Effect of cattle manure and mycorrhiza 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 micorrizas sobre los cambios fisiológicos y bioquímicos en soya [*Glycine max* (L.) Merr.] cultivada bajo estrés por déficit hídrico

Amin TAHERIANFAR [1], Hamid Reza TOHIDI MOGHADAM [1] and Hossein ZAHEDI [2] ✉

[1]Department of Agronomy, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran and [2]Department of Agronomy and Plant Breeding, Eslamshahr Branch, Islamic Azad University, Tehran, Iran.
E-mail: hzahedi2006@gmail.com ✉ Corresponding author

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: 10/14/2013

ABSTRACT

In order to study the effects of organic (cattle manure) and bio-fertilizer (mycorrhiza) applications on physiological and biochemical changes in soybean [*Glycine max* (L.) Merr.] grown under conditions of water stress, an experiment was conducted at Varamin, Iran during 2011 growing season. 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 t per hectare) and mycorrhiza application (with and without). The results showed that water stress significantly decreased relative water content and electrolyte leakage while increased antioxidant enzyme activity, malondialdehyde content, dityrosine and hydroxyguanosine. By contrast, mycorrhiza 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, it is concluded that mycorrhiza and manure application can ameliorate growth conditions for soybean plants grown under water deficit stress.

Key words: Soybean, cattle manure, mycorrhiza, water stress, enzyme activity, malondialdehyde

RESUMEN

Con el fin de estudiar los efectos de la fertilización orgánica (estiércol de ganado) y biofertilizante (micorrizas) sobre los cambios fisiológicos y bioquímicos de soya [*Glycine max* (L.) Merr.] 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 micorrizas (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 antioxidante, el contenido de malondialdehído, ditirosina e hidroxiguanosina. Por el contrario, la aplicación de las micorrizas 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 micorrizas 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, micorrizas, estrés hídrico, actividad enzimática, malondialdehído

INTRODUCTION

Water stress frequently limits growth and yield in crops (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), signalling pathways (Chaves *et al*., 2003), gene expression (Bray, 2002), and leaf photosynthesis (Flexas *et al*., 2004), have been studied extensively. It is well documented that mycorrhiza symbiosis can improve plant water relations or enhance drought resistance of host plants, thus protecting host plants against detrimental effects caused by drought stress (Augé, 2001). 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 induction of ROS-scavenging enzymes, such as superoxide dismutase, catalase, peroxidase and ascorbate peroxidase is the most common mechanism for detoxifying ROS synthesized during stress response. Activation of antioxidant capacity of host plant by mycorrhiza symbiosis may account for growth stimulation effects of mycorrhization under salinity. Reports on the response of antioxidant defence system to stress factors in mycorrhizal plants are contradictory; increase, no change, or even decrease in the activity of superoxide dismutase, catalase, peroxidase and ascorbate peroxidase were reported in mycorrhizal soybean (Porcel *et al*., 2003) subjected to drought and tomato subjected to salinity (He *et al*., 2007; Hajiboland *et al*., 2010). However, the exact role of the symbiosis in improving reactive oxygen metabolism remains unclear, though mycorrhiza-induced increases in the activities of several antioxidant enzymes are often associated with mycorrhiza-induced increases in growth and shoot (Alguacil *et al*. 2003).

On the other hand additional mechanisms have been proposed, such as enhanced osmotic adjustment and leaf hydration or reduced oxidative damage caused by the ROS generated during drought (Ruiz-Lozano, 2003). In fact, it has been shown that mycorrhizal colonization and drought interact in modifying free amino acid and sugar pools in roots (Auge´ *et al*., 1992). Diverse studies have demonstrated the protection mechanisms that mycorrhizal plants have against the detrimental effects of drought stress: increased plant leaf gas exchange, photosynthetic rate and water use efficiency (Querejeta *et al*., 2003).

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 micro organism 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 mycorrhiza 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 mycorrhizal 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 t per hectare) and mycorrhiza 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.

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 was purchased from Pars Abad Oil Seed Company and inoculated with *Rhizobium japonicum* and mycorrhiza and the sown in certain experimental plots with 10 cm apart each other. Irrigation was performed

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

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). RWC were calculated by following formula:

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

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 H2O2 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 H2O2 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 mycorrhiza application were significant on all measured traits (Table 2). In addition, interaction between these three factors on all traits except for chlorophyll content was statistically significant at 0.01 probability level. Interaction between cattle manure application and mycorrhiza inoculation was significant on chlorophyll content (Table 2). As can be seen from Table 3, water stress significantly decreased relative water content. The results showed that cattle manure application or mycorrhiza inoculation had not significant effect on RWC under normal irrigation conditions. By contrast, under stress conditions, mycorrhiza and cattle manure application improved RWC. However, increase in cattle manure up to 30 t per hectare 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).

