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RESEARCH ARTICLE

Interaction Effects of Different Soil Moisture levels, Arbuscular Mycorrhizal Fungi and Three Phosphate Levels on: II- Mineral ions, Protein and Amino Acids contents of Garden Cress (*Lepidium sativum* L.) plant.

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Abstract

The contents of mineral ions, protein and amino acids in seeds of (*Lepidium sativum* L.) were determined in response to water stress (85, 55 and25% depletion of the available soil water), Arbuscular mycorrhizal fungi and three phosphate levels (control (without mycorrhiza and phosphorus), zero phosphorus + mycorrhiza (P0), 25% recommended phosphorus + mycorrhiza (P1), 50% recommended phosphorus + mycorrhiza (P2) and 100% recommended phosphorus + mycorrhiza (P3)). Treatments were arranged in a randomized complete blocks design with five replicates. The results revealed significant decrease in protein and mineral ions contents of L. sativum seeds with increