Short Communication

Effect of zeolite and selenium foliar application on growth, production and some physiological attributes of three canola (*Brassica napus* L.) cultivars subjected to drought stress

Efecto de la zeolite y la aplicación foliar de selenio sobre el crecimiento, producción y algunos caracteres fisiológicos de tres cultivares de canola (*Brassica napus* L.) sujetos a estrés hídrico

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ABSTRACT

In order to study effect of zeolite soil application and selenium foliar application on growth, seed yield and some physiological attributes of three canola cultivar under conditions of drought stress an experiment was conducted in two growing season in 2006 and 2007. Site of study was located in Seed and Plant Improvement Institute, Karaj, Iran. The experimental design was a randomized complete block arranged in factorial split plot with three replications. Irrigation factor was chosen at two levels, included complete irrigation and irrigation holding at stem elongation stage. Zeolite was used at two levels, non application and 10 ton per hectare. Also selenium was sprayed at three concentrations, 0, 15 and 30 g per liter from sodium selenate. These treatments were randomized in main plots while three canola cultivars (Zarfam, Okapi and Sarigol) were randomized in sub plots. According to obtained results Zarfam cultivar showed the lowest electrolyte leakage and the highest biological yield as result of soil zeolite application and 30 g per liter selenium. On the other side the highest chlorophyll content and seed yield was related to Okapi cultivars under the same treatments while Sarigol cultivar was in the next rank.

Key words: Biological yield, canola, drought stress, intracellular electrolyte leakage, seed yield, selenium, zeolite

RESUMEN

Con el fin de estudiar el efecto de la aplicación al suelo de zeolita y la aplicación foliar de selenio sobre el crecimiento, el rendimiento de semillas y algunos caracteres fisiológicos de tres cultivares de canola en condiciones de sequía se llevó a cabo un experimento en dos épocas de crecimiento en 2006 y 2007. El sitio del estudio se encuentra en el Seed and Plant Improvement Institute, Karaj, Irán. El diseño experimental fue de bloques completos al azar dispuestas en un factorial de parcelas divididas con tres repeticiones. El factor de riego fue seleccionado a dos niveles incluido el riego completo y la restricción de riego en la fase de alargamiento del tallo. La zeolita fue utilizada a dos niveles, sin aplicación y la aplicación de 10 t/ha. También, el selenio fue asperjado a tres concentraciones 0, 15 y 30 g/l de selenato de sodio. Estos tratamientos fueron asignados al azar en las parcelas principales, mientras que los tres cultivares de canola (Zarfam, Okapi y Sarigol) fueron asignados aleatoriamente a las subparcelas. De acuerdo a los resultados obtenidos, el cultivar Zarfam mostró el menor lixiviado de electrolitos y el mayor rendimiento biológico como resultado de la aplicación de la zeolita al suelo y 30 g/l de selenio. Por otra parte, el mayor contenido de clorofila y del rendimiento de semillas estuvo relacionado al cultivar Okapi bajo los mismos tratamientos mientras el cultivar Sarigol estuvo en el próximo rango.

Palabras clave: Rendimiento biológico, canola, estrés hídrico, pérdida de electrolitos intracelular, rendimiento de semilla, selenio, Zeolita.

INTRODUCTION

In the arid and semi arid environment of Iran, rainfall and thus soil moisture are the most important factors affecting crop production. Water stress causes some biochemical mad physiological changes in plants (Pattangual and Madore, 1999). Previous studies have shown that water stress significantly decreases biological yield and seed yield especially at flowering stages (Deepak and Wattal, 1995). The most important effect of environmental stresses is cell membrane degradation. This event decreases membrane selective permeability and increases intracellular electrolyte leakage. Amount of electrolyte leakage would be measured as an index for determining stress intensity. On the other hand, chlorophyll content has close and negative correlation with water stress and so chlorophyll measurement can be a useful index to explanation of stress intensity (Shen *et al*., 2008). Moreover membrane lipid peroxidation occurs due to generated reactive oxygen species as result of water stress. Lipid peroxidation decreases cell membrane elective permeability dramatically (Basaga, 1989). Chloroplasts, mitochondria and peroxisomes are intracellular generators of activated oxygen species such as H_2O_2, superoxide and hydroxyl radicals in the plant cells (Salin, 1991). Today, most researches focused on the effects of calcium and potassium on the cell membrane stability and increase of resistance to environmental stress (Yu *et al*., 1998; Shen *et al*., 2008). While there are evidences based on beneficial effects of selenium on plants. Selenium as trace element is known as most important element in animals and plants. Selenium plays an important role in enzyme activity such as glutathione peroxidase (Gladyshev *et al*., 1998). It has been reported that selenium improves plant growth and increases antioxidant capacity (Seppanen *et al*., 2003).