Colla *et al*. (2008) proved that the inoculation of mycorrhiza fungi under stress can increase leaf relative water content of the squash. He (2007) reached the same result on tomato seedlings by inoculating mycorrhiza fungi. Increase in RWC due to mycorrhiza application could be attributed to primary drought-avoidance mechanisms, such as the active water transfer from mycorrhiza fungi to the host (Porcel and Ruiz-Lozano, 2004) or increased water uptake related to mycorrhizal changes in root morphology (Ibrahim *et al*., 1990) or the greater water-retention properties of an amended soil (Caravaca *et al*., 2002). The highest catalase activity was obtained when soybean plants were exposure to water stress and not treated with manure or mycorrhiza, while the lowest activity was related to those plants which were treated with mycorrhiza and cattle manure under normal irrigation conditions (Table 3).

It is notable that under limited irrigation conditions, when mycorrhiza was applied along with 30 t 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 mycorrhiza 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. It has been reported that mycorrhizal soybean plants subjected to drought had lower oxidative damage to lipids and proteins in nodules than non-mycorrhizal plants, and this was linked to protection against nodule senescence (Ruiz-Lozano *et al*., 2001; Porcel *et al*., 2003). In this study mycorrhiza and cattle manure application decreased antioxidant enzyme activity. A similar response was observed by Porcel *et al*., (2003) in mycorrhizal

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 mycorrhiza.

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	**	*	**	**	**	*	*	**	**
Error (a)	2									
Cattle manure (CM)	2	**	**	**	**	**	**	**	**	*
Mycorrhiza (M)	1	*	**	**	**	**	**	**	**	**
WS x CM	2	ns	ns	**	**	*	**	**	**	**
WS x M	1	ns	ns	**	**	ns	ns	ns	**	**
CM x M	2	**	**	**	**	*	**	**	**	**
WS x CM x M	2	**	ns	**	**	**	**	**	**	**
Error (b)	20									
C.V (%)		2.08	12.10	7.45	9.29	6.35	7.72	7.51	11.96	5.66

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 mycorrhiza on physiological and biochemical attributes of soybean [*Glycine max* (L.) Merr.] cv. Williams.

WS	CM	M	RWC	Cat	GP	SD	Mal	Dit	Hyd	EL
No stress	0 t ha^{-1}	No	76.77ab	34.87b	15.55b	15.57b	15.7c	8.75d	6.48ef	747.5d
		Yes	77.79ab	20.67c	9.68c	10.43c	22.2b	13.91c	8.76de	755.5d
	15 t ha^{-1}	No	78.08ab	20.87c	8.42c	10.44c	21.03b	13.59c	8.81de	717.5de
		Yes	79.06a	12 d	5.52d	7.57d	15.57c	8.53d	5.4f	640.67e
	30 t ha^{-1}	No	77.09ab	12.2d	4.99d	7.81d	17.47c	9.88d	7.73ef	717.67de
		Yes	76.12ab	13.7d	3.24e	4.7e	15.87c	8.23d	5.99f	638.67e
Water stress	0 t ha^{-1}	No	67.21d	49.97a	22.2a	19.31a	36.63a	22.52a	20.74a	1386.5a
		Yes	75.47b	35.2b	16.73b	15.42b	36.33a	21.49a	17.86b	911c
	15 t ha^{-1}	No	70.3c	36.13b	17.17b	15.76b	37.43a	22.89a	19.1ab	1287a
		Yes	75.94b	23.17c	9.5c	10.08c	23.7b	16.25b	10.75cd	1147.67b
	30 t ha^{-1}	No	67.14d	22.3c	9.72c	10.25c	23.5b	15.25bc	18.68ab	1336a
		Yes	70.36c	12.9d	5.33d	7.67d	36.17a	22.78a	12.43c	1310a

WS: Water stress: CM: Cattle manure; M: Mycorrhiza; RWC: Relative water content (%); 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}).
Treatment means followed by the same letter within each common are not significantly different ($p < 0.05$) according to Duncan's Multiple Range test.

soybean plants that showed lower ascorbate peroxidase activity values relative to the corresponding non-mycorrhizal plants. The decrease in antioxidant enzymes observed in both plants inoculated with mycorrhiza 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.

The highest malondialdehyde content was observed in water stressed plants (Table 3). 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. With the water stress, leaf malondialdehyde content increased. However, the malondialdehyde content in mycorrhizal plants remained lower than that in non mycorrhizal plants, which shows that the presence of the mycorrhiza fungus could alleviate the peroxidation of membrane lipids. 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). Mycorrhiza 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 mycorrhiza application decreased electrolyte leakage when 0 or 15 t per hectare cattle manure was applied. Similar results were obtained under normal irrigation conditions (Table 3). EI-Tohamy *et al*. (1999) indicated no significant effects of electrolyte leakage in mycorrhizal and non mycorrhizal beans under low temperature stress. However, in this study, there were significant differences between mycorrhizal and non mycorrhizal plants in leaf membrane relative permeability under water stresses. Moreover, membrane relative permeability of mycorrhizal plants was lower than that of non mycorrhizal plants. All this shows that mycorrhiza fungi can lower the plant's membrane permeability and alleviate the damage on the cell membrane caused by water stress.

