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Physiological response of Fenugreek plant to the application of proline under different water regimes.

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ABSTRACT

The aim of the present work is to study the effect of foliar application with proline at different water holding capacities (WHCs) on growth, some biochemical compounds, yield components and some antioxidant components of the yielded seeds of Fenugreek plants and also to raise the efficiency of Fenugreek plants to resist water stress by reducing the water amount used for irrigation. The experiment was carried out in the Greenhouse of National Research Centre using Fenugreek cv. Giza 30. Three concentrations of proline were sprayed at 30 days from sowing. The plants were raised at different levels of WHC 100%, 75% and 50%. Data revealed that irrigation of Fenugreek plants with low WHC 75% and 50% leads to decrease in all growth parameters and yield components accompanied with the decrease in IAA and photosynthetic pigments. However, free amino acids and proline contents were increased under low level of WHCs. Growth of Fenugreek plant under different WHC which pretreated with proline led to increases in all growth parameters and yield components concomitantly with increases in the levels of IAA, photosynthetic pigments content, free amino acids and proline contents of Fenugreek plants. In addition, proline 25.0 mM treatment increased antioxidant components of the yielded seeds grown under different WHC as compared with control plant. As a conclusion, cultivation of Fenugreek plant with foliar treatment of proline was effective in overcoming on the inhibitory effect of low WHC.

Key words: Fenugreek, Water regimes, Proline, Antioxidant activity, Flavonoids, Tannins, IAA., andfree amino acids.

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INTRODUCTION

Abiotic stress conditions such as drought, heat, salinity, etc. are major threats to agriculture and reduce growth and cause severe loss of crop yield due to different physiological, morphological and molecular level changes (Boyer, 1992). Among the different a biotic stresses drought is one of important factor which restrict growth and yield of plant (Tas&Tas, 2007) . The decrease in water content, decreased water potential of leaf and loss of turgor and cell enlargement decrease all of these caused drought stress. The decline of photosynthesis, metabolism disturbance and finally the death of the plant are the result of high water stress(Jaleel *et al.,* 2008). In addition, the response of plants to water stress vary significantly depending on the duration and intensity of stress as well as species of plant and the growth stage of plant (Dacosta& Huang, 2007).

One of the viable strategies of enhancing stress tolerance to adverse environmental stresses is by using osmoprotectants. Treatment with osmoprotectants as seed soaking or as a foliar treatment can be an economically viable strategy to enhance stress tolerance under adverse environmental conditions (Ali *et al*., 2007; Ashraf and Foolad, 2007; Ali and Ashraf, 2011 delhamid *et al*., 2013 and Sadak & Mostafa., 2015). Proline is one of the compatible osmolytes induced by stress in plants. Several functions are proposed for the accumulation of proline in tissues exposed to stress: C and N reserves for growth after stress relief(Hayashi *et al*., 2000), anti-oxidation(Hoque*et al*., 2007), the stabilisation of proteins and membranes and the protection of macromolecules from denaturation (Hamilton &Heckathorn, 2001), osmoprotection (Kishor*et al.*, 1995), and as a reducing power and available energy source (Stewart *et al*., 1974). The effect of proline is dependent on its concentration. So, it is important to determine the optimum concentration of proline exogenously applied which lead to improvement of plant tolerance of different crops as Fenugreek under a biotic stress. Futhermore, the effectiveness of proline applied as a foliar spray depends on the type of species, plant developmental stage, time of application and on the concentration[6]. Water stress resulted in a significant accumulation of free proline content in leaves of groundnut cultivars (Ranganayakulu et al., 2015).

Fenugreek (*Trigonellafoenumgraecum* L.) is an annual herb that belongs to the family *Leguminosae* widely grown in Egypt and Middle Eastern countries, and is a widely known medicinal semi-arid crop, cultivated worldwide (Al-Saady*et al.*, 2012 and Sadak 2016)**.** Due to its strong flavor and aroma fenugreek is one of such plants whose leaves and seeds are widely consumed as a spice in food preparations, and as an ingredient in traditional medicine. It is rich source of calcium, iron, â-carotene and other vitamins (Sharma *et al*., 1996). Both leaves and seeds should be included in normal diet of family, especially diet of growing kids, pregnant ladies, puberty reaching girls and elder members of family because they have haematinic (i.e. blood formation) value (Ody, 1993). The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol absorption and to help lower sugar levels. In addition, all extracts of the fenugreek seeds (methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate) exhibit antioxidant activity because of phenolic acids and Flavonoidsss, the phenolic compounds ranged from 1.35 to 6.85 mg/g and total Flavonoidsss are in the range from 208 to 653 µg/g according to the extract type(Bukhari *et al*., 2008)**.** Furthermore, intercropping fenugreek with faba bean can reduce *Orobanchecrenata*infection **(**Fernández-Aparicio*et al*., 2006).

