Effect of cattle manure and zeolite applications on physiological and biochemical changes in soybean [*Glycine max* (L.) Merr.] grown under water deficit stress

Efecto de la aplicación de estiércol de ganado y zeolita sobre los cambios fisiológicos y bioquímicos en soya [*Glycine max* (L.) Merr.] cultivada bajo estrés por déficit hídrico

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Received: 03/08/2013 First reviewing ending: 08/28/2013 First review received: 08/30/2013
Second reviewing ending: 09/11/2013 Second review received: 09/13/2013 Accepted: 10/15/2013

ABSTRACT

In order to study the effects of cattle manure and zeolite applications on physiological and biochemical changes in soybean grown under conditions of water stress, an experiment was conducted at Varamin, Iran during 2011 growing season. The experimental design was carried out in a randomized complete blocks with a split factorial arrangement of treatments in three replications. Main factor was water stress (normal irrigation and irrigation withholding after 50% flowering) and sub factors were included cattle manure (0, 15 and 30 ton per hectare) and zeolite application (with and without). The results showed that water stress significantly decreased relative water content and electrolyte leakage while increased antioxidant enzyme activity. By contrast, zeolite and cattle manure application had positive effect on relative water content and improve of electrolyte leakage. These treatments alleviate enzyme activity and lipid or protein peroxidation. In general, we concluded that zeolite and manure application can ameliorate growth conditions for soybean plants grown under water deficit stress.

Key words: Soybean, cattle manure, zeolite, water stress, enzyme activity

RESUMEN

Con el fin de estudiar los efectos de la aplicación de estiércol de ganado y zeolita sobre los cambios fisiológicos y bioquímicos de soya cultivada bajo condiciones de estrés hídrico se realizó un experimento de campo en Varamin, Irán durante la temporada de cultivo del 2011. El diseño experimental fue bloques completos al azar en parcelas divididas con tres repeticiones. El factor principal fue el estrés hídrico (riego normal y la supresión de riego después del 50% de floración) y los sub factores fueron estiércol de ganado (0, 15 y 30 t/ha) y la aplicación de zeolita (con y sin). Los resultados mostraron que el estrés hídrico disminuyó significativamente el contenido relativo de agua y la estabilidad de la membrana mientras que incrementó la actividad enzimática. Por el contrario, la aplicación de zeolita y estiércol de ganado tuvieron un efecto positivo sobre el contenido relativo de agua y mejoraron la estabilidad de la membrana. Estos tratamientos aliviaron la actividad enzimática y la peroxidación de lípidos o proteínas. En general, se concluye que la aplicación de zeolita y estiércol puede mejorar las condiciones de crecimiento de las plantas de soya cultivadas bajo estrés por déficit hídrico.

Palabras clave: Soya, estiércol de ganado, zeolita, estrés hídrico, actividad enzimática

INTRODUCTION

Drought stress significantly limits plant growth and crop productivity (Heatherly and Spurlock, 1999; Pandey *et al*., 2000; Deblonde and Ledent, 2001; De Costa and Shanmugathasan, 2002). Effects of water stress on a plant's physiology, including growth (McDonald and Davies, 1996), signaling pathways (Chaves *et al*., 2003), gene expression (Bray, 2002), and leaf photosynthesis (Flexas *et al*., 2004), have been studied extensively. Water stress induces oxidative stress in plants (Hajiboland and Joudmand, 2009). Plant cells contain an array of protection mechanisms and repair systems that can minimize the occurrence of oxidative damage caused by reactive oxygen species (ROS) (Abdel Latef, 2010).

The ROS 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 (Mittler, 2002).The reaction of plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development (Chaves *et al*.,2003). Mechanisms of active oxygen species detoxification exist in all the plants and include activation of enzymatic (superoxide dismutase, catalase, ascorbate peroxidase, peroxidase, glutathione reductase) (Johnson *et al*., 2003). Several studies have pointed out that drought-tolerant species increased their antioxidant enzyme activities and antioxidant contents in response to drought treatment, whereas drought-sensitive species failed to do so (Shalata *et al*., 2001; Demiral and Türkan, 2005).

Efficient management of soil moisture is important for agricultural production in the light of scarce water resources. Soil conditioners contribute significantly to provide a reservoir of soil water to plants on demand in the upper layers of the soil where the root systems normally develop. Zeolite apart from improving the soil physical properties, also serve as buffers against temporary drought stress and reduce the risk of plant failure during establishment. This is achieved by means of reduction of evaporation through restricted movement of water from the sub-surface to the surface layer. Amendment of clinoptilolite zeolite to sandy soils has been reported to lower nitrogen concentrations in leachate and to increase moisture and nutrients in the soil, due to increased soil surface area and cation exchange capacity (He, 2002).

