Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2017, 7(4), 325–329, doi: 10.15421/2017_123

ORIGINAL ARTICLE

The effect of mycorrhiza and vermicompost bio-fertilizers on some physiological characteristics of sweet basil plant (*Ocimum basilicum* L.) under the stress condition caused by water deficit

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Submitted: 02.11.2017. Accepted: 11.12.2017

One of the main limiting factors in crop production in different regions of Iran is water deficit stress. Mycorrhiza and vermicompost fertilizer may be help plants to uptake more water. Therefore, a greenhouse experiment was carried out in factorial design that was carried out to evaluate the effect of Mycorrhiza, vermicompost fertilizer and water deficit stress on some physiological traits of sweet basil (*Ocimum basilicum* L.). Factors included Mycorrhiza (inoculation and non-inoculation), Vermicompost fertilizer (0, 30, 50, and 70 percentage of pod volume), and water deficit stress factor included irrigating up to 60% of F.C as water deficit stress treatment, up to 75% of F.C as mild water deficit stress treatment and up to 90% of F.C as well irrigated treatment. The results showed that sugars and proline increased with increasing water deficit stress, while higher vermicompost consumption decreased the severity of the increase. Merging vermicompost, and mycorrhiza, had a synergistic effect on the catalase enzyme and chlorophyl a+b. Significant negative and positive relationships were found between shoot dry weight and proline with water deficit stress. In addition, basil plant physiological responses to drought stress showed that this stress-sensitive plants, tried to adjust to stress, through osmotic adjustment and increasing antioxidant activity. The results totally showed that merging mycorrhizal inoculation and vermicompost mitigate the effects of drought stress in Basil.
Key words: Water deficit stress; sugars; catalase enzyme; peroxidase enzyme; dry matter.

Introduction

Basil is a plant of the family Lamiaceae, which is used in the pharmaceutical, food, cosmetic and health industries.
Soluble sugars accumulate in dry conditions and act as protective factors in plants. In stress conditions, sugars protect the cells through osmotic adjustment and maintain turgor and stability of membranes and proteins. During the dehydration of cells, sugars cause tolerance to drought, through the cytoplasm being glass (Bartels and Sunkar, 2005). Proline and soluble sugars accumulation is important as a defense mechanism in osmotic adjustment in plants (Irigoyen et al., 1992; Chegeni-Bahrami et al., 2013; Mattioni et al., 1997). Leaf proline content increases by reducing soil moisture (Chegeni-Bahrami et al., 2013; Hassani et al., 2003). Various studies have shown that stress causes an increase in proline levels in Pelargonium graveolens (Khalid et al., 2006), amaranth (Amaranthus) (Cunhua et al., 2011), and basil (*Ocimum basilicum* L.) (Chegeni-Bahrami et al., 2013). Water stress induces severe degradation in protein content of soybean leaves (Purcell and King, 1996). Water channel proteins such as acupurine and macromolecules such as proteins, have the role of protective factors, increased with drought stress (Boyer et al., 1997), while dehydration cause protein degradation and destruction of complex macromolecules and to stop construction typical set of proteins in the translation stage (Kafi and Damghani, 2003). Irigoyen et al., (1992) reported that protein degradation in mature leaves cause reduction in protein, and causing an increase in free amino acid concentrations including proline. Enzymatic and non-enzymatic mechanisms against oxidative stress are other plant protection mechanisms to cope with drought stress (Tian and Lee, 2006). Peroxidase activity increases under various stress factors such as drought, cold, wounding, salinity, air pollutants and pathogens, and in any case do specific protective action. Peroxidase absorption of H2O2 and organic peroxidases that are formed under different stress conditions, are important (Vu et al., 1987). The aim of this study was to investigate the physiological response of basil to mycorrhizal inoculation, vermicompost fertilizer and water deficit stress.

Materials and methods

The Experimental Site

The experiment was carried out in 2015, in greenhouse of Lorestan Agricultural and Natural Resources Research Center, Khorramabad, Iran situated in the West of Iran, which lies at latitude 33°29' N, longitude 48°18' E, and an altitude of 1195 m above sea level.