This study was conducted to investigate the effects of exogenous applications of proline on various growth parameters, on the concentrations of endogenous proline, photosynthetic pigments, yield and some biochemical compounds of the yielded seeds in fenugreek plants grown under three levels of irrigation water (drought stress). The aim was to improve our understanding of the mechanisms of drought stress tolerance in proline-treated plants.

MATERIALS AND METHODS

Experimental Conditions, Plant Materials, Growth and Treatment Conditions:

Seeds of Fenugreek (*Trigonellafoenumgraecum*) cv. Giza 30 were obtained from Agriculture Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Seeds were grown in Pots (diameter 30 cm) at two successive seasons (2012/2013 and 2013/2014), filled with clay and sand soil with the ratio of 2:1.

Treatments of WHC were started after 45 days from sowing. Irrigation treatments were given to plants with different levels of water holding capacity (W HC) 100%, 75% and 50%. Proline concentrations (0, 12.5, 25.0 and 50.0 mM) were sprayed after 30 and 37 days of sowing. Fertilization with super phosphate (5 g / pot), potassium sulfate (2.5 g / pot) and urea (6 g / pot) were used.

Experimental Design, Growth and Yield & Yield components:

The pot experiment was conducted in the greenhouse of Botany Department, National Research Center. Experimental design was complete randomized blocks. Samples were taken after 60 days after sowing to analyze crop performed in terms of growth parameters, indole acetic acid (IAA), photosynthetic pigment comtent (chlorophyll a, chlorophyll b and carotenoids), total free amino acids and proline contents. Each treatment was replicated four times and each replicate had three plants. Three healthy plants were left in each pot to determine number of pods/ plant, number of seeds/pod weight of seeds/ plant, and seed index were determined. Air dried seeds were ground into a fine powder and kept in desiccators for chemical analysis.

Chemical Analysis:

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) of fresh leaves were determined according to Moran (1982). Indole acetic acid content were extracted and analyzed by the method of Larsen *et al*., (1962). Free amino acid content was extracted according to the method described by Vartanain*et al*. (1992). Free amino acid was determined with the ninhydrin reagent method (Yemm and Cocking, 1955). Proline was assayed according to the method described by Bates *et al*. (1973).

Seed chemical analysis: Seeds were powdered to determine proteins, carbohydrates, phenolic, Flavonoidsss and tannins contents. Protein contents were determined by microkjeldahl method according to Miller and Houghton (1945). Total carbohydrates were determined calorimetrically according to the method of Dubois *et al.* (1956). Total phenol content was measured as described by Danil and George (1972). Total Flavonoidss contents were measured by the aluminum chloride colorimetric assay as described by Ordoñez*et al*. (2006). Tannins were determined using the modified vanillin hydrochloric acid (MV-HCl) as reported by Maxson and Rooney (1972). The free radical scavenging activity was determined according to Brand- Williams *et al*. (1995) using the 1.1-diphenyl-2-picrylhydrazil (DPPH) reagent.

Statistical Analysis:

The results were statistically analyzed using MSTAT-C (1988) software. The mean comparisons among treatments were determined by Duncan's multiple range test at 5 $P \leq 0.05$.(Gomez and Gomez, 1984).

RESULTS

Plant Growth:

Exposure of plants to 75% and 50% of WHC leads to marked decreases in all vegetative parameter studied (plant height, leaves number, fresh and dry weight of shoots) when compared to plants grown under the level of 100% water holding capacity (Table 1). Treatment of Fenugreek plants with different concentrations of proline increased all growth criteria under different water levels at (100, 75 &50% WHC) as compared with the untreated plants. The maximum increases in all the growth criteria were obtained by using 100% water holding capacity.25.0 mM proline treatment was the most effective treatment in the studied parameters of plant growth under all water holding capacity.

Yield and Yield Components:

Decreasing WHC decreased yield components (no of pods/plant, wt of pods / plant, wt of seeds / plant and seed index) (Table 2). Spraying Fenugreek plant with proline showed that different concentrations of prolineincreased yield components of the Fenugreek as compared with the corresponding water stress levels. The most effective treatment was 25mM of proline under different water holding capacity levels.

Biochemical analysis of Fenugreek plant:

Photosynthetic Pigments:

Data in Table (3) show the response of photosynthetic pigment of Fenugreek leaves sprayed with different concentrations of proline and subjected to different levels of WHC. The decrease of the WHC up to 50% led to significant decreases in (chlorophyll a and chlorophyll b and total pigments), meanwhile increased carotenoids contents significantly as compared to control plants. Results also reveal that, significant increases in all photosynthetic pigment contents in response to treatment with different concentrations of proline,under WHC condition or under water stress. The most effective treatment was 25 mM proline as it gave the highest increases in all photosynthetic pigments.

Table (1): Effect of different concentrations of proline on morphological criteria of Fenugreek plants subjected to different levels of water holding capacity (at 60 days after sowing, means of two seasons).