Xiubin and Zhanbin (2001) showed that zeolite improved water retention capacity and cation exchange capacity in arable soils. Leggo (2000) studied the response of wheat to poultry manure amended by zeolite and found out that crop faced a better growth rate when zeolite was applied in poultry manure, and reported that the increase of growth and yield is due to nitrogen availability by zeolite. Kavoosi (2007) showed that the application of 10 t ha^{-1} of zeolite significantly increased rice grain yield. Zahedi and Tohidi-Moghadam (2011) reported that soil zeolite application decreased antioxidant enzymes activity. It seems that zeolite increases water retention capacity and thus water stress intensity will be decreased.

Cattle manure is a good source of nutrients for vegetation especially when supplemented with commercial nitrogen fertilizer. Cattle manure application improves physical and chemical properties of soil, In addition, increases microorganism activity and water retention capacity (Gupta *et al*., 2004). Crop yield is usually increased by manure application because of the increased nutrient availability and the improved soil structure (Matsi *et al*. 2003). Eghball and Power (1999) found that application of cattle manure resulted in maize yield similar to that from commercial fertilizer application.

The aim of this study was to investigate the effect of cattle manure and zeolite on some physiological and biochemical changes such as relative water content, chlorophyll content, membrane lipid peroxidation, antioxidant enzyme activity, dityrosine, hydroxyguanosine and electrolyte leakage of soybean under water stress, in order to further understand drought tolerance mechanisms in plants.

MATERIAL AND METHODS

The experiment was conducted at research field of Azad University, Varamin, Iran during 2011 growing season. Site of study was situated at 31° 519 E and 20° 359 N and 1050 m above sea level. Before beginning of experiment, soil samples were taken in order to determine the physical and chemical properties. A composite soil sample was collected at a depth of 0-30 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 blocks with a split factorial arrangement of treatments in three replications. Main factor was water stress (normal irrigation and irrigation withholding after 50% flowering) and sub factors were included cattle manure (0, 15 and 30 ton per hectare) and zeolite application (with and without). The 16 m^2 plots were prepared with 4 m long and consisted of five rows, 0.65 m apart. Between all main plots, 2 m alley was kept to eliminate all influence of lateral water movement.

Table 1. Soil properties of the experimental site at Varamin, Iran during 2011.

Depth	EC (ds m^{-1})	pH	OC (%)	TNV (%)	K (ppm)	P(ppm)	Total N (%)	Texture
0-30 cm	4.1	7.4	0.71	< 10	368	25.9	0.079	Clay loam

EC: Electrical conductivity; OC: Organic carbon and TNV: Total neutralizing value

Cattle manure was applied before seed sowing so that manure was spread on the soil surface and then was mixed into the soil manually. Soybean seeds [*Glycine max* (L.) Merr.] cv. Williams were purchased from Pars Abad Oil Seed Company and inoculated with *Rhizobium japonicum* and the sown in certain experimental plots with 10 cm apart each other. Irrigation was performed immediately after seed sowing and after seedling establishment irrigation was done every week. Non-stressed plants were irrigated after reaching 80-mm evaporation from Class A pan. At 5-leaf stage plants were thinned to 150000 plants ha^{-1} density. Weeds were controlled manually at 5-leaf stage, stem elongation and flowering stage. In order to stress induction irrigation was stopped at 50% flowering stage in stressed plots till end of growing stage. One week after water stress initiation leaf samples were collected and physiological and biochemical changes were assayed.

Relative water content assay

Relative water content (RWC) was measured, from each plant leaf discs were taken and weighted (fresh weigh, FW). The discs were then placed in distilled water for 5 h at 25°C and then their saturated weights (SW) were measured. The discs were then dried in oven at 70 °C for 24 h to calculate dry weight (DW). Relative water contents were calculated by following formula (Gupta, 2004):

$$RWC = \frac{FW - DW}{SW - DW}$$

Chlorophyll assay

The first fully expanded leaf blades were taken to determine chlorophyll (Chl) contents after 30 h of salt stress. For the chlorophyll assay, leaf discs were ground with 10 of 80% acetone (v/v). The amount of chlorophyll a and b was determined spectrophotometrically at 663 and 645 nm, using the method of Arnon (1949).

Antioxidant enzymes activity assay

Catalase activity was estimated by the method of Cakmak and Horst (1991). The reaction mixture contained 100 µl crude extract, 500 µl 10mm H_2O_2 and 1400 µl 25mm sodium phosphate buffer. The decrease in the absorbance recorded at 240 nm for 1 min by a spectrophotometer.