Clifton (1985) has been reported that zeolites are microporous, aluminosilicate minerals widely used in industry for water purification, as catalysts, and in nuclear reprocessing. They are also used in medicine and in agriculture. Zeolites have a porous structure that can accommodate a wide variety of cations, such as Na^+, K^+, Ca^{2+}, Mg^{2+} and others. These positive ions are rather loosely held and can readily be exchanged for others in a contact solution. The most famous and abundant type of zeolites called clinoptilolite which has been discovered in 1890. Zeolite application into the soil leads to increase water retention capacity. In addition zeolite acts as a chemical sieve allowing some ions to pass through while blocking others (Mumpton, 1999). Zeolite because of its high cation exchange capacity decreases nutrient leaching; especially nitrate and so play an important role in agriculture. Zeolite application in sandy soils subjected to drought stress can improve final yield via increase of soil water holding (Zahedi *et al*., 2009). Selective absorption and controlled release of nutrients by zeolite helps to plant growth under poor conditions (Putnam *et al*., 1993). Unique features of zeolite such as high cation exchange capacity, selective absorption, structure stability and being abundant and finally inexpensive make it a suitable substance as soil amendment in order to conquest against water stress and fertilizer optimizing (Mumpton,1999).

In this experiment we studied effect of zeolite soil application and selenium foliar application on growth, seed production and some physiological attribute of canola plants under conditions of water deficit stress.

MATERIALS AND METHODS

This study was conducted at the experimental field of Seed and Plant Improvement Institute (SPII), Karaj, Iran, (35º 59′ N latitude, 50º 75′ E longitude and altitudes of 1313 m) on three canola cultivar (*Brassica napus* L. C.V Zarfam, Okapi and Sarigol), in 2006 and 2007 growing season. The yearly average precipitation (30-years long term period) which is mostly concentrated during the autumn and winter months was 244 mm.

Before the beginning of experiment, soil samples were taken in order to determine the physical and chemical properties. A composite soil sample was collected from depth of 0-30 and 30-60 cm. It was air dried, crushed, and tested for physical and chemical properties. The research field had a clay loam soil. Details of soil properties are shown in Table 1. After plow and disk, plots were prepared. The experimental design was laid out in a Randomized Complete Block with a factorial split plot arrangement of treatments in three replications. The treatments were included; irrigation at two levels, complete (I1) and limited

Table 1. Physical and chemical properties of soil collected from the experimental field of Seed and Plant Improvement Institute (SPII), Karaj, Iran.

Year	Depth (cm)	EC ($ds.m^{-1}$)	pH	Organic Carbon (%)	Saturated percentage (%)	N (%)	P (ppm)	K (ppm)	T.N.V (%)	Texture
2006	0-30	1.36	7.8	0.47	30.58	0.05	4.9	171	8.25	Clay loam
	30-60	1.76	7.7	0.35	30.84	0.04	2	132	10.69	
2007	0-30	1.42	7.8	0.51	36.01	0.06	3.1	205	9.81	Clay loam
	30-60	1.44	7.9	0.40	37.12	0.06	2	150	10.53	

irrigation (I2) at early of stem elongation. Zeolite application at two levels that is 0 (Z1) and 10 ton per hectare (Z2) and selenium foliar application at three concentration 0, 15 and 30 $g.liter^{-1}$ as sodium selenate, S1, S2 and S3, respectively. These treatments were applied on three canola cultivars. According to soil analysis chemical fertilizers and zeolite were distributed on the soil surface and incorporated with the soil in depth of 30 cm. The plots were 5 m long and consisted of six rows, 0.3 m apart. Between blocks and main plots, 6 m and 2.4 m alley was kept to eliminate all influence of lateral water movement.