Treatment		Shoot length (cm)	Leaves no/plant	Shoot dry wt (g)
WHC	Proline (mM)			
100%	0	22.00	8.33	0.97
	12.5	23.33	10.33	1.283
	25.0	32.33	13.00	2.84
	50.0	25.33	11.00	2.26
75%	0	20.33	8.00	0.67
	12.5	22.00	9.33	0.76
	25.0	26.67	12.00	1.53
	50.0	24.00	9.67	1.078
50%	0	17.33	7.67	0.41
	12.5	18.33	8.33	0.81
	25.0	22.00	12.67	1.47
	50.0	20.67	11.67	0.97
LSD at 5%		2.25	0.88	0.14

Table (2): Effect of different concentrations of proline on yield components of Fenugreek plants subjected to different levels of water holding capacity. (At 60 days after sowing, means of two seasons).

Treatment		Pods	Seeds no/pod	Seeds wt/plant	Seed index
WHC	Proline (mM)	no/plant			
100%	0	6.67	5.33	2.689	5.154
	12.5	7.67	6.33	3.717	5.687
	25.0	9.33	8.33	5.727	6.321
	50.0	8.33	6.67	4.104	5.325
75%	0	5.33	5.00	1.57	4.986
	12.5	6.33	5.67	2.787	5.321
	25.0	8.67	7.67	3.983	6.024
	50.0	7.67	5.67	3.069	4.325
50%	0	4.00	3.67	0.859	3.254
	12.5	5.33	4.33	1.532	4.035
	25.0	6.67	6.33	2.637	4.987

	50.0	6.00	5.67	1.973	3.986
LSD at 5%		0.246	0.354	0.175	0.187

Table (3): Effect of different concentrations of proline on photosynthetic pigments of Fenugreek plants subjected to different levels of water holding capacity. (At 60 days after sowing).

Treatment		Chlo a	Chlo b	Carot	Total pigments
WHC	Proline (mM)				
100%	0	1.489	0.806	0.231	2.5206
	12.5	1.652	0.802	0.262	2.716
	25.0	1.851	0.941	0.261	3.053
	50.0	1.661	0.867	0.248	2.776
75%	0	1.292	0.706	0.261	2.259
	12.5	1.366	0.743	0.263	2.372
	25.0	1.483	0.756	0.254	2.493
	50.0	1.338	0.743	0.259	2.340
50%	0	0.985	0.668	0.287	1.940
	12.5	0.991	0.687	0.268	1.946
	25.0	1.143	0.702	0.275	2.120
	50.0	0.985	0.698	0.267	1.950
LSD at5%		0.234	0.068	0.065	0.849

Endogenous IAA:

Data in Fig (1) show the variation in growth promoter (indole acetic acid) in response to spraying Fenugreek plant with different concentrations of proline which subjected to different levels of WHC. The WHC decreased from 100% to 50% caused marked significant decreases in IAA, as compared with those of the corresponding controls. Spraying Fenugreek plants with different concentrations of proline significantly increased growth promoter IAA especially at 25 mM as compared with the corresponding control plants.

Free amino acids content:

The obtained data presented in Fig (1) showed the effect of proline treatment on free amino acids of Fenugreek plant grown under low WHC. Data clearly show that lowering of WHC to 75% and 50% caused significant increases in free amino acid content, foliar treatment with different concentrations of proline caused significant increases of free amino acids contents as compared with the corresponding WHC levels. The most effective treatment was 25 mM proline especially at 50% WHC.

Proline content:

Fig (1) shows that WHC levels (75% & 50%) caused significant increases in the proline contents of Fenugreek plant compared with control plants. In addition, proline treatments at 100% WHC increased proline contents at all concentrations. Meanwhile at lower WHC it caused significant decreases in proline contents as compared with the corresponding WHC (controls).