Superoxide dismutase activity was determined by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitro blue tetrazolium according to the method of Giannopolitis and Ries (1977). The reaction mixture contained 100 µl l µm riboflavin, 100 µl 12 mM L-methionine, 100 µl 0.1 mM EDTA (pH 7.8), 100 µl 50 mM Na_2CO_3 (pH 10.2), 100 µl 75 µM nitro blue tetrazolium in 2300 nitro blue tetrazolium 25mM sodium phosphate buffer (pH 6.8) and 200 µl crude enzyme extract, in a final volume of 3 ml. Glass test tubes that contained the reaction mixture were illuminated with a fluorescent lamp (120 W), and identical tubes that were not illuminated served as blanks. After illumination for 15 min, absorbance was measured at 560 nm. One unit of Superoxide dismutase activity was defined as the amount of enzyme which caused 50 % inhibition of photochemical reduction of nitro blue tetrazolium.

Glutathione peroxidase activity was measured according to method of Paglia and Valentine (1997) in which 0.56 M (pH=7) phosphate buffer, 0.5 M EDTA, 1mM NaN3, 0.2mM NADPH were added to the extracted solution. Glutathione peroxidase catalyses the oxidation of glutathione by cumene hydroperoxide in the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with the concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm was measured with a spectrophotometer.

Malondialdehyde assay

The level of membrane damage was determined by measuring MDA as the end product of peroxidation of membrane lipids (De Vos *et al*., 1991). In brief, samples were homogenized in an aqueous solution of trichloroacetic acid (10% w/v), and aliquots of filtrates were heated in 0.25% trichloroacetic acid. The amount of MDA was determined from the absorbance at 532 nm, followed by correction for the non-specific absorbance at 600 nm. The content of MDA was determined using the extinction coefficient of MDA ($\varepsilon = 155\ \mu M^{-1}\ cm^{-1}$).

Dityrosine assay

1.2 grams of fresh tissue material were homogenized with 5 ml of ice-cold 50 mM HEPES-KOH, pH 7.2, containing 10 mM EDTA, 2 mM PMSF, 0.1 mM p-chloromercuribenzoic acid, 0.1 mM

DL-norleucine and 100 mg polyclar AT. The plan tissue homogenate was centrifuged at 5000 g for 60 min to remove debris. Purification of o,o0-dityrosine in the clear tissue homogenized supernatant fluid was accomplished by preparative HPLC. o,o0-Dityrosine was recovered by gradient elution from the C-18 column (Econosil C18, 250 mm · 10 mm) (Orhanl *et al*., 2004). The composition of fluent varied linearly from acetonitrile–water–TFA (1:99:0.02) to acetonitrile–water–TFA (20:80:0.02) over 25 min. The gradient was started 5 min after the injection. A flow rate of 4 ml/min was used. o,o0-Dityrosine was analyzed by reversed-phase HPLC with simultaneous UV-detection (280 nm) and fluorescence-detection (ex. 280 nm, em. 410 nm). A phenomenex inertsil ODS 2 (150 mm · 4.6 mm, 5 lm) HPLC column (Bester, Amsterdam, The Netherlands) equipped with a guard column was used for these analyses. A gradient was formed from 10 mM ammonium acetate, adjusted to pH 4.5 with acetic acid, and methanol, starting with 1% methanol and increasing to 10% over 30 min. The flow rate was 0.8 ml/min. A standard dityrosine sample was prepared according to Amado *et al*. (1984). Dityrosine was quantified by assuming that its generation from the reaction of tyrosine with horseradish peroxidase in the presence of H_2O_2 was quantitative (using the extinction coefficient e315 = 4.5 mM^{-1} cm^{-1} at pH 7.5).

Hydroxyguanosine assay

Hydroxyguanosine (8OH-2'dG) was measured in the leaves essentially as described previously (Bogdanov *et al*., 1999). Briefly, an automated column switching method for 8OH-2'dG is based on the unique selectivity of the integral porous carbon column for purines. Samples were injected onto a C8 column and the band containing 8OH-2'dG was then quantitatively trapped on a carbon column. The selectivity of the carbon column for 8OH-2'dG allows elimination of interfering peaks by washing the column with a second mobile phase and then eluting 8OH-2'dG to an analytical C18 column with an identical mobile phase containing adenosine to displace 8OH-2'dG. Detection with series colorimetric electrodes provides qualitative certainty for 8OH-2'dG peak by response ratios.