Experimental Design and Treatments

A factorial design with three factors was used with three replications in greenhouse. Mycorrhiza factor included inoculation and non-inoculation (control). Vermicompost fertilizer factor included 0, 30, 50, and 70 percentage of pod volume, and water deficit stress factor included irrigating up to 60% of F.C as water deficit stress treatment, up to 75% of F.C as mild water deficit stress treatment and up to 90% of F.C as well irrigated treatment.

Pod Preparation and Seed Sowing

Before the beginning of experiment, soil samples were taken in order to determine the physical and chemical properties. Soil and vermicompost fertilizer were air dried, crushed, and tested for physical and chemical properties. Details of soil and vermicompost fertilizer properties are given in Tables 1 and 2, respectively.

Each plastic pod consisted of 20 cm diameter and 15 cm height. The sweet basil plant (*Ocimum basilicum* L.) seeds were sown with an average depth of 1 cm with 3 seeds per hole on october in 2015. Thinning was performed after at 2-4 leafy stage until the density reached to 8 plants pod^{-2}. All other agronomic practices were the same between treatments during the study.

Soil Water Content Measurement

Irrigation treatments were applied after 6-8 leafy stage. Prior to imposing irrigation treatments, all plots were irrigated as the same based on 90% of F.C.

The amount of water for each round of irrigation was determined using following formula to reach soil water content in root zone to field capacity:

$$V_W = (FC - \theta) \times BD \times A \times D / Ea \qquad (1)$$

Where: V_W: Water volume (m ^{3}), F.C: weight percentage of soil moisture at field capacity state (28%), θ: weight percentage of soil moisture during the irrigation (10%), BD: soil bulk density (1.3 g cm ^{3}), A: Pot area (200 cm^2), D: Root depth (m), Ea: water irrigation efficiency of 90 percent. After calculating the amount of water needed, pods were irrigated. The moisture content of the soil was determined using moisture meter before irrigation. After determining the moisture content, the certain amounts of water were transferred to the pod. At flowering stage, non-structural carbohydrates, proline (Bates et al., 1973), total protein (Bradford, 1976), peroxidase and catalase (Chance and Maehly, 1955), and chlorophyll a+b (Dere *et al.*, 1998) were measured in shoot.

Statistical Analysis

The recorded data were statistically analyzed using the software SAS (SAS, 2003). Comparisons of means were performed using Graphpad Prism 5.

Results and discussion

Proline and Total Protein

Proline was significantly affected by vermicompost, water deficit stress, and vermicompost and water deficit stress interaction (Table 3). Amount of proline increased significantly by increasing water deficit stress. The amount of proline in vermicompost consumption of 0, 30, 50, and 70 levels, increased 42, 11, 12, and 40 percentage from normal condition (90% F.C) to high water deficit stress (60% F.C), respectively (Fig. 1a). It means that this increasing decreased with higher amount of vermicompost fertilizer consumption. Total protein decreased by increasing water deficit stress in all levels of vermicompost consumption (Table 3, Fig. 1b). Because higher vermicompost consumption reduced stress, sweet basil plant didn't need to increase proline for osmotic adjustment. Our results are in agreement with findings of Aracon et al. (2004) who investigated the effect of vermicompost to reduce water deficit stress. Water deficit stress increased proline (Khalid, 2006; Aslani et al., 2009) and decreased total protein (Boyer et al., 1997; Good and Stonet, 1994) as many previous studied have reported.

Non Structural Sugars

Vermicompost, and vermicompost and water deficit stress interaction effects significantly on non-structural sugar (Table 3). The lowest sugar achieved from irrigation up to 90% F.C in different levels of vermicompost, and increased significantly by water deficit stress. This increasing was 33% in control treatment and achieved to 27% in high fertilizer consumption (Fig. 1c).