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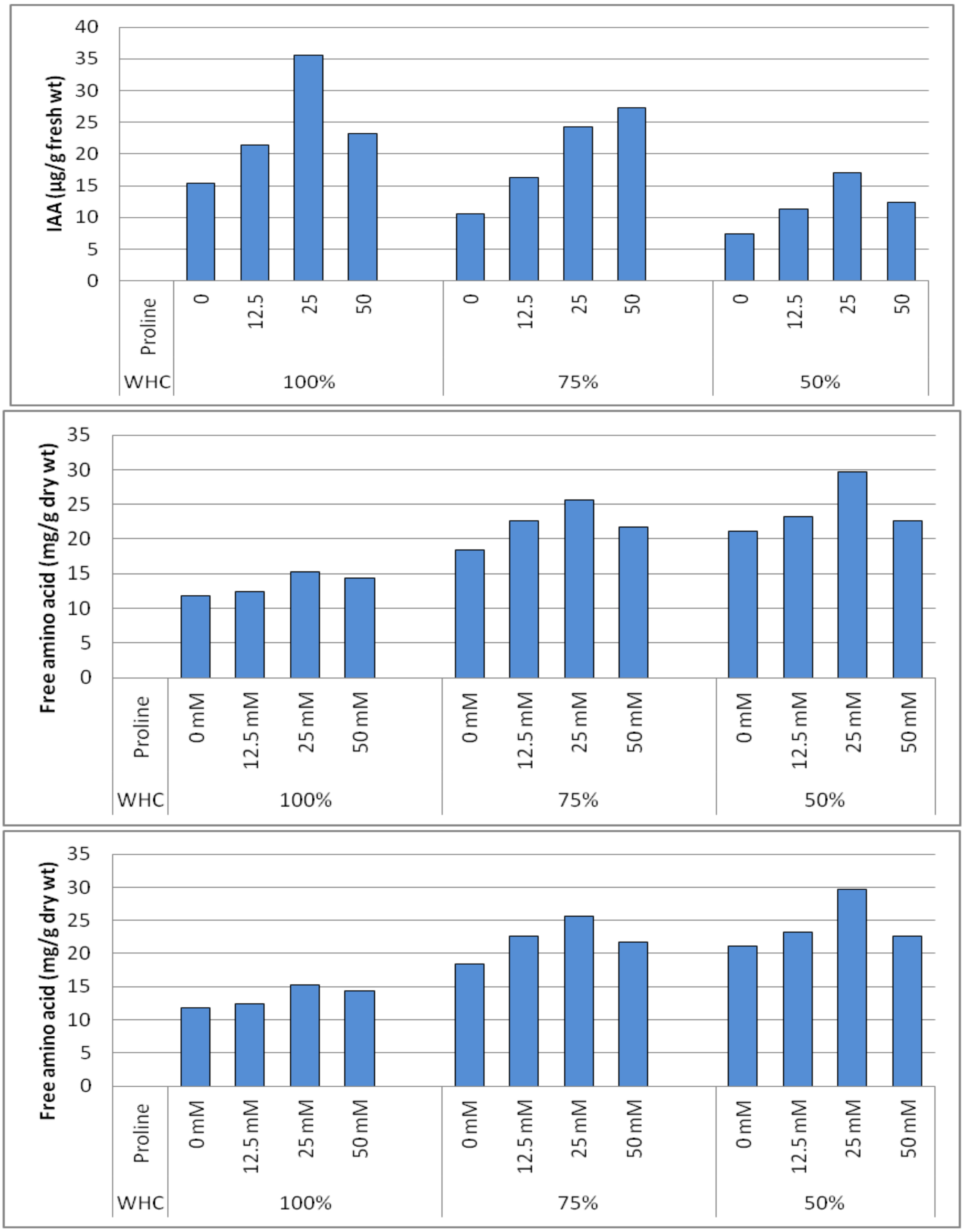


Fig (1): Effect of different concentrations of proline on IAA as µg/g fresh wt, free amino acids mg/g dry wt. and proline as mg/g dry wt.of Fenugreek plants subjected to different levels of water holding capacity. (LSD at 5%: IAA: 1.46, free amino acids: 2.35and proline: 0.08)

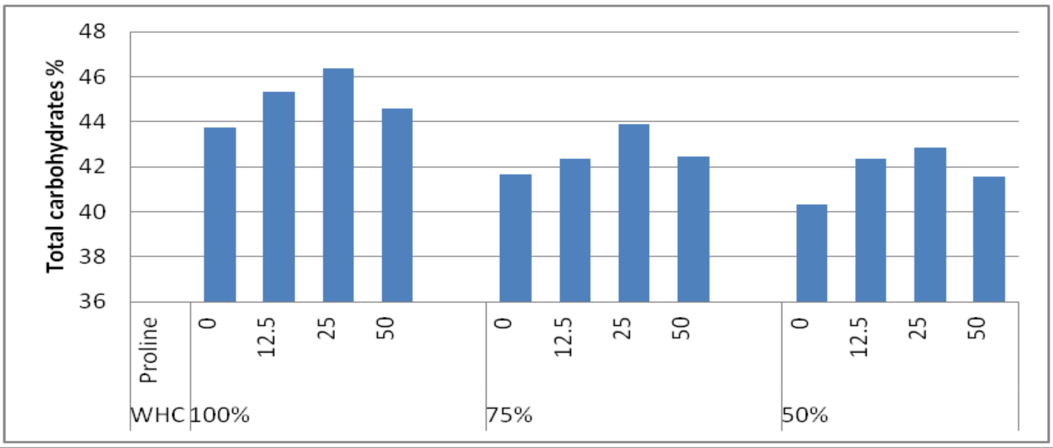
Biochemical constituents of the yielded Fenugreek seeds:

Carbohydrate Contents:

Data in Fig (2) demonstrate that, field capacity up to 50% led to marked decrease in total carbohydrates of the yielded seeds compared to plants grown under 100% of field capacity. Foliar proline treatment of Fenugreek plant under different WHC (100%, 75% and 50 WHC) led to marked increases in total carbohydrates when compared with the corresponding controls especially at 100% WHC.