Electrolyte leakage assay

For the determination of Injury index 15 leaf pieces (2 cm in length) were cut from stressed and control plants and immersed in 15 ml distilled water at room temperature. Conductivity of the solutions was measured periodically during a 24-h-period as previously described (Kocheva et al., 2005).

Statistical analysis

All data were analyzed from analysis of variance (ANOVA) using the GLM procedure in SAS ($p \leq 0.05$ and $p \leq 0.01$). The assumptions of variance analysis were tested by insuring that the residuals were random, homogenous, with a normal distribution about a mean of zero. Duncan's multiple range tests was used to measure statistical differences between treatment methods and controls ($p \leq 0.05$).

RESULTS AND DISCUSSION

Analysis of variance showed that the effects of water stress, cattle manure and zeolite application were significant on all measured traits except for dityrosine (Table 2). In addition, interaction between these three factors on all traits except for malondialdehyde was statistically significant at 0.01 probability level but for relative water content was statistically significant at 0.05 probability level. As can be seen from Table 3, water stress significantly decreased relative water content. The results showed that cattle manure application or zeolite application had not significant effect on RWC under normal irrigation conditions. By contrast, under stress conditions, zeolite application and cattle manure application improved RWC. However, increase in cattle manure up to 30 ton per hectare and zeolite application decreased RWC again (Table 3). RWC is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit, while water potential is an estimate of plant water status and it is useful in dealing with water transport in the soil-plant-atmosphere continuum (Kramer, 1988).

The highest chlorophyll content was related to those plots which were treated with zeolite and cattle manure. It is worth mentioning that application of 30 ton per hectare cattle manure could produce the highest chlorophyll content (Table 3). Chlorophyll content in live plants is an important factor in determining photosynthetic capacity. Decreased or unchanged chlorophyll level during drought stress has been observed in other species, depending on drought duration and severity (Rensburg and Kruger, 1994; Kyparissis *et al*., 1995; Zhang and Kirkham,1996; Jagtap *et al*.,1998). Changes in leaf chlorophyll content with drought and heat injury may involve a severe chlorophyll photooxidation mediated by oxy-radicals (Wise and Naylor,1987). Follet *et al*. (1981) reported that chlorophyll coloration is related to the amount of nutrients absorbed by the plant from the soil. Sivasubramaniawn (1992) related the drought resistance of plants to the chlorophyll stability index. Organic and inorganic fertilizers applied to the soil supply plant nutrients for crop growth and affect the plant's physiological processes, which serve as important instruments in chlorophyll synthesis and yield development.

The highest catalase activity was obtained when soybean plants were exposure to water stress and not treated with manure or zeolite, while the

Table 2. Analysis of variance of physiological and biochemical attributes of soybean [*Glycine max* (L.) Merr.] cv. Williams affected by water stress, cattle manure and zeolite.

Sources of variation	d.f.	RWC	Chl	Cat	GP	SD	Mal	Dit	Hyd	EL
Replication	2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Water stress (WS)	1	**	**	*	**	**	**	ns	*	**
Error (a)	2									
Cattle manure (CM)	2	**	**	**	**	**	**	**	**	**
Zeolite (Z)	1	*	**	**	**	**	**	**	ns	**
WS x CM	2	**	**	**	**	**	**	**	**	**
WS x Z	1	**	ns	**	*	**	**	ns	**	*
CM x Z	2	**	**	ns	**	**	**	**	**	**
WS x CM x Z	2	*	**	**	**	**	ns	**	**	**
Error (b)	20									
C.V (%)		2.08	4.13	3.91	7.97	4.51	4.12	5.60	1.29	2.52

d.f.: Degree of freedom; CV: Coefficient of variation; RWC: Relative water content (%): Chl: Chlorophyll (mg g^{-1} fresh weight); Cat: Catalase (u mg^{-1} protein); GP: Glutathione peroxidase (u mg^{-1} protein); SD: Superoxide dismutase (u mg^{-1} protein); Mal: Malondialdehyde (nm mg^{-1} protein); Dit: Dityrosine (nm mg^{-1} protein); Hyd: Hydroxyguanosine (nm mg^{-1} protein) and EL: Electrolyte leakage (µs cm^{-1}).
* Significant ($p \leq 0.01$); ** Significant ($p \leq 0.05$) and ns significant ($p > 0.05$).

Table 3. Interaction among water stress, cattle manure and zeolite on physiological and biochemical attributes of soybean [*Glycine max* (L.) Merr.] cv. Williams.