The canola seeds were disinfected and sown on early of October. The distance between plant rows was 30 cm and the plant density was around 1,000,000 plants per hectare at sowing time. Irrigation was carried out as similar in all of plots until flowering stage. Non stressed plants were irrigated according to 80 mm evaporation from class A pan evaporation. Weeds were effectively controlled by hand. In plots which were exposure to drought stress criteria of water stress was electrical conductivity (Soil Moisture Meter) and gypsum block was used for soil moisture measurement. Before initiate of experiment, a separate plot was prepared and then gypsum block was put in depth of 0.4 m, irrigation was performed until soil was completely saturated. Electrical conductivity and soil moisture percentage were measured daily and then soil moisture carve was drawn. When electrical conductivity was equal 0 soil moisture was 8%. In main plots gypsum block was inserted in the soil and moisture was estimated. Thus, when electrical conductivity was 60 the plants were wilted and soil moisture was 12%. Selenium foliar application from sodium selenate was carried out in three concentrations (0, 15 and 30 $gr.liter^{-1}$) by engine backpack sprayer at late of stem elongation stage.

Intracellular electrolyte leakage assay

To this purpose five fully mature and expanded leaves of each treatment were clipped. Leaf disks were cut and immersed in 20 ml manitole in test tube (-2 bar osmotic potential). After 24 h darkness overnight, electrical conductivity of samples was measured.

Chlorophyll content assay

Leaf samples (1g) were extracted in 10 ml 100% acetone by mortar and pistil. Homogenate was filtered and then centrifuged at 2000 rpm for 2 min. One ml of supernatant was pipetted and mixed with 9 ml 80% acetone. Absorbance of diluted samples was read by spectrophotometer at 663 and 647 nm. Chlorophyll a, b and total chlorophyll were calculated according to below equations (Chdolvadova *et al*., 1999; Sestak and Catasky, 1966).

$$\text{chl.a } (mg.l^{-1}) = (12.25 \times A663 - 2.79 \times A647) \times D$$

$$\text{chl.b } (mg.l^{-1}) = (21.5 \times A647 – 5.1 \times A663) \times D$$

$$\text{chl.a + b } (mg.l^{-1}) = (7.15 \times A663 – 1871 \times A647) \times D$$

Where:

D: thickness of used cuvette (cm)

At and of growing season, biological yield and seed yield were measured.

All data were analyzed from analysis of variance (ANOVA) using the GLM procedure in SAS (SAS Institute, 2002). The assumptions of variance analysis were tested by insuring that the residuals were random, homogenous, with a normal distribution about a mean of zero. LSMEANS command was used to comparison of means at $P<0.05$ probability.

RESULTS AND DISCUSSION

Combined analysis of variance over years showed that effect of year was not significant on evaluated traits (Table 2). In addition, in most cases, interaction among treatments was not significant. It is worth mentioning that quadripartite interaction had significant effect on intracellular electrolyte leakage and chlorophyll content (Table 2). Comparisons of means are given in table 3. Comparisons of means were performed by LSMEANS command in SAS software so that comparisons were done among different canola cultivars under different treatment condition. The highest intracellular electrolyte leakage was obtained from Zarfam cultivar under conditions of full irrigation and no zeolite or selenium application. Furthermore, the highest chlorophyll content was observed from Okapi cultivar. There was no significant difference among canola cultivars in respect of seed yield while the highest biological yield was produced in Zarfam cultivar. Under conditions of water deficit stress, the highest and the lowest electrolyte leakage occurred in Zarfam and

Okapi cultivars, respectively because of their differences in sensitivity to water stress. In these conditions, the lowest chlorophyll content was related to Zarfam cultivar (Table 3). Increase of electrolyte leakage represents cell membrane degradation and also chloroplast destruction leads to chlorophyll content reduction. In addition, Sarigol cultivar produced the highest seed yield while the highest biological yield was observed in Zarfam cultivar. Under such conditions, zeolite application increased seed yield in Zarfam and Okapi cultivars and improved biological yield in Okapi cultivars (Table 3). Under full irrigation conditions and selenium foliar application (15 per liter) but lack of zeolite, Zarfam cultivar had the highest intracellular electrolyte leakage and the lowest chlorophyll content while inverse results were obtained from Okapi cultivar. In general, in all conditions, Zarfam cultivar had the highest and the lowest electrolyte leakage and chlorophyll content, respectively (Table 3).