The highest chlorophyll content was related to those plots which were treated with mycorrhiza and 15 or 30 t per hectare cattle manure. It is worth mentioning that application of 30 t per hectare cattle manure could produce the highest chlorophyll content without mycorrhiza application (Figure 1). The literature reports that mycorrhiza fungi play a very important role in terms of chlorophyll content (Paradis *et al*. 1995). 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.

CONCLUSIONS

The effects of water stress, cattle manure and mycorrhiza application were significant on all traits. In addition, interaction between these three factors on all traits except for chlorophyll content was statistically significant. Interaction between cattle manure application and mycorrhiza inoculation was significant on chlorophyll content. The results showed that cattle manure application or mycorrhiza inoculation had no significant effect on relative water content under normal irrigation conditions. By contrast, under stress conditions, mycorrhiza and cattle manure application improved relative water 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 mycorrhiza and cattle manure has been applied. The highest malondialdehyde, dityrosine and hydroxyguanosine were observed in water stressed plants. The highest electrolyte leakage was occurred

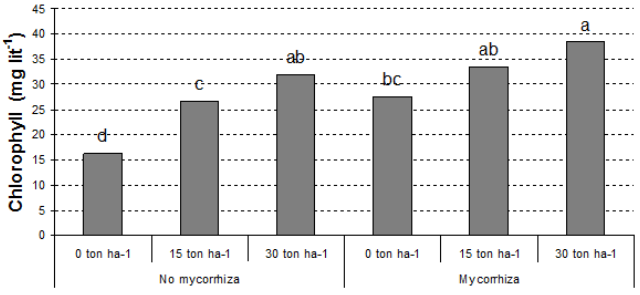

Figure 1. Interaction between cattle manure and mycorrhiza application on chlorophyll content of soybean [*Glycine max* (L.) Merr.] cv. Williams Treatment means followed by the same letter within each common are not significantly different ($p < 0.05$) according to Duncan's Multiple Range test.

when soybean plants were stressed. The highest chlorophyll content was related to those plots which were treated with mycorrhiza and 15 or 30 t per hectare cattle manure. It is concluded that mycorrhiza and manure application can ameliorate growth conditions for soybean plants grown under water deficit stress.

LITERATURE CITED

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

Alguacil, M. M.; J. A. Hernández, F. Caravaca, B. Portillo and A. Roldán. 2003. Antioxidant enzyme activities in shoots from three mycorrhizal shrub species afforested in a degraded semi-arid soil. Plant Physiology 118: 562-570.

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.

Augé, R. M.; J. G. Foster, W. H. Loescher and A. W. Stodola. 1992. Symplastic sugar and free amino acid molality of Rosa roots with regard to mycorrhizal colonization and drought. Symbiosis 12: 1-17.

Augé, R. M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3-42.

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

Caravaca, F.; J. M. Barea, D. Figueroa and A. Roldan. 2002. Assessing the effectiveness of inoculation with mycorrhizal fungi and soil compost addition for reforestation with *Olea europaea* subsp. *sylvestris* through changes in soil biological and physical parameters. Appl. Soil Ecol 20: 107-118.

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.

Colla, G.; Y. Rouphael, M. Cardarelli, M. Tullio, C. M. Rivera and E. Rea. 2008. Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. Biol. Fertil. Soils 44: 501-509.

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.

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.

EI-Tohamy, W.; W. H. Schnitzler, U. EI-Behairy and M. S. EI-Beltagy. 1999. Effect of VA mycorrhiza on improving drought and chilling tolerance of bean plants (*Phaseolus vulgaris*). J Appl Bot 73:178-183.

Flexas, J.; J. Bota, F. Loreto, G. Cornic and T. D. Sharkey. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 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.; C. He, Z. Zhang, Z. Zou and H. Wang. 2007. Changes of antioxidative enzymes and cell membrane osmosis in tomato colonized by arbuscular mycorrhiza under NaCl stress. Colloids Surf. B: Biointerfaces 59: 128-133.

Ibrahim, M. A; W. F. Campbell; L. A. Rupp and E. B. Allen. 1990. Effects of mycorrhizae on sorghum growth, photosynthesis, and stomatal conductance under drought conditions. Arid Soil Res. Rehab 4: 99-107.

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

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.

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.

Paradis, R; Y. Dalpé and C. Charest .1995. The combined effect of arbuscular mycorrhiza and short-term cold exposure on wheat. New Phytol 129:637-642.

Porcel, R.; J. M. Barea and J. M. Ruiz Lozano. 2003. Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. New Phytol 157: 135-143.

Porcel, R. and J. M. Ruiz Lozano. 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. J. Exp. Bot 55: 1743-1750.

Querejeta, J. I.; J. M. Barea, M. F. Allen, F. Caravaca and A. Roldán. 2003. Differential response of d13C and water use efficiency to arbuscular mycorrhizal infection in two arid land woody plant species. Oecologia 135: 510-515.

Ruiz Lozano, J. M.; C. Collados, J. M. Barea and R. Azcón. 2001. Arbuscular mycorrhizal symbiosis can alleviate drought-induced nodule senescence in soybean plants. New Phytologist 151: 493-502.

Ruiz Lozano, J. M. 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress: new perspectives for molecular studies. Mycorrhiza 13: 309-317.

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