WS	CM	Z	RWC	Chl	Cat	GP	SD	Dit	Hyd	EL
No stress	0 t ha^{-1}	No	76.7ab	18.7gh	36.8c	11.4b	31.1c	35.6a	29.6b	884.6c
		Yes	79 a	18.6gh	27.7d	5.4e	23.1d	27 b	24.7de	677.6de
	15 t ha^{-1}	No	77.8ab	21.4f	27.8d	7.2d	19.6e	27.2b	26.4cd	648.3e
		Yes	77.1ab	32.5b	26.6d	8.7c	19.4e	19.7de	23.9e	458.6h
	30 t ha^{-1}	No	78 ab	25.2d	19.2e	5.4e	14.8f	17.1fg	21.2fg	588f
		Yes	76.1b	38.3a	15f	5e	7g	15.7g	18.6h	274i
Water stress	0 t ha^{-1}	No	67.2d	12.2j	47.9a	13.8a	39.3b	36a	33.3a	1042a
		Yes	70.3c	17.3hi	45.2b	11.3b	46.5a	35.5a	31.8a	946b
	15 t ha^{-1}	No	75.4b	16.9i	36.9c	10.7b	32.6c	28.5b	27.2c	883.6c
		Yes	67.1d	23.4e	26.5d	8.4c	24.2d	22.3c	24e	700.6d
	30 t ha^{-1}	No	75.9c	19.7g	27d	8.8c	24.8d	21.3cd	22.9ef	876.6c
		Yes	70.3c	30.7c	16.2f	5.4e	14.7f	18.3ef	20.6g	539g

WS: Water stress: CM: Cattle manure; Z: Zeolite; RWC: Relative water content (%); Chl: Chlorophyll (mg g^{-1} fresh weight); Cat: Catalase (u mg^{-1} protein); GP: Glutathione peroxidase (u mg^{-1} protein); SD: Superoxide dismutase (u mg^{-1} protein); Dit: Dityrosine (nm mg^{-1} protein); Hyd: Hydroxyguanosine (nm mg^{-1} protein) and EL: Electrolyte leakage (µs cm^{-1}).
Treatment means followed by the same letter within each common are not significantly different ($p < 0.05$) according to Duncan's Multiple Range test.

lowest activity was related to those plants which were treated with zeolite and cattle manure under normal irrigation conditions (Table 3). It is notable that under limited irrigation conditions, when zeolite was applied along with 30 ton per hectare cattle manure, no significant difference was observed between this treatment and no stressed plants. Similar results were obtained regarding glutathione peroxidase and superoxide dismutase activity. The highest activity was observed in stressed plants without any further treatment while glutathione peroxidase and superoxide dismutase activity significantly decreased when zeolite and cattle manure has been applied (Table 3). Under environmental stress conditions, such as drought stress, an increase in the generation of ROS has been described (Munné-Bosch and Peñuelas, 2003) that may cause damage to cells. Tohidi-Moghadam *et al.* (2009) reported that drought-tolerant species increased their antioxidant enzyme activities and antioxidant contents in response to drought treatment.

In this study it was found that zeolite and cattle manure application decreased antioxidant enzyme activity. Obtained results are in accordance with findings of Zheng *et al*., (2008). This finding can be related to the ability of the crops against different intensities of water deficit stress. In other words, when crops are exposed to water deficit stress, their antioxidant defensive mechanism is activated and the content of antioxidants will rise. Results of this research indicate the same trend, too. Thus, the content of all three measured antioxidants increased in soybean plant. Our results suggest that drought stress directly or indirectly leads to production of oxygen radicals, which results in increased lipid peroxidation and oxidative stress in the plant. Drought stress may also lead to stomata closure, which reduces CO_2 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 (Johnson *et al*., 2003). The increase in SOD activity was reported in tolerance basmati rice variety (Wang *et al*.,1991). In our study, zeolite decreased the activity of these enzymes maybe by elimination of free radicals. Previous finding explains that glutathione peroxidase activities were increased when wheat and cotton plants were subjected to water stress (Sestak and Catasky, 1966). Increase of glutathione peroxidase activities leads to ferredoxin electron absorption by NADP and super oxide ion concentration will be decreased (Semirnoff and Cumbes, 1998). The results showed that soil zeolite application and cattle manure decreased antioxidant enzyme activities (Table 3). It seems that zeolite application and cattle manure increase water retention capacity and thus water stress intensity will be decreased. The decrease in antioxidant enzymes observed in both plants applied with zeolite and the plants grown with cattle manure could be explained partially by the fact that these plants may be submitted to a lower oxidative stress under both control and drought stress conditions.