Table 2. Summary of combined F significance from analysis of variance of irrigation, zeolite, selenium and cultivar in 2006 and 2007.

Source of Variation	df	Cellular electrolyte leakage	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Seed yield	Biological yield
Y	1	ns	ns	ns	ns	**	**
R(Y)	4	**	**	**	**	ns	ns
I	1	**	**	**	**	**	**
S	2	**	**	**	**	**	**
Z	1	**	**	**	**	**	**
I*S	2	**	**	**	**	**	**
I*Z	1	**	**	**	**	**	**
I*Y	1	ns	ns	ns	ns	**	*
S*Y	2	ns	ns	ns	ns	ns	ns
Z*Y	1	ns	ns	ns	ns	ns	ns
S*Z	2	**	**	**	**	**	ns
I*S*Z	2	**	**	**	**	**	**
I*S*Y	2	ns	ns	ns	ns	ns	ns
I*Z*Y	1	ns	ns	ns	ns	ns	ns
S*Z*Y	2	ns	ns	ns	ns	ns	ns
I*S*Z*Y	2	ns	ns	ns	ns	ns	ns
R*I*S*Z(Y)	44	**	**	**	**	ns	ns
C	2	**	**	**	**	**	**
I*C	2	**	**	**	**	ns	ns
S*C	4	**	**	**	**	**	**
Z*C	2	**	**	**	**	**	**
C*Y	2	ns	ns	ns	ns	ns	ns
I*C*Y	2	ns	ns	ns	ns	ns	ns
S*C*Y	4	ns	ns	ns	ns	ns	ns
Z*C*Y	2	ns	ns	ns	ns	ns	ns
I*S*C	4	**	**	**	**	ns	**
S*Z*C	4	**	**	**	**	ns	**
I*Z*C	2	**	**	**	**	*	ns
I*S*C*Y	4	ns	ns	ns	ns	ns	ns
I*S*Z*C	4	*	**	**	**	**	**
S*Z*C*Y	4	ns	ns	ns	ns	ns	ns
I*Z*C*Y	2	ns	ns	ns	ns	ns	ns
I*S*Z*C*Y	4	ns	ns	ns	ns	ns	ns
C.V. (%)		0.19	0.81	1.29	0.80	11.49	8.64

df: Degree of freedom; Y: Year; I: irrigation; S: Selenium; Z: Zeolite; C: Cultivar; C.V.: Coefficient of variation
ns: Not significant, * and **: significant at $P<0.05$ and $P<0.01$, respectively

Thus Zarfam cultivar was known as most sensitive cultivar. In the same treatment, increase of selenium concentration increased b chlorophyll content in Zarfam and Sarigol cultivars. In addition, zeolite application under full irrigation condition and selenium foliar application (30 g per liter) in compare with lack of selenium significantly increased measured traits (Table 3). Under conditions of water deficit stress, the highest electrolyte leakage was found in Zarfam cultivar while this cultivar produced the highest chlorophyll content. This represents high level of resistance to water stress in this cultivar

Table 3: Effects of irrigation, zeolite and selenium treatments on seed yield, biological yield, harvest index and oil yield three canola cultivar in 2006 and 2007.