Protein Contents:

Fig (2) show that, the decrease of WHC (up to 75% and 50%) of soil led to marked decreases in protein content of yielded seeds compared to plants grown under 100% of WHC. The results showed stimulatory effects of proline treatment especially at 25mM under normal or under different levels of water stress on protein contents compared to untreated plants.



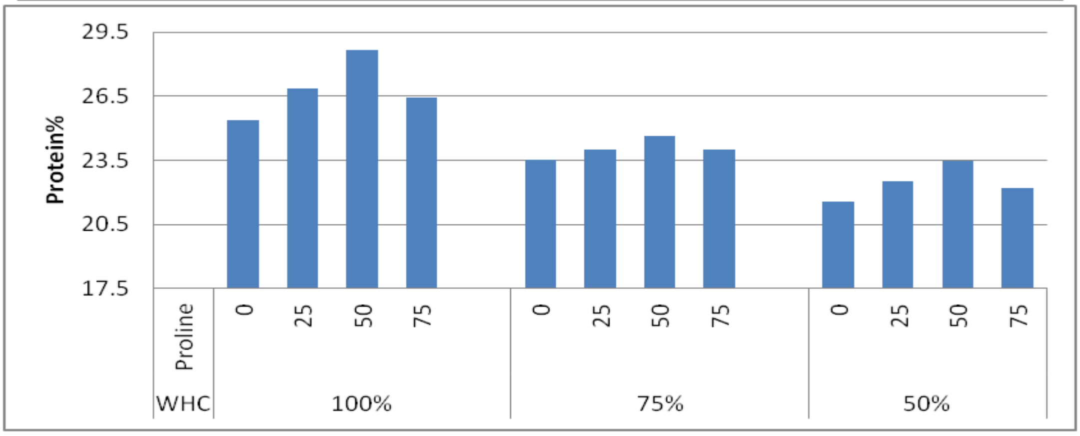


Fig (2): Effect of different concentrations of proline on total carbohydrate% and protein% of Fenugreek seeds subjected to different levels of WHC.
(LSD at 5%: total carbohydrate% : 1.587and protein%: 0.847)

Antioxidant Substances of the Yielded Seed and its Activity:

Data in Fig (3) show the variation in antioxidant substances of the yielded seeds of Fenugreek plant in response to spraying with different concentrations of proline and subjected to different levels of WHC. The WHC decreases at 75% & 50% caused marked significant increases in both total phenolic contents and Flavonoidsss content, as well as tannin contents as compared with those of the corresponding controls. Spraying Fenugreek plants with different concentrations of proline increased significantly total phenolic contents, Flavonoidsss and tannins content as compared with the corresponding control plants. Regarding to antioxidant activity as WHC decreased from 100% to 75% or 50% caused significant increases at all treatments. Foliar treatment with proline treatment with different concentrations caused significant increases in antioxidant activity of the yielded seeds of Fenugreek plant grown under different WHC as compared with the corresponding WHC.