Hydroxyguanosine is a nucleoside which is an oxidative derivative of guanosine. Measurement of the levels of hydroxyguanosine is used as a biomarker of oxidative stress. The highest dityrosine and hydroxyguanosine were observed from stressed plants (Table 3). Cattle manure and zeolite application significantly decreased hydroxyguanosine under water stress conditions.

According to Table 3 the highest electrolyte leakage was occurred when soybean plants were stressed. The results indicated that zeolite application decreased electrolyte leakage when manure was applied. Similar results were obtained under normal irrigation conditions (Table 3). However, in this study, there were significant differences between zeolite and non zeolite application in plant's electrolyte leakage under water stress. All this shows that zeolite can lower the plant's electrolyte leakage and alleviate the damage on the cell membrane caused by water stress.

Interaction between water stress and cattle manure application was significant on malondialdehyde (Figure 1) malondialdehyde decreased of on account of cattle manure in drought stress condition (Figure 1). Interaction between water stress and zeolite application was significant on malondialdehyde (Table 2). Zeolite application decreased malondialdehyde in water stress conditions (Figure 2). Also in drought stress conditions, the lowest malondialdehyde was obtained when 30 ton per hectare cattle manure was applied with zeolite application (Figure 3). The highest malondialdehyde content was observed in water stressed plants. Malondialdehyde is often regarded as the product and a reflection of the degree of membrane lipid peroxidation (Ali *et al*. 2005). Therefore, malondialdehyde content in the leaves soybean plants was measured under water stress and other environmental stress. With the water stress, leaf malondialdehyde content increased. However, the malondialdehyde content in zeolited plants remained lower than that in non zeolited plants, which shows that the presence of the zeolite could alleviate the peroxidation of membrane lipids.

CONCLUSIONS

All data shown that the effects of water stress, cattle manure and zeolite applications were significant on all measured traits except for dityrosine. In addition, interaction between these three factors on all traits except for malondialdehyde and relative water content was statistically significant. Water stress significantly decreased relative water content. It is worth mentioning that application of 30 ton per hectare cattle manure could produce the highest chlorophyll content. The highest chlorophyll content was related to those plots which were treated with zeolite and cattle manure. It is worth mentioning that application of 30 ton per hectare cattle manure could produce the highest chlorophyll content. The highest antioxidant enzymes activity was observed in stressed plants without any further treatment while glutathione peroxidase and superoxide dismutase activity significantly decreased when zeolite and cattle manure has been applied. The highest malondialdehyde, dityrosine and hydroxyguanosine were observed in water stressed plants.The results indicated that zeolite application decreased electrolyte leakage when manure was applied. We concluded that zeolite and manure application can improve growth conditions for soybean plants grown under water deficit stress.

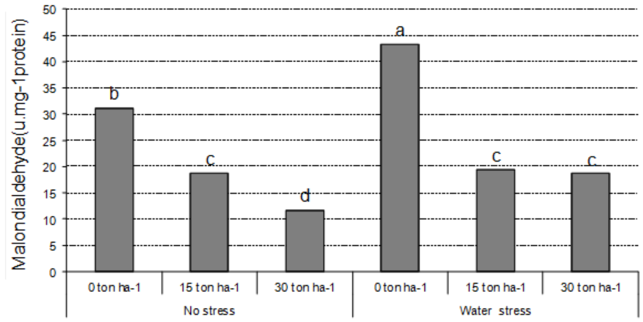

Figure 1. Interaction between water stress and cattle manure application on malondialdehyde content of soybean [*Glycine max* (L.) Merr.] cv. Williams. Treatment means followed by the same letter within each common are not significantly different ($p < 0.05$) according to Duncan's Multiple Range test.

Figure 2. Interaction between water stress and zeolite application on malondialdehyde content of soybean [*Glycine max* (L.) Merr.] cv. Williams. Treatment means followed by the same letter within each common are not significantly different ($p < 0.05$) according to Duncan's Multiple Range test.

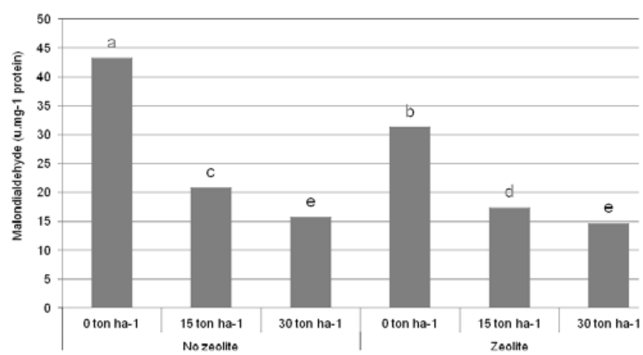

Figure 3. Interaction between cattle manure and zeolite application on malondialdehyde content of soybean [*Glycine max* (L.) Merr.] cv. Williams. Treatment means followed by the same letter within each common are not significantly different ($p < 0.05$) according to Duncan's Multiple Range test.