Table 1. Chemical characteristics of substrate soil

Texture	K	P	Organic carbon (%)	T. N. V	mg kg^{-1}	mg kg^{-1}	pH	F.C (%)	EC × 10^3
Silt loam	230	11	1.22	28			7.3	27	0.55

Table 2. Chemical characteristics of vermicompost fertilizer

Cu	Zn	Mn	Fe	Mg	Ca	K	P	N	EC	PH
Mg/kg									ds/m	
30	125	650	1500	1200	33000	4400	4230	6600	4.9	8.03

Table 3. Analysis of variance for some physio logical traits i n basil

SOV	df	MS				
		Proline	Total protein	Non structural sugar	Catalase	Peroxidase
Mycorrhiza (M)	1	2.27E-05	2.97E-05	0.000154	0.011**	0.0058**
Vermicompost (V)	3	0.0046**	0.00118	0.0086**	0.016**	0.055**
Water deficit stress (S)	2	0.057**	0.04**	0.104919	0.0036**	0.002*
M*V	3	0.000757	0.000329	0.002812	0.0032**	0.000326
M*S	2	0.001423	0.000674	0.000223	0.00059*	5.7E-06
V*S	6	0.0054**	0.0047**	0.0098**	0.00055**	0.000179
M*V*S	6	0.000288	0.000785	0.001944	0.00015	0.000272
Ea	48	0.000519	0.000853	0.001379	0.000175	0.00042
CV (%)		7.81	9.91	7.37	2.44	2.6

*and ** significant at 5 and 1 percentages, respectively.

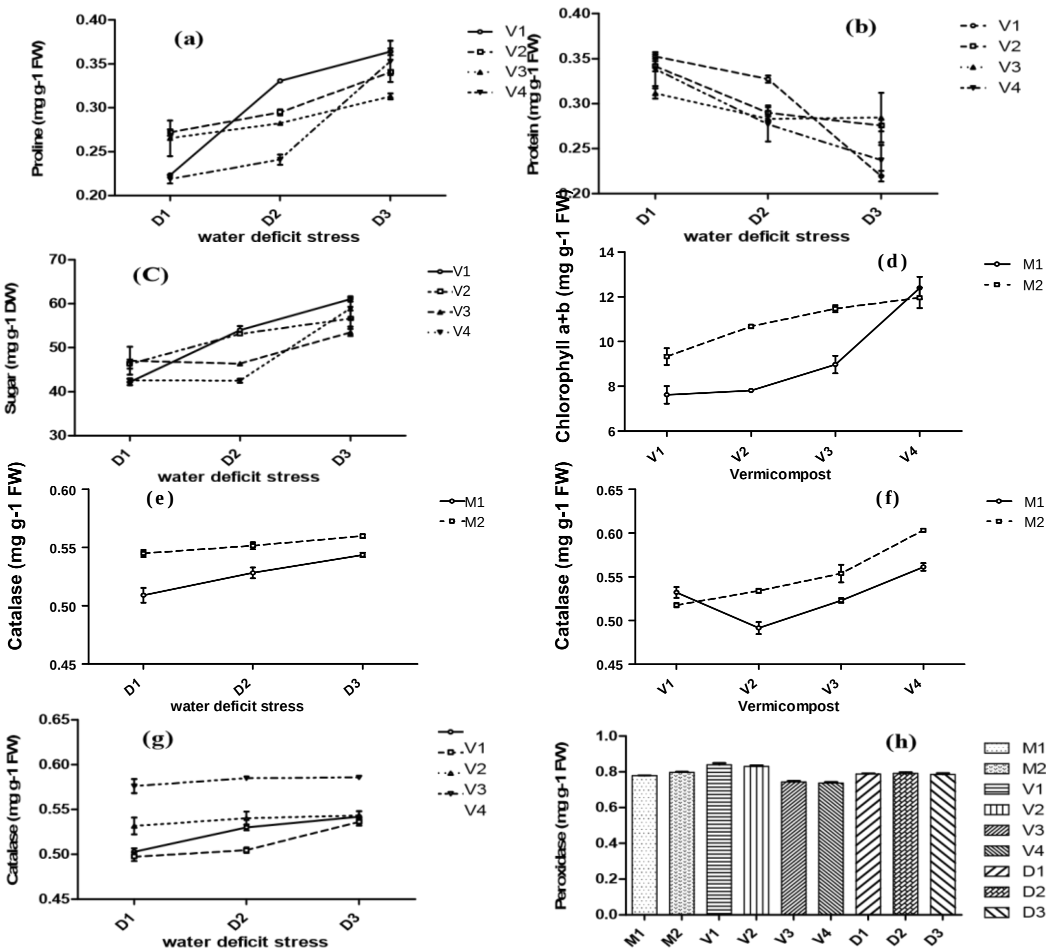

Fig. 1. Effect of mycorrhiza, vermicompost and water deficit stress on some biochemical traits of basil. M1 and M2: mycorrhiza inoculation and non-inoculation (control), respectively. V1, V2, V3 and V4: 0, 30, 50, and 70 vermicompost fertilizer percent of pod volume, respectively. D1, D2 and D3: water deficit stress factor included irrigating up to 60%, 75% and 90% of F.C, respectively

