete block design in three replicates. Treatments comprised two forms of selenium (sodium selenate or sodium selenite) at various concentrations (0, 18 and 36 g/ha) on wheat (Azar 2 cultivar) and barley (Abidar cultivar). Certified, healthy seeds of wheat and barley were obtained from the Agriculture Jihad of Aligudarz city. The seeds were sown by hand, spaced 15 cm apart and in 6-m rows, with a 1m border between the plots. The soil condition was poor according to selenium (Se = 0.29 ppm in 2011 and 2012, ND in 2014) content as determined by the results of soil experiments that had been done three years ago. Fertilizer 115.7 kg/ha P was applied before the seedling stage and mixed with the soil, and an application of 46.2

Table 1.  
Metrological data in Arak, Iran, during the growing season of 2014–2015

Months	Mean of temperature (°c)	Precipitation (mm)	Evaporation (mm)	Relatively humidity (%)
October	18	47	194.8	39.5
November	8.3	24.9	38.1	60
December	5.5	13.4	-	66
January	3.6	8.9	-	60.9
February	7.3	18.1	-	48.5
March	5.9	41.8	-	55
April	12.2	68.2	135.6	48
May	18.4	9.8	247.6	33
June	24.8	4.5	331.5	24

kg/ha of N at tillering and at stem elongation as a top-dressing. Foliar application of Se was applied at the start of stem extension (ZGS 35) and heading emergence at (ZGS 57) 18 pm.

### Determination of physiological characteristics

Measurements were taken for physiological and biochemical characteristics of wheat and barley at heading (ZGS 59); five fully developed flag leaves were harvested at 12: 00 h at heading and complete emergence stages.

Measurement of relative water content was determined from fifteen disks (3 cm in diameter) from leaves that were prepared immediately and weighed to measure fresh leaf weight in the laboratory at 25 °C. Then disks were placed in distilled water for about 24 h until completely saturated. At the end of this stage, leaf disks were dried with dry paper towels and reweighed. 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):

$$RWC(\%) = \frac{W_f - W_d}{W_s - W_d} \times 100 \quad (\text{Equation 1})$$

RW C= relative water content,  $W_f$  = fresh leaf weight,  $W_d$  = dry leaf weight, and  $W_s$  = saturated leaf weight.

To calculate cell membrane stability, five fully developed flag leaves were harvested and fifteen disks were prepared from lamina. Then, the drive test tube containing 10 cc manitol solution of -2 bar osmotic potential was moved. After 24 h, electrical conductivity of each tube was measured at the temperature of 25 °C by an electrical conduction device. The manitol solution with -2 bar osmotic potential was calculated from the Van't Hoff equation (Martinez et al., 2004).

$$\psi_s = -CmIRT \quad (\text{Equation II})$$

$\psi_s$  = osmotic potential solution (MPa),  $C_m$  = molarity of solution (g/L),  $I$  = ionization coefficient (1 for manitol),  $R$  = gas constant equal 0.083 (Mpa/mol. K), and  $T$  = temperature (K).

Evaluations of photosynthetic pigments were calculated by the method presented in Arnon (1949). Samples collected from five fully developed flag leaves and 0.5 g leaves were ground in 80% acetone for determination and absorbance of the resulting extracts, recorded at 480, 510, , and 663 nm with a spectrophotometer (PerkinElmer, Lambda 25).

### Determination of biochemical charachtristises

For measuring CAT activity, GSH, and MAD contents of wheat and barley, fifteen fully developed flag leaves were harvested at 12:00 h at heading complete mergence (ZGS 59), then washed, frozen, and stored in liquid  $N_2$  at -80 °C prior to the analysis. Determinations of protein content were made for each sample according to the method cited in Bradford (1976) using BSA as standard.

Catalase activity was determined based on the method of Aebi (1984). Accordingly, 10  $\mu$ l texture extract was added to a cell containing 1.995  $\mu$ l  $H_2O_2$  and mixed. Destruction rate of  $H_2O_2$ , in terms of time taken was assessed by spectrophotometerat 240 nm.

The assay for glutathione was determined according to the method described by Ellman (1959). Samples were homogenized in a phosphate buffer (pH 7.4). 30  $\mu$ l from samples and 50  $\mu$ l phosphate buffer (pH 7.4, 0.1 M) were added to each hole plate and then 40  $\mu$ l stock DTNB (2 mg/ml) was added. Absorbance was measured by reading the plate apparatus at 412 nm.