LITERATURE CITED

Abdel Latef, A. A. 2010. Changes of antioxidative enzymes in salinity tolerance among different wheat cultivars. Cereal Res. Comm 38: 43-55.

Ali, M. B.; E. Hahn and K. Paek. 2005. Effects of temperature on oxidative stress defense systems, lipid peroxidation and lipoxygenase activity in *Phalaenopsis*. Plant Physiol Biochem 43:213-223.

Amado, R.; R. Aeschbach and H. Neukom. 1984. Dityrosine: in vitro production and characterization. Methods Enzymol 107:377-388.

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts, polyphennoloxidase in *Beta vulgaris*. Plant Physiol 24: 1-150.

Bogdanov, M. B.; M. F. Beal, D. R. McCabe, R. M. Griffin and W. R. Matson. 1999. A carbon column-based liquid chromatography electrochemical approach to routine 8-hydroxy-2′-deoxyguanosine measurements in urine and other biologic matrices: a one-year evaluation of methods. Free Radic Biol Med 27: 647-666.

Bray, E. 2002. Abscisic acid regulation of gene expression during water deficit stress in the era of the Arabidopsis genome. Plant Cell Environ 25: 153-161.

Cakmak, I. and W. Horst. 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (*Glycine max* L.). Plant Physiol 83: 463-468.

Chaves, M. M.; J. P. Maroco and J. Pereira. 2003. Understanding plant responses to drought from genes to the whole plant. Funct Plant Biol 30: 239-264.

Deblonde, P. M. K. and J. F. Ledent. 2001. Effects of moderate drought conditions on green leaf number stem height, leaf length and tuber yield of potato cultivars. Eur. J. Agron 14: 31-41.

De Costa, W. A. J. M. and K. N. Shanmugathasan. 2002. Physiology of yield determination of soybean [*Glycine max* (L.) Merr.] under different irrigation regimes in the sub-humid zone of Sri Lanka. Field Crops Res 75: 23-35.

Demiral, T. and I. Turkan. 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot 53: 247-257.

De Vos, C.; H. M. Schat, M. A. De Waal, R. Vooijs and W. Ernst. 1991. Increased to copper-induced damage of the root plasma membrane in copper tolerant *Silene cucubalus*. Plant Physiol 82: 523-528.

Eghball, B. and J. F. Power. 1999. Composted and non-composted manure application to conventional and no-tillage systems: corn yield and nitrogen uptake. Agron J 91:819-825.

Flexas, J.; J. Bota, F. Loreto, G. Cornic and T. D. Sharkey. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biol 6: 269-279.

Follet, R. H.; L. S. Murphy and R. L. Donalue. 1981. Soil-fertilizer-plant relationship. Fertilizer Soil Amendment 6(16): 478-481.

Giannopolitis, C. and S. Ries. 1977. Superoxide dismutase occurrence in higher plant. Plant Physiol 59: 309-314.

Gupta, S.; E. Munyankusi. J. Monerief, F. Zvomuya and M. Hanewall. 2004. Tillage and manure application effects mineral nitrogen leaching from seasonally frozen soils. J. of Environ. Qual 33: 1239-1246.

Hajiboland, R. and A. Joudmand. 2009. The K/Na replacement and function of antioxidant defense system in sugar beet (*Beta vulgaris* L.) cultivars. Acta Agric. Scand B Soil Plant Sci 59: 246-259.

Heatherly, L. G. and S. R. Spurlock. 1999. Yield and economics of traditional and early soybean production system (ESPS) seedings in the mid southern United States. Field Crops Res 63: 35-45.

He, Z. L. 2002. Clinoptilolite zeolite and cellulose amendments to reduce ammonia volatilization in a calcareous sandy soil. Plant and Soil 247(6):253-260.

Jagtap, V.; S. Bhargava, P. Streb and J. Feierabend. 1998. Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) Moench. J. Exp. Bot 49:1715-1721.

Johnson, S. M.; S. J. Doherty and R. R. D. Croy. 2003. Biphasic superoxide generation in potato tubers: a self amplifying response to stress. Plant Physiol 13:1440-1449.

Kavoosi, M. 2007. Effects of zeolite application on rice yield, nitrogen recovery, and nitrogen use efficiency. Communications in Soil Science and Plant Analysis 38(2): 69-76.