Sugar increased significantly by water deficit stress, while the intensity increasing decreased with higher amount of vermicompost fertilizer consumption. It indicated that higher amount of vermicompost fertilizer decreased water deficit stress damages due to higher water conservation capacity (Dorzi and Hajseyedhadi, 2003), so sweet basil plant didn't need to increase nonstructural sugars for osmotic adjustment. Increase in nonstructural sugars due to water deficit stress is also reported by Khalid (2006), Hasani et al. (2003), and Aslani et al. (2009). Mycorrhiza inoculation had no effect on non-structural sugars in this study.

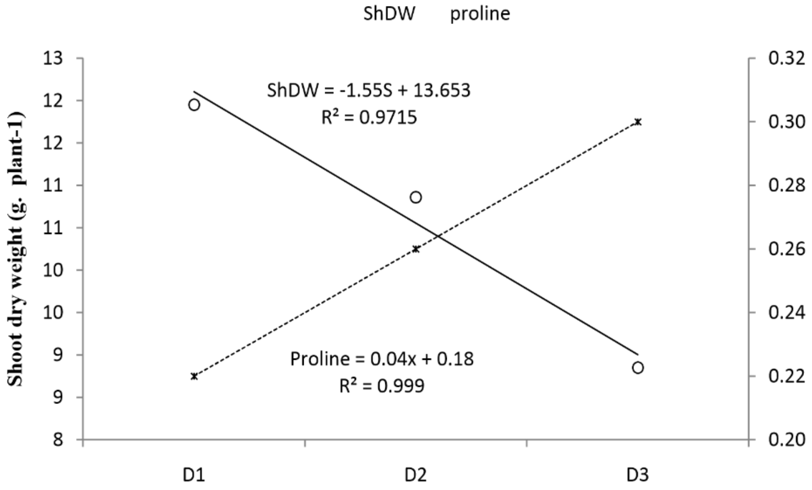

Fig. 2. Relationship between shoot dry weight and proline as affected by water deficit stress. D1, D2 and D3: water deficit stress factor included irrigating up to 60%, 75% and 90% of F.C, respectively

Antioxidants Shoot Dry Weight and Proline Relationship

Peroxidase levels significantly affected by mycorrhiza, vermicompost and water deficit stress (Table 3). Mycorrhiza inoculation increased levels significantly from 0.78 to 0.80 mg. g-1 FW, but using vermicompost fertilizer decreased levels from 0.844 to 0.734 mg. g-1 FW. Water deficit stress increased peroxidase levels significantly from 0.69 in normal condition to 0.71 mg. g-1 FW in stressful condition (Fig. 1h). Our results are in agreement with findings of Tian and Lee (2006) who found increasing peroxidase in water deficit stress condition.

(Khalid, 2006; Aslani et al., 2009) which are in agreement with our findings.

Conclusion

By increasing drought stress, the amount of sugar and proline increased, while this increasing decreased with higher amount of vermicompost fertilizer consumption. Drought stress decreased chlorophyll a + b. Merging mycorrhizal inoculation and vermicompost consumption had synergistic effect on the catalase and chlorophyll a + b. Peroxidase and catalase were increased by mycorrhiza inoculation in basil. Vermicompost consumption decreased peroxidase enzyme in the plant. Significant negative and positive relationships were found between shoot dry weight and proline with water deficit stress. The results totally showed that merging mycorrhizal inoculation and vermicompost mitigate the effects of drought stress in Basil.

Competing interests

Authors have declared that no competing interests exist.

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Citation:
Azin Ghavami, Vahid Abdossi, Masoud Rafiee, Ahmad Khalighi (2017). The effect of mycorrhiza and vermicompost bio-fertilizers on some physiological characteristics of sweet basil plant (Ocimum basilicum L.) under the stress condition caused by water deficit.
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