Treatments	Cultivar	Cellular electrolyte leakage	Chlorophyll a	Chlorophyll b	Chlorophyll a + b	Seed yield	Biological yield
$I_1S_1Z_1$	Zarfam	1391.16a	1.60c	0.93b	2.52c	4277.8a	18813a
	Okapi	1336.00c	1.68a	0.98a	2.65a	3521.7a	15528b
	Sarigol	1357.66b	1.65b	0.96c	2.59b	4300.8a	15229b
$I_1S_1Z_2$	Zarfam	1097.66a	1.96c	1.14c	3.08c	5915.7a	21201a
	Okapi	1076.00c	2.04a	1.19a	3.21a	5627.7a	21799a
	Sarigol	1085.00b	2.01b	1.17b	3.16b	5584.0a	22097a
$I_1S_2Z_1$	Zarfam	1305.66a	1.73c	1.00c	2.71c	4827.8a	19111a
	Okapi	1260.16c	1.82a	1.05a	2.86a	4980.5a	16424a
	Sarigol	1280.33b	1.78b	1.03b	2.80b	4522.2a	15826a
$I_1S_2Z_2$	Zarfam	1054.16a	2.09c	1.21c	3.29c	6615.3a	23740a
	Okapi	1043.66a	2.17a	1.26a	3.40a	6386.3a	23590a
	Sarigol	1046.16a	2.12b	1.23b	3.34b	5599.3a	23292a
$I_1S_3Z_1$	Zarfam	1230.00a	1.86c	1.08a	2.92c	5079.8a	19858a
	Okapi	1202.33c	1.92a	1.12a	3.03a	5125.7a	17320b
	Sarigol	1217.83b	1.89b	1.09a	2.97b	5064.7a	18812ab
$I_1S_3Z_2$	Zarfam	1021.83a	2.21b	1.28b	3.47b	7180.5a	31205a
	Okapi	1011.66b	2.28a	1.32a	3.58a	7585.3a	25083b
	Sarigol	1017.50ab	2.26a	1.31a	3.55a	6011.8b	19708c
$I_2S_1Z_1$	Zarfam	1555.66a	0.95b	0.55b	1.50b	1588.8b	10750a
	Okapi	2484.16c	1.05a	0.61a	1.66a	1680.7b	8062.5c
	Sarigol	2514.83b	1.02a	0.59a	1.62a	2276.3a	9257.0b
$I_2S_1Z_2$	Zarfam	2049.66a	1.30c	0.75c	2.04c	3368.8a	16125.0a
	Okapi	1979.83c	1.37a	0.79a	2.15a	3246.5a	14631.7ab
	Sarigol	2007.00b	1.34b	0.78b	2.11b	3063.2a	13586.8b
$I_2S_2Z_1$	Zarfam	2432.33a	1.07b	0.62b	1.69b	3025.0a	14632.1a
	Okapi	2386.66c	1.11a	0.64a	1.74a	2612.5a	11048.6b
	Sarigol	2400.50b	0.99c	0.58c	1.56c	2620.2a	11496.6b
$I_2S_2Z_2$	Zarfam	1892.66a	1.40c	0.82c	2.20c	3437.5a	16573a
	Okapi	1853.83c	1.49a	0.86	2.23a	3368.8a	15379ab
	Sarigol	1869.50b	1.45b	0.84b	2.28	3162.5a	14035b
$I_2S_3Z_1$	Zarfam	2342.83a	1.12b	0.65a	1.76b	2803.3ab	12243a
	Okapi	2303.50c	1.14a	0.66	1.80a	3468.0a	9227b
	Sarigol	2322.66b	1.05c	0.60b	1.65c	2360.5b	10451ab
$I_2S_3Z_2$	Zarfam	1834.66a	1.52c	0.88c	2.40c	3158.5a	15453.0a
	Okapi	1782.00c	1.60a	0.93a	2.51a	2887.5ab	12989.7b
	Sarigol	1797.50b	1.56b	0.90b	2.45b	2734.7b	11645b

I_1: complete irrigation; I_2: limited irrigation at stem elongation stage; S_1: 0 $gr.liter^{-1}$ Selenium; S_2 15 $gr.liter^{-1}$ Selenium; S_3: 30 $gr.liter^{-1}$ Selenium; Z_1: no zeolite; Z_2: 10 $ton.ha^{-1}$ zeolite. For a given means within each column of each section followed by the same letter are not significantly different ($p < 0.05$).

(Table 3). There was no significant difference among cultivars in seed yield and biological yield when they were treated by 15 mg per liter selenium, under conditions of full irrigation and zeolite application.