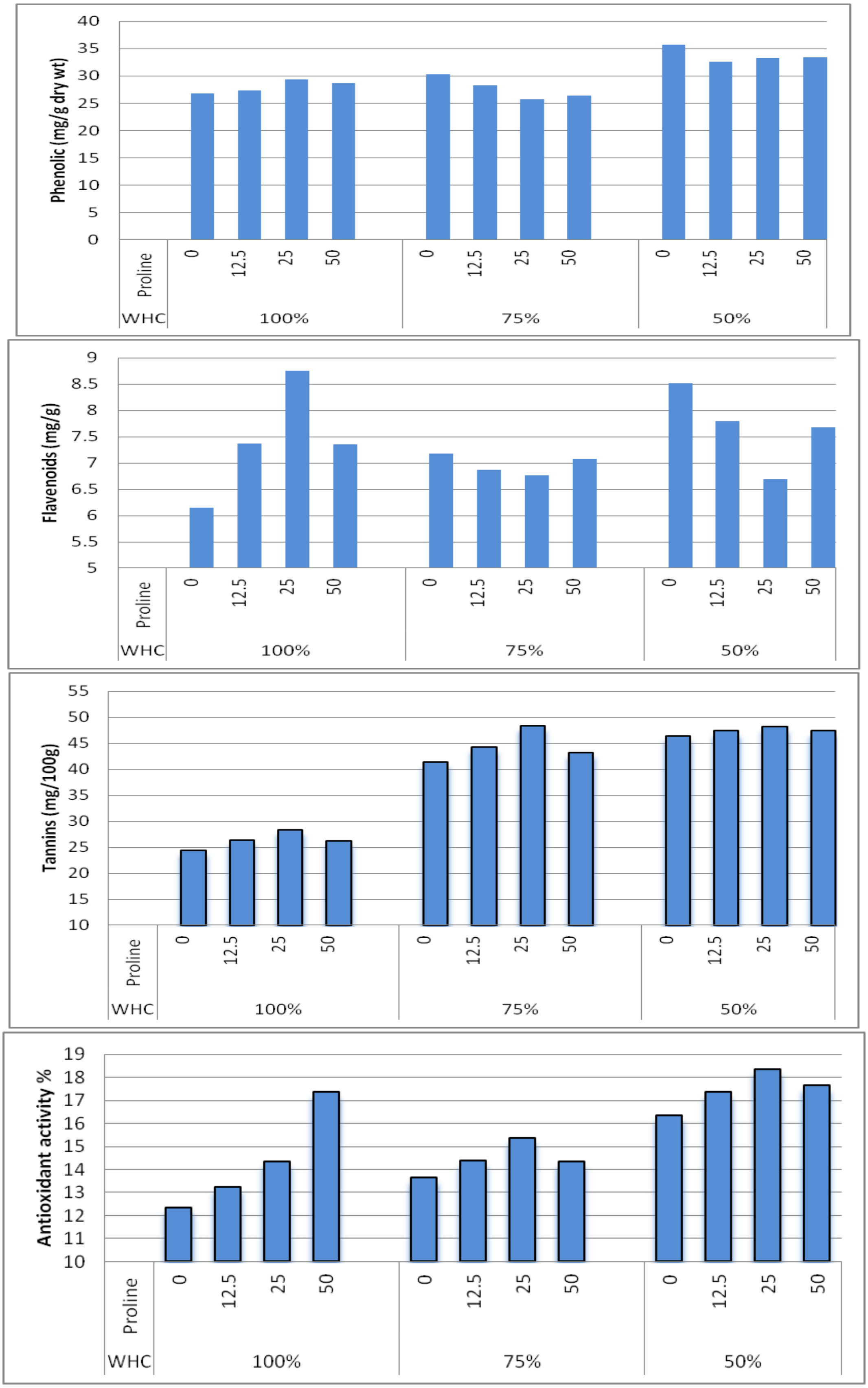


Fig (3): Effect of different concentrations of proline on antioxidant contents (phenolic contents, Flavonoids, tannins) and antioxidant activity of Fenugreek seeds subjected to different levels of water holding capacity. (LSD at 5%: phenolic contents: 0.498, Flavonoids: 0.945,tannins: 2.585 and antioxidant activity: 2.043)

DISCUSSION

Plant Growth and Yield:

Plant responses to water stress include growth parameters and biochemical changes that lead first to acclimation and later, as water stress become more severe leading to damage and the loss of plant parts (Chaves et al., 2003).Water deficits affect plants in different ways, slowly developing water deficits decrease growth, by slowing rates of cell division and expansion due to loss of turgor (Lawlor and Cornic, 2002 and Bakry et al., 2012) and/or resulted from the osmotic effect of water stress which caused disturbances in water balance of stressed Fenugreek plant leading to decreases in photosynthetic pigments (Table 3) and consequently retarded growth rate (Table 1). The decrease in dry weight of shoots by increasing water stress could be ascribed to the decrease in photosynthetic output as indicated by the significant decreases of chlorophylls (Table 3) in water stressed Fenugreek plants. Other authors concluded that, reduction of dry weight might be due to turgor limitation **(**Farooq et al., 2009) or cell wall hardening by limited extension growth (Chazen and Neumann, 1994).The reasons may be the nonavailability of nutrients and the expenditure of energy to counteract the toxic effects of stress. During the acclimation phase, water stress typically results in slower growth rates because of the inhibition of cell expansion, the reduction in carbon assimilation and the resultant effect on carbon partitioning (Hsiao and Xu, 2000). Water stress reduced the yield and yield components (no of pods/plant, wt of seeds / plant and seed index) in Fenugreek plant. Similar results were obtained by Ali et al., (2011), Hussein & Safi-naz (2013) and Dawood and Sadak (2014). The increases in growth characters caused by different proline concentrations especially at 25mMmight be due to the role of proline in protecting enzymes, 3D structures of proteins and organelle membranes and also supplies energy for growth and survival thereby helping the plant to tolerate stress (Hoque et al., 2007; Ashraf and Foolad, 2007). It is probable that proline would have been absorbed by the developing seedlings, where it maintained water status by increasing the influx of water and reducing the efflux of water under water-limiting conditions (Chen and Murata, 2008) and thus increased growth and yield of Fenugreek plantlets. The obtained results of proline treatment in accordance with those obtained by Abdelhamidet al., (2013) on common bean,Taie et al., (2013) on faba bean and Dawood et al., (2014) on faba bean plants and Sadak & Mostafa (2015) on sunflower plant.

