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Effect of Drought Stress and Selenium Spraying on Superoxide Dismotase Activity of Winter Rapeseed (*Brassica napus* L.) Cultivars

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Abstract**—**In the other to Study of drought stress and Selenium spraying effect on superoxide dismotase (SOD) activity of rapeseed **(***Brassica napus* L.) cultivars in Shahr-e-Rey region, an experiment carried out in Split factorial design in the basis of randomized complete blocks with 4 replications in 2006. Irrigation in two levels: Normal irrigation and irrigation with drought stress when the soil electrical conductivity reached to 60 as main factor and rapeseed cultivars in 3 levels Zarfam, Okapi, Opera and selenium spraying at the beginning of flowering stage in 3 levels: 0, 16 and 21 g/ha as sub factor.

The results showed that the simple and interaction effect of irrigation, selenium and cultivars on SOD activity had significant difference. In this case Zarfam cultivar with 2010 $u.mg^{-1}$ protein and Opera with 1454 $u.mg^{-1}$ protein produced maximum and minimum amounts of SOD activitiy. Interaction effect of irrigation and variety showed that, normal irrigation in Opera with 1115 $u.mg^{-1}$ protein and drought stress in Zarfam with 2784 $u.mg^{-1}$ protein conducted to and minimum and maximum amounts of SOD activity.

Interaction effect of irrigation, cultivar and selenium on SOD indicated that drought stress condition and 21 gr/ha selenium spraying in Zarfam variety with 3146 $u.mg^{-1}$ protein gained to highest activities of SOD.

Keywords**—**Drought stress, Rapeseed, Selenium, Superoxide dismutase.

I. INTRODUCTION

THE AOS such as O_2, H_2O_2 and OH radicals, can directly attack membrane lipids, inactive metabolic enzymes and damage the nucleic acids leading to cell death [12]. Being toxic for cells, AOS are efficiently eliminated by nonenzymatic (α-tocopherol, β -carotene, phenolic compounds, ascorbate, glutathione) and enzymatic antioxidants [10]-[17]. The enzymatic antioxidant system is one of the protective mechanisms including superoxide dismutase (SOD: EC 1.15.1.1), which can be found in various cell compartments and it catalyses the isproportion of two O_2 radicals to H_2O_2 and O_2 [5]-[14].

In the conditions of drought stress and because of that the aperture are close for preventing from the transpiration it would have concluded the reduction in the CO_2's absorption and would have the less producing the amount of dry substance and it would use the food elements with the less efficiency. This counter effect for the amount of humidity and nutriment elements are bilateral[1]-[9].

Plants have developed the scavenging mechanism of ROS categorized as enzymatic and non- nzymatic[2]-[16]. When ROS increases, chain reactions start, in which Superoxide Dismutase (SOD) catalyzes the dismutation of O_2^{-} radicals to molecular O_2 and H_2O_2. The H_2O_2 is then detoxified in the ascorbate-glutathione cycle [12]**.**

Selenium is an essential trace element for animals and humans [20] but its role in plants is still unclear [4]. Most cereal crops and fodder plant are relatively weakly able to absorb selenium, even when grown on soils with higher selenium content. Selenium is chemically similar to sulphur, this may cause a non-specific replacement of S by selenium in proteins and other sulphur compounds [11]. There are indications that it can also play a positive biological role in higher plants [3]. Selenium could increase the tolerance of plants to UV-radiation induced oxidative stress, delay senescence, and promote the growth of ageing seedlings [22]. The Results showed that plant growth promoted by selenium is due to the increased starch accumulation in chloroplasts[13]. Recently it has been shown that selenium can regulate the water status of plants under conditions of water deficiency and thereby performs its protective effect [7].

Se also decreased the activity of superoxide dismutase (SOD) and lowered the amount of tocopherols in some cases. It was suggested that while Se promotes H_2O_2 scavenging, by increasing GPX activity, it also enhances spontaneous disproportion of superoxide radicals to H_2O_2 and thus decreases the need for high SOD activity [22]. Se can alleviate oxidative stress in chloroplasts. The responses of potato to Se supplementation were investigated by monitoring chlorophyll

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fluorescence and the transcription of antioxidative enzymes [15].