On the other side foliar application by 30 mg per liter selenium along with full irrigation but without zeolite showed that different cultivars are the same in case of seed yield while biological yield in Zarfam cultivars increased in compare with two other cultivars. By contrast, selenium foliar application (30 mg per liter) along with full irrigation and also zeolite application led to increase of seed yield in Okapi and Zarfam cultivars. In addition, the highest biological yield was observed in Zarfam cultivar. Under conditions of water deficit stress without any treatment the Sarigol cultivar produced the highest seed yield whilst the highest biological yield was related to Zarfam cultivar. Under such conditions zeolite application increased seed yield in Zarfam and Okapi cultivars and also improved biological yield in Okapi. Under conditions of water deficit stress, lack of zeolite and application of 15 mg per liter selenium, there was no significant difference among cultivars in case of seed yield but by comparison with lack of zeolite in the same treatment, seed yield in Zarfam and Okapi significantly increased. Furthermore, biological yield increased affected by selenium foliar application under water deficit stress conditions. The highest harvest index was obtained from Okapi and Sarigol cultivars when these cultivars were subjected to water stress and selenium (15 mg per liter). Comparison of means revealed that there was no significant difference among cultivars regarding seed yield when they were treated by zeolite and selenium (15 mg per liter) under water stress conditions. The results demonstrated that selenium use (15 mg per liter) under conditions of water stress and zeolite application in compare with lack of zeolite under similar conditions had no significant effect on seed yield and biological yield. The highest seed yield was achieved from Okapi under conditions of water deficit stress without zeolite soil application but 30 mg per liter selenium while the highest biological yield was related to Zarfam cultivar (Table 3). The highest and the lowest seed yield were obtained from Zarfam and Sarigol under conditions of water deficit stress, zeolite use and selenium foliar application (Table 3).

Reproductive stage including stem elongation, flowering, pollination and seed filling are the most sensitive stages to water deficit stress in canola (Thomas *et al*., 2004) and water stress at these stages leads to loss of yield (Wright *et al*., 1995). It seems that zeolite application improves growth and seed yield through keeping water into the soil. Positive effect of zeolite on plant height, number of branches, yield and yield components can be due to decrease of nitrogen leaching and increase of nitrogen availability (Polat *et al*., 2004). Nonetheless plant response to water stress is so variable and it is depends on stress intensity, stress duration and plant growth stage (Chaves *et al*., 2003). It has been reported that water stress decreases relative water content, chlorophyll and cell membrane stability all over the growth period (Chandrasekar *et al* ., 2000). Increase of cellular electrolyte leakage on account of water stress is due to cell membrane degradation. Kumar *et al*., 1993 showed that electrical conductivity in canola leaves is dependent on relative humidity and turgor potential while this parameter is dependent on relative humidity in mustard. Under conditions of mild stress, chlorophyll concentration increases because of leaf area reduction. Mild water stress increases protoplasm concentration and decreases leaf extension while severe stress inhibits chlorophyll synthesis completely. Ward *et al*., have been reported that, loss of cell water content increases chlorophyll concentration in leaves. In general, effect of water stress on chlorophyll content is erratic and it is depends on environmental conditions and genetic of the plant. In some species, water stress increases chlorophyll concentration and in some other decrease. Increase of stress intensity leads to chlorophyll degradation (Kumar and Paul, 1997). Water stress leads to hasty senescence, chloroplast break down and chlorophyll degradation (Lawlor and, 1985). Deepak and Wattal (1995) showed that chlorophyll content decreased significantly when canola plants were subjected to water stress. Kumar and Paul (1997) showed that water stress at flowering and seed filling stage significantly decreases chlorophyll a and b. In this regard Due *et al* (1994) have been reported that, when soil water potential reached to -1.5 MPa, chlorophyll content decreased by 82% due to pigment degradation. Zeolite with high cation exchange capacity acts as a sink for nutrient such as ammonium and thus improves plant growth especially in sandy soils (Polat *et al*., 2004). Water can penetrate easily into the zeolite structure and thud zeolite application increases soil water retention capacity (Shaw and Andrews, 2001). According to above mentioned features, zeolite application on sandy soils is rational. In addition selenium foliar application increase relative water content and improve water uptake from roots (Kuznestsov *et al*., 2003).

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