Photosynthetic Pigments:

Water stress may reduce leaf net photosynthetic assimilation by both stomatal and metabolic limitations (Ripley et al., 2007). . Decreasing WHC decreased chlorophyll contents (Table 3). These results are in harmony with those obtained by Mittler (2002) who suggested that, the source of reducing energy for ROS scavenging during stress accompanied by suppression of photosynthetic apparatus. In addition, Murkute et al., (2006) attributed the reduction in chlorophyll content to the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments. Proline application to Fenugreek plants significantly increased chlorophyll a, and chorophyll b content, with no significant change in carotenoid level under low WHC environment**.** Ali et al. (2007) mentioned that the exogenous application of 30 mM proline increased photosynthetic pigments of maize plants grown under water deficit stress. Proline treatments had the ability to alleviate the adverse effects of salinity on photosynthetic pigments. Yan et al., (2011) mentioned that proline not only functioned as a nutrient but also possessed some defensive mechanisms for damaged plants under salt stress. These mechanisms were, promoting photosynthesis, maintaining enzyme activity and scavenging ROS. Ali et al., (2007) explained the beneficial effect of proline applied was due to its promotive effects on photosynthetic capacity by overcoming stomata limitations, enhancing biosynthesis of photosynthetic pigments, or protecting photosynthetic pigments from water stress-induced degradation.

Endogenous IAA:

Results shown in (Fig 1) indicated that, the decrease in the water holding capacity of the soil caused marked significant decreases in IAA content, as compared with those of the control. The decreases in IAA might be due to, as soil dries out and soil water potential becomes more negative, plants must activate their defense system to be sure to absorb water as much as they can. In view of the results obtained from this study, foliar application of proline counteracted water stress induced decline in the concentration of IAA in Fenugreek shoots, as compared with the corresponding untreated plants (Fig 1). The increase in IAA contents in shoot tissues treated with proline concurrent with the increase in growth rate (Table1) indicates the role of the endogenous hormones in stimulation the cell division and/or cell enlargement and subsequently growth (Taiz and Zeiger, 1998).

Free amino acids contents:

Free amino acids have been reported to accumulate in higher plants under low drought stress. Moreover, Fenugreek plants irrigated with different WHC and subjected to the exogenous application with three proline levels showed marked increases in free amino acids compared with controls. The obtained results of **stress** are in harmony with those obtained with those of Kala and Godar, (2011), Radhika and Thind (2013) Dawood and Sadak (2014) and Bakhoum & Sadak (2016) on different plant species. It was noted that plants treated with three proline doses and irrigated with different WHC levels showed significant increase in free amino acids relative to the corresponding WHC levels. Taie et al (2013)reported that faba bean amino acids under low WHC in agreement with the present obtained results on Fenugreek plants.

Proline content:

Proline accumulates in larger amounts than other amino acids in stressed plants (Taie et al., 2013 and Abdelhamid et al.,2013). Its accumulation in plants could be due to de novo synthesis, to decreased degradation, or both and depends on the type of species and the extent of the stress (Kavi-Kishor et al., 2005). The accumulation of proline in plant tissues in response to different abiotic stresses may play an important role against oxidative damages caused by ROS due to its action as a single oxygen quencher (Alia et al., 2001), participating in cellular osmotic adjustment (Yamada et al., 2005), buffering the cellular redox potential, stabilising the membrane and protein 3D structure, and protecting the subcellular structures, such as mitochondria and chloroplasts, under adverse environmental conditions (Ashraf and Foolad, 2007), as well as participating in the induction of stress responsive genes (Chinnusamy et al., 2005). In view of some earlier reports who suggested that exogenously proline applied might have enhanced endogenous proline accumulation under water stress conditions which not only protects enzymes, 3D structures of proteins and organelle membranes, but it also supplies energy for growth and survival thereby helping the plant to tolerate stress (Hoque et al., 2007). Fig (1) illustrates that proline treatments at 12.5, 25 and 50 mM caused significant increases in proline contents(mg/g) in leaves of Fenugreek plants that irrigated with 100% WHC relative to control. Special attention must be paid to that Fenugreek plants treated with proline and irrigated with low WHC at two levels (75 and 50%) showed significant reduction in proline content than corresponding controls. In contrast, Abd El-Samad et al. (2011), Abdelhamid et al. (2013) and Dawood et al., (2014) mentioned that the spraying of proline under all WHC levels was accompanied by decreases in the proline concentration compared with control values. These reductions may be explained by proline degradation mechanisms that are induced after stress recovery, and this process leads to the generation of reducing equivalents (Hare et al., 1998). Moreover, proline degradation can provide carbon, nitrogen and energy sources. Upon oxidation, one proline molecule can release 30 ATPs (Kavi-Kishor et al., 2005).