II. Materials and Methods

In the other to Study of drought stress and Selenium spraying on CAT and SOD activities of rapeseed (*Brassica napus* L.) cultivars in Shahr-e-Rey region, an experiment carried out in Split factorial design in the basis of randomized complete blocks with 4 replications in 2006. Irrigation in two levels: Normal irrigation and irrigation with drought stress when the soil electrical conductivity reached to 60 as main factor and rapeseed cultivars in 3 levels Zarfam, Okapi, Opera and selenium spraying at the beginning of flowering stage in 3 levels : 0 , 16 and 21 g/ha as sub factor.

Seeds planted on 6 rows in each plots, the rows distance was 25 cm and the plant distance on each row was 4.5 cm, it had used 150 kg/ha pure nitrogen fertilizer and 60 kg/ha phosphorus and potassium, for maximum exact, beginning and end of each plots closed, with regarding area of each plots, irrigation intervals and water amounts used in each irrigation, volume of water for each plot determined.

In this research for uniform selenium spraying of each plot at first sprayer machine calibrated with water and sodium selenit treatment (0, 16 and 21 g/ha) sprayed by sodium selenit (Na2O3Se.5H2O) form in early flowering stage.

First and last rows of each plot considered as edge effect and sampling for SOD enzyme activity did from 4 central rows (the area 4 m2 of each plot). SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD was defined as the amount of enzyme activity that was able to inhibit by 50% the photo reduction of NBT to blue Formosan. The SOD activity of the extract was expressed as SOD units per milligram of PROT. Peroxides activity was determined by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm (Hernandez *et al*., 2000). The reaction mixture contained 100 µL crude enzyme, 500 µL H_2O_2 5 mM, 500 µL guaiacol 28 mM and 1900 µL potassium phosphate buffer 60 mM (pH 6.1). POX activity of the extract was expressed as POX units per mg [18].

III. Results and Discussion

Simple effect of irrigation, cultivar and foliar application of Selenium on SOD activity had significant difference at %1 level of probability (Table I). In this case drought stress condition with 2289.9 u.mg^{-1}protein, Zarfam cultivar with 2010 u.mg^{-1}protein and 21 g/ha Se application with 1973.0 u.mg^{-1}protein showed maximum amounts of SOD activity (Table II).

TABLE I
Analysis of Variance for SOD Activity

Factor	M.S	
	Degree of freedom	SOD
Replication	3	80429.389^{ns}
Irrigation	1	18974853.389**
Error	3	9155.759
Cultivar	2	199694.347**
Selenium	2	1237121.347**
cultivar * Irrigation	9	1267009.847**
Selenium * Irrigation	9	459804.514**
Selenium * Cultivar	4	9243.701*
Selenium *Cultivar *Irrigation	4	1952.785 *
Error	48	5754.022
Total	71	-
C.V(%)	-	7.27

ns , * and **:Nonsignificant and significant at %5 and %1 level of probability respectively

TABLE II
Mean Comparison of Irrigation, Variety and Selenium Effect on SOD Activity

Factor	SOD (u.mg^{-1} protein)
Irrigation (I)	
Normal irrigation(I1)	1263.2 b
Drought stress(I2)	2289.9 a
Cultivar (C)	
Zarfam(C_1)	2010 a
Okapi (C_2)	1865 b
Opera (C_3)	1454 c
Selenium(g/ha)	
0 (S0)	1528 c
16 (S1)	1829 b
21 (S2)	1973 a

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test

Interaction effect of Irrigation and cultivar showed drought stress condition in Zarfam with 2784 u.mg^{-1}protein gained to maximum SOD activity and normal irrigation in Opera located at the lowest part of the Average comparison table (Table III). Drought stress may also lead to stomata closure, which reduces CO2 availability in the leaves and inhibits carbon fixation. This exposes the chloroplast to excessive Excitation energy, which in turn could increase the generation of free radicals and induce oxidative stress [6].