The degree of lipid peroxidation in leaf tissue was assessed by MAD. Malondialdehyde content was determined by the double heating method. Based on this method, the measure of spectrophotometry violet-pink paint was obtained from the TBA with MAD reaction. 80  $\mu$ l texture extract was mixed with 100  $\mu$ l TCA10% (w/v). The mixture was placed in a water bath at 100 °C for 15 min and then cooled until room temperature and centrifuged at 3000 rpm at 4 °C for 10 min. The supernatant was then transferred to Eppendorf tubes containing 150  $\mu$ l TCA 10% (w/v). The Eppendorf tubes were placed in a water bath at 100 °C for 15 min until appearance of a violet-pink

Table 2

The effects of selenium forms and rates on photosynthetic pigments content in wheat and barley

Treatments	Chl a (mg/g fw)	Chl b (mg/g fw)	Chl a+b (mg/g fw)	Chl a/b (mg/g fw)	Carotenoid (mg/g fw)	Total Chl (mg/g fw)
Selenium rates						
without	b 1.19	0.56a	1.75b	2.18a	0.50a	1.83a
18g/ha	a 1.31	0.60a	1.92a	2.19a	0.52a	1.97a
36g/ha	b 1.19	0.55a	1.75b	2.22a	0.50a	1.92a
Plant						
wheat	1.17b	0.54b	1.77b	2.21a	0.47b	1.75b
barlay	1.29a	0.61a	1.91a	2.18a	0.54a	2.06a

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

Table 3

The effects of selenium forms and rates on physiological, biochemical traits and grain yield in wheat and barley

Treatments	Electrolyte leakage (%)	Relative water content (%)	Leaf catalase (Unit/g protein)	GSH ( $\mu$ mol/g protein)	MAD ( $\mu$ mol/g protein)	Grain yield (kg/ha)
Selenium sources						
selenate	a 492.12	58.39a	0.132a	233.86a	26.04a	1691.91a
selenite	b 457.31	41.52a	0.116b	232.78a	25.51a	1778.59a
Selenium rates						
without	527.27a	35.43b	0.106b	238.10a	26.45a	a 1776.31
18g/ha	452.88b	44.20a	0.115b	230.27a	25.62b	a 1889.92
36g/ha	443.99b	42.01a	0.151a	231.59a	25.26b	b 1539.62
Plant						
wheat	476.82a	37.16b	0.141a	230.18a	25.83a	b 1644.36
barlay	472.61a	43.94a	0.107b	236.46a	25.72a	1826.14a

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

colour. After cooling, absorbance was read at 532 nm (Draper and Hadley, 1990).

### Grain yield

At the final harvest, 1 m<sup>2</sup> was harvested from the middle of each plot and grain yield was evaluated.

### Statistical Analysis

Data were subjected to analysis of variance using SAS. Means were compared using the Duncan's multiple range test at the confidence level of  $p \leq 0.05$ .

### Results

Results showed that foliar application of 18 g/ha Se increased the Chl *a* content from 1.19 to 1.31 mg/g fw. The highest Chl (*a* + *b*) content

(1.92 mg/g fw) was obtained from the foliar application 18 g/ha Se. The foliar application of 18 g/ha Se increased Chl (*a* + *b*) content by 9.7% compared to the control. Evaluations of Chl *a*, Chl *b*, Chl (*a* + *b*), carotenoid, and total Chl contents for barely were 10, 12.9, 11.69, 14.8, and 17.7% more than those for wheat, respectively (Table 2). The highest Chl *a* content 1.29 mg/g fw in wheat and 1.45 mg/g fw in barley were obtained from foliar application of 18 g/ha sodium selenate. The foliar application of 18 g/ha sodium selenate increased the Chl *a* content by 16.2 and 11.5% in wheat and barley respectively, compared to the control. The foliar application of 18 g/ha sodium selenite increased the Chl *a* content in wheat by 17% compared to the control (Table 5). Foliar applications of 18 and 36 g/ha Se increased relative water content by 24.75 and 18.57%, respectively compared with the control. The relative water content in barley was 18.24% more than that in wheat (Table 3).

Table 4

The effects of selenium forms and rates on physiological, biochemical traits and grain yield in wheat and barley

Treatments		Electrolyte leakage (%)	Relative water content (%)	Leaf catalase (Unit/g protein)	GSH ( $\mu\text{mol/g}$ protein)	MAD ( $\mu\text{mol/g}$ protein)	Grain yield (kg/ha)
Selenium sources	Selenium rates						
	without	542.20a	35.16b	0.115c	240.44a	26.78a	a 1758.46
selenate	18g/ha	461.25bcd	41.82a	0.118c	230.17a	26.07ab	a 1908.01
	36g/ha	512.33ab	41.78a	0.165a	230.98a	25.27b	b 1409.24
	without	444.52cd	35.71b	0.098d	235.76a	26.12ab	a 1794.05
selenite	18g/ha	472.92cd	46.59a	0.111c	230.36a	25.17b	a 1871.75
	36g/ha	415.07d	42.25a	0.138b	232.20a	25.24b	ab1670.01
Selenium sources	Plant						
	Wheat	460.36bc	35.53b	0.143a	234.07a	26.00a	a 1594.761
selenate	Barley	523.89a	43.64a	0.122b	233.66a	26.08a	a 1789.07
	Wheat	493.29ab	38.80b	0.138a	226.30a	25.66a	a 1693.88
selenite	Barley	421.32c	44.24a	0.93c	239.25a	25.36a	a 1863.3
Plant	Selenium rates						
	without	499.70b	34.77c	0.125b	238.35a	26.53a	bc1679.66
Wheat	18g/ha	479.35bc	37.03c	0.118b	229.52a	25.80ab	ab1872.85
	36g/ha	451.42bc	39.68bc	0.180a	226.69a	25.16b	bc1740.23
	without	554.83a	36.10c	0.088c	241.86a	26.37ab	a 2039.55
Barley	18g/ha	426.42c	51.38a	0.111b	231.01a	25.44ab	c 1513.21
	36g/ha	436.57c	44.35b	0.123b	236.50a	25.35ab	bc1566.34

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

Results showed that the increase in relative water content in plants treated with sodium selenate or selenite was significant (Table 4). Results also showed that foliar applications of 18 and 36 g/ha sodium selenate or selenite increased relative water content in both wheat and barley (Table 5).

The effect of rate and form of Se on electrolyte leakage was significant. Sodium selenite decreased electrolyte leakage by 7.6% compared to selenate sodium. Foliar application of 18 and 36 g/ha Se decreased electrolyte leakage by 16.4 and 18.7%, respectively compared to the control (Table 3). The effect of two-way interaction of treatments showed that the foliar application of Se decreased electrolyte leakage in wheat and barley (Table 4). Results showed that foliar applications of 18 and 36 g/ha sodium selenate or selenite decreased electrolyte leakage contents in both wheat and barley (Table 5).

Catalase activity showed a significant increase in leaf tissue in Se-treated plants. The effect of foliar application of sodium selenate

on catalase activity was more than that of sodium selenite. The foliar applications of 18 and 36 g/ha Se increased catalase activity by 8.4 and 42.4%, respectively compared with the control. Catalase activity in wheat leaf tissue was 31.7% more than that in barley (Table 3). Foliar application of 18 and 36 g/ha sodium selenate or selenite increased catalase activity by 2.6, 43.4, 13.2, and 40.8%, respectively compared to the control (Table 4). Results showed that foliar applications of 36 g/ha sodium selenate and selenite increased catalase activity both in wheat and barley (Table 5). Effect of treatments on glutathione was not significant (Tables 3 and 4).

Malondialdehyde content decreased under foliar spray applications of Se in both wheat and barley. The foliar spray applications of 18 and 36 g/ha Se decreased MAD content by 3.2 and 4.7, respectively compared with the control (Table 3). The Se additions of 18 and 36 g/ha sodium selenate or selenite decreased MAD content in both wheat and barley (Table 4).

Results showed that foliar application of 18 g/ha Se increased grain yield by 6.3% compared to

Table 5  
Effects of Selenium forms and rates on physiological, biochemical traits, and grain yield in wheat and barley

Plant	Selenium sources	Selenium rates	Chlorophylla )mg/g fw(	Electrolyte leakage (%)	Relative water content (%)	Leaf catalase (Unit/g protein)	Grain yield (kg/ha)
Wheat	selenate	without	1.11cd	470.40bcd	33.51d	0.116cd	1611.06ab
		18g/ha	1.29b	486.17bcd	36.62cd	0.110de	ab1757.45
		36g/ha	1.19bcd	424.50de	36.45cd	0.203a	b 1415.83
	selenite	without	1.05d	529.00b	36.03cd	0.133c	ab1748.26
		18g/ha	1.23bc	472.53bcd	37.45cd	0.126cd	ab1722.9
		36g/ha	1.17bcd	478.33bcd	42.92bc	0.156b	ab1610.60
Barley	selenate	without	1.30b	614.00a	36.80cd	0.113cde	1905.86ab
		18g/ha	1.45a	436.33dc	47.02b	0.126cd	a 2058.56
		36g/ha	1.08cd	512.33bc	47.12b	dc 0.126	1402.66b
	selenite	without	1.30b	495.67 bcd	35.40cd	0.063f	ab1839.83
		18g/ha	1.28b	416.50de	55.74a	0.096e	a 2020.56
		36g/ha	1.31b	351.80e	42.92bc	0.120cd	ab1729.37

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

the control, but foliar spray of 36 g/ha Se decreased grain yield compared to the control. Grain yield in barley was 11% more than that in wheat (Table 3). The effect of sodium selenate on grain yield was more than that of sodium selenite. Foliar application of 18 g/ha sodium selenate or selenite to the treated plants increased grain yield by 10.7 and 4.3%, respectively (Table 4). Moreover, foliar application of 18 g/ha sodium selenate on wheat and 18 g/ha sodium selenate or selenite on barley increased grain yield which was not significant (Table 5).

## Discussion

Selenium is beneficial for some plants and could increase plant tolerance to environmental stress (Djanaguiraman et al., 2005). Several studies have reported that Se application had a positive effect on plant pigments. In the present study, Se significantly improved Chl *a* and Chl (*a* + *b*) contents in wheat and barley under drought condition. Increased Chl *a* and Chl *b* contents may have been due to the protective effect of Se on chloroplast enzymes and biosynthesis of photosynthetic pigments (Pennanen et al., 2002). Results of a pot experiment demonstrated that addition of Se significantly enhanced Chl *a* and Chl (*a* + *b*) contents in wheat seedlings treated

with Se under drought stress. Oraghi Ardebili et al. (2014) reports that Se had a significant effect on Chl *a*, carotenoid, and Chl *a/b* contents in treated plants. The study demonstrated that application of Se restored the apparatus of photosynthesis, which could have been caused by enhanced nutrition and/or antioxidant system activity. In this study, photosynthetic pigment contents in barley were more than those in wheat. The increased total Chl in barley from Se-treated plants resulted in a rise in Chl *a*, Chl *b*, and carotenoid contents. Li et al. (2010) reports a protective effect of carotenoids, xanthophyll cycle dependent energy dissipation against photoinhibition.

In this study, Se-treated plants showed higher relative water content under drought stress condition compared to the control, an indication that application of sodium selenate or selenite improved water status of the stressed plants. Ajiboso et al. (2012) reports that Se-application to cowpea and maize resulted in the increased relative water content under both normal and water deficit stress.

Selenium application could have a beneficial effect on growth and stress tolerance of plants by increasing antioxidant activity (Rios et al., 2009), and thereby reducing overproduction of reactive oxygen species (Feng et al., 2013). In this

study, results showed a significant increase in CAT activity in plants supplemented with Se, in both forms, sodium selenate or selenite. This finding is consistent with results reported in Yao et al. (2009). Optimal Se supply significantly increased activity of peroxidase and catalase enzymes in wheat seedlings during drought conditions. It was reported that foliar application of Se increased activity of catalase and glutathione peroxidase in cowpea and maize under both normal and drought stress conditions (Ajiboso et al., 2012).

Foliar application of Se decreased the MAD content and electrolyte leakage in wheat and barley under drought stress. MAD is an index for determining level of lipid peroxidation (Varshney and Kale, 1990) and membrane integrity (Khan and Naqvi, 2013). The results of this study indicate that Se could reduce oxidative stress caused by drought in wheat and barley and alleviate damage to cell membrane under stress conditions. It was reported that selenium decreased MAD content in wheat under drought stress (He et al., 1995). Habibi (2013) reported that application of 30 g/ha Se decreased MAD content in spring barley under drought stress. This result indicates that selenium had antioxidant properties. In our previous experiment, Se application increased activity of CAT, glutathione peroxidase, and proline content in rain-fed wheat cultivars compared with the control while it significantly decreased MDA content (Sajedi and Boojar, 2013).

In the present study, foliar application of 18 g/ha sodium selenate or selenite increased grain yield while addition of 36 g/ha Se dramatically reduced grain yield compared to the control. It was reported that foliar application of 10 g/ha Se had an insignificant increase on grain yield but that Se addition at the rate of 40 g/ha decreased grain yield in the year 2011-2012 (Rodrigo et al., 2013). In our previous study, seed pre-treatment with 1 and 2 mg/L Se along with foliar application of 18 g/ha Se in wheat increased grain yield by 16.7 and 19% under rainfed conditions compared to the control (Sajedi, 2015). It was reported that increasing of Se to 50 g/ha increased grain yield in rice. The study demonstrates that addition of Se increased rate of photosynthesis, intercellular CO<sub>2</sub> concentration, and transpiration efficiency which in turn enhanced photosynthesis and increased grain yield (Zhang et al., 2014).

## Conclusion

It is concluded that foliar application of 18 g/ha sodium selenate or selenite both in wheat and barley increased photosynthetic pigments, the relative water content, CAT enzyme activity, while it decreased electrolyte leakage and MAD content and therefore protection of photosynthesis apparatus improved grain yield under rain-fed condition. The effect of sodium selenate was more effective than that of sodium selenite. Accordingly, the foliar application of 18 g/ha sodium selenate is recommended at the start of stem extension and heading emergence in barley and especially wheat in semi-arid or arid regions.

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