Biochemical constituents of the yielded Fenugreek seeds:

Carbohydrate Contents:

Water rejemes (75% and 50% WHC) resulted in the decrease of in total carbohydrates in Fenugreek yielded seeds. (Fig 2). These results are in harmony with those reported by Taie et al., (2013) and Dawood et al., (2014) who mentioned that salinity stress decreased total carbohydrates of faba bean plant. These decreases may be due to inhibition of photosynthesis which is associated with decline in total pigments content (Table 3), Treated plants with different levels of proline and irrigated with 100%WHC exhibited significant increase in total carbohydrates compared with control plant. Meanwhile, treatment of Fenugreek plant with proline at 75% and 50% WHC showed significant increases in total carbohydrate % relative to untreated plants. Proline application might counteract the negative effects of stress on carbohydrate metabolism, which, consequently, could promote the entire plant growth, as noted by Abd El- Samad et al. (2011) and Dawood et al., (2014). This result could be due to the role of proline in minimising the adverse effects of stress, which are associated with the increase in photosynthetic pigments in Fenugreek plants.

Protein contents:

Data presented in Fig (2) showed that lower WHC decreased protein contents of Fenugreek seeds. Frechill et al., (2001) reported that water stress interferes with nitrogen (N) acquisition and utilization by influencing different stages of N metabolism, such as, NO uptake and reduction and protein synthesis. It could be concluded that the inhibitory effect of water stress on the Fenugreek plants was alleviated by treatment of proline through enhancing the biosynthesis of free amino acids and their incorporation into protein. These results added support to the results obtained by Dawood et al., (2013) and Tai et al., (2014)on faba bean plant. Thus, it can be concluded that prolinetreatments not only alleviated the inhibitory effect of water stress, via osmotic adjustment or by conferring some desiccation resistance to plant cell, but also stimulated the accumulation of protein contents over those in the control plants. Moreover, proline might act as activators of protein synthesis via significant alteration in the enzymes related to protein metabolism (Cuin and Shabala, (2007)

Antioxidant Substances of the Yielded Seed and its Activity:

Synthesis of secondary nonenzymatic metabolites are a unique capabilities of plants to tolerate stress. These compounds as phenolics and flavonoids have antioxidative role in ROS scavenging (Reddy et al., 2004). Biotic and a biotic stress factors induced the synthesis of phenolic and flavonoid compounds. Phenolic, Flavonoids and tannins concentrations were significantly increased under drought stress (Fig 3). Phenolic contents protect cells from potential oxidative damage, increase the stability of cell membranes (Burguieres et al., 2006), and mitigate stress injuries. The accumulation of phenolic compounds in response to abiotic stress would be attributed to the activation of phenylalanineammonialyase (PAL) (Rivero et al., 2001). These obtained increases in these antioxidant compounds are in harmony with those obtained by Kirakosyan et al., (2004) who observed enhancement in the levels of polyphenolics in the leaves of two hawthorn sp due to water deficit. According to, Sharafzadeh and Zare (2011) tannin like phenolics are defence metabolites increased under stress condition. Pritchard et al. 1997 reported increase in total phenolic and tannins due to elevated CO_2 under both water stressed and well-watered conditions. It is observed that total flavanoids increase with increasing water stress treatments. Yaginuma et al. (2003) noticed that under the stressed conditions content of Flavonoidss glucosides in foliage of safflower seedlings markedly increased. Awate and Gaikwad (2014) observed that polyphenols, tannins, alkaloid and Flavonoidss contents of Simaroubaglauca were increased with increasing water stress treatments. The accumulation of various isoflavone suggests that drought stress activates the phenylpropanoid pathway leading to increases in the synthesis of isoflavones in Fenugreek seeds. The foliar spraying of proline resulted in significant decreases in the total phenolic and Flavonoidss concentration compared with the control. Moreover, Fenugreek plants treated with an exogenous application of proline under drought stress showed significant decreases in phenolic and Flavonoidsss concentrations compared with controls. These reductions may be due to their oxidation by antioxidant enzymes, which withdrew phenols as their substrate (Burguieres et al., 2006), and may be due to the decline in their biosynthesis and the activation of their degradation.

CONCLUSION

On the basis of the present results, it can be concluded that, foliar application of 25 mM proline alleviated water regimes inhibitory effects which induced decreases in growth parameters, photosynthetic pigments, IAA, free amino acids and proline contents in Fenugreek plant. Water stress reduced the yield, yield components and some chemical components and antioxidant compounds of the yielded seeds of Fenugreek. Foliar application with 25 mM proline counteracted such effects. It can be concluded that the exogenous application of proline partially alleviated the inhibitory effect of low WHC on Fenugreek plants through increasing amounts of antioxidants contents which played important role in defiance against low water holding capacity. Moreover, application of proline increased amounts of, carbohydrate%, protein%, tanninsandantioxidant activity of Fenugreek seeds subjected to different levels of water holding capacity in yielded seeds.

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