TABLE III
MEAN COMPARISON INTERACTION EFFECT OF IRRIGATION AND CULTIVARS EFFECT ON SOD

Factor		SOD ($u.mg^{-1}$ protein)
Irrigation	**Cultivar**	
Normal irrigation	**Zarfam**	**1237 cdef**
	Okapi	**1438 bc**
	Opera	**1115 f**
Drought stress	**Zarfam**	**2784 cde**
	Okapi	**2293 def**
	Opera	**1793 f**

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test

In the case of Irrigation and selenium, Interaction effect of drought stress condition and 21 g/ha selenium application with 2620 $u.mg^{-1}$protein gained to highest SOD activity (Table IV). The enzymatic antioxidant system is one of the protective mechanisms including superoxide dismutase (SOD: EC 1.15.1.1), which can be found in various cell compartments and it catalyses the isproportion of two $O2^{\tilde{}}_{.}$ radicals to H_2O_2 and O2 [5]–[14].

TABLE IV
MEAN COMPARISON INTERACTION EFFECT OF IRRIGATION AND SELENIUM EFFECT ON SOD

Factor		SOD ($u.mg^{-1}$ protein)
Irrigation	**Selenium (g/ ha)**	
Normal irrigation	**0**	**1157 e**
	16	**1307 d**
	21	**1325 d**
Drought stress	**0**	**1899 c**
	16	**2351 b**
	21	**2620 a**

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test

Interaction effect of selenium and cultivar showed Zarfam has high potential for SOD production and increase in selenium spraying to 21 g/ha helps this matter (Table V). Selenium is chemically similar to sulphur, this may cause a non-specific replacement of S by selenium in proteins and other sulphur compounds [11]. There indicates that it can also play a positive biological role in higher plants [3]. Selenium could increase the tolerance of plants to UV-radiation induced oxidative stress, delay senescence, and promote the growth of ageing seedlings [22]. After study on 3 experimental factor observed that drought stress 21 g/ha selenium spraying in Zarfam variety with 3146 $u.mg^{-1}$ protein gained to maximum SOD activity. Plant growth promoted by selenium is due to the increased starch accumulation in chloroplasts [13].

TABLE V
MEAN COMPARISON INTERACTION EFFECT OF VARIETY AND SELENIUM ON SOD

Facor		SOD ($u.mg^{-1}$ protein)
Cultivar	**Selenium (g/ ha)**	
Źarfam	**0**	**1749 d**
	16	**2057 b**
	21	**2224 a**
Okapi	**0**	**1587 ef**
	16	**1940 c**
	21	**2069 b**
Opera	**0**	**1248 g**
	16	**1490 f**
	21	**1625 e**

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test

Interaction effect of irrigation, cultivar and selenium on SOD activity had significant difference at %5 level of probability (Table I). The results showed that drought stress and 21 g/ha foliar application of Se in Zarfam variety with 3146 $u.mg^{-1}$protein gained to highest SOD activity and normal irrigation and non Selenium spraying in Opera cultivar with 1042 $u.mg^{-1}$protein produced lowest SOD activity (Table VI). It seems that in the case of SOD activity in varieties there were positive correlation to selenium uses and 21 g/ha of selenium application was more effective than 16 g/ha. Recently it has been shown that selenium can regulate the water status of plants under conditions of water deficiency and thereby performs its protective effect [7]. Selenium can alleviate oxidative stress in chloroplasts. The responses of plant to selenium supplementation were investigated by monitoring chlorophyll fluorescence and the transcription of antioxidative enzymes [15]. When selenium was applied to Trigonella foenum-graecum seedlings, mitochondrial oxygen uptake increased concomitantly with enhanced mitochondrial SOD (superoxide dismutase) activity [19].

The result shows that selenium as foliar application can improve yield under conditions of drought stress and it can be suggested for these lands in arid and semi arid regions [23].

TABLE VI
MEAN COMPARISON INTERACTION EFFECT OF IRRIGATION, CULTIVARS AND SELENIUM ON SOD

Factor			SOD ($u.mg^{-1}$ protein)
Irrigation	**Variety**	**Selenium g/ ha**	
Normal irrigation	**Zarfam**	**0**	**1124 cdef**
		16	**1283 bc**
		21	**1303 f**
	Okapi	**0**	**1305 ab**
		16	**1487 a**
		21	**1521 ef**
	Opera	**0**	**1042 bcd**
		16	**1153 ab**
		21	**1152 f**
Drought stress	**Zarfam**	**0**	**2375 a**
		16	**2831 cde**
		21	**3146 def**
	Okapi	**0**	**1868 f**
		16	**2393 ab**
		21	**2617 a**
	Opera	**0**	**1458 ef**
		16	**1828 bcd**
		21	**2098 cdef**

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test

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