Kocheva, K. V.; G. I. Georgiev and V. K. Kochev. 2005. A diffusion approach to the electrolyte leakage from plant tissues. Physiol Plant 125: 1-9.

Kramer, P. J. 1988. Measurement of plant water status: Historical perspectives and current concerns. Irrig. Sci 9: 275-287.

Kyparissis, A.; Y. Petropoulun and Y. Manetas. 1995. Summer survival of leaves in a soft-leaved shrub *(Phlomis fruticosa* L., Labiatae) under Mediterranean field conditions: Avoidance of photoinhibitory damage through decreased chlorophyll contents. J. Exp. Bot 461:1825-1831.

Leggo, P. J. 2000. An investigation of plant growth in an organo-zeolite substrate and its ecological significant. Journal of Plant and Soil 219 (7): 135-146.

Matsi, T.; A. S. Lithourgidis and A. A. Gagianas. 2003. Effects of injected liquid cattle manure growth and yield of winter wheat and soil characteristics. Agron J 95:592-596.

Mc Donald, A. J. S. and W. J. Davies. 1996. Keeping in touch: responses of the whole plant to deficits in water and nitrogen supply. Adv Bot Res 22: 229-300.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7: 405-410.

Munné-Bosch, S. and J. Peñuelas. 2003. Photo and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. Planta 217: 758-766.

Orhanl, H.; N. P. E. Vermeulen, C. Tump, H. Zappey and J. H. N. Meerman. 2004. Simultaneous determination of tyrosine, phenylalanine and deoxyguanosine oxidation products by liquid chromatography–tandem mass spectrometry as non-invasive biomarkers for oxidative damage. Journal of Chromatography B 799: 245-254.

Paglia, D. E. and W. N. Valentine. 1997. Studies on the quantitative and quantitative traits of glutathione peroxidase. Dase. J. Lab. Med 70: 158-165.

Pandey, R. K.; J. W. Maranville and M. M. Chetima. 2000. Deficit irrigation and nitrogen effects on maize in a Sahelian environment. II. Shoot growth, nitrogen up-take and water extraction. Agric. Water Manage 46: 15-27.

Rensburg, L. V. and G. H. J. Kruger. 1994. Evaluation of components of oxidative stress metabolism for use selection of drought tolerant cultivars of *Nicotiana tabacum*. L. J. Plant Physiol 143:730-737.

Semirnoff , N. and Q. J. Cumbes. 1998. Drought influence on the activity of enzymes of the chloroplast hydrogen peroxide system. J. Exp. Bot 39 : 1097-1108.

Sestak, Z. and J. Catasky. 1966. Method study photo synteticke produkee rostlin. Praha. Academia 2: 5-7.

Shalata, A.; V. Mittova, M. Volokita, M. Guy and M. Tal. 2001. Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress: The root antioxidative system. Physiol. Plant 112: 487-494.

Sivasubramaniawn, K. 1992. Chlorophyll stability index: methods for determining drought Hardness of Acacia species. Nitrogen Fixing Tree Research Reports 10:111-112.

Tohidi Moghadam, H. R.; A. H. Shirani Rad, G. Nour Mohammadi, D. Habibi and M. Mashhadi Akbar-Boojar, 2009. Effect of superabsorbent application on antioxidant enzyme activities in canola (*Brassica napus* L.) cultivars under water stress conditions. Amer. J. Agric. and Biol. Sci 4: 215-223.

Wang, S. Y.; H. Jiao and M. Faust. 1991. Changes in ascorbate, glutathione and related enzyme activities during thiodiazuron-induced bud break of apple. Plant Physiol 82: 231-236.

Wise, R. R. and A. W. Naylor. 1987. Chilling-enhanced Photo-oxidation. The peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. Plant Physiol 83:278-282.

Xiubin, H. and H. Zhanbin. 2001. Zeolite application for enhancing infiltration and retention in loess soil. Resource, Conservation and Recycling 34 (3): 45-52.

Zahedi, H. and H. R. Tohidi Moghadam. 2011. Effect of drought stress on antioxidant enzymes activities with zeolite and selenium application in canola cultivars. Research on crops 12 (2): 388-392.

Zhang, J. and M. B. Kirkham. 1996. Antioxidant response to drought in sunflower and sorghum seedlings. New Phytol 132: 361-373.

Zheng, L.; M. Y. Su, X. Wu, C. Liu, C. X. Qu, L. Chen, H. Huang, X. Q. Liu and F. S. Hong. 2008. Antioxidant stress is promoted by nano-anatse in spinach chloroplast under UV-B radiation. Biol. Trace Elem. Res 121 (1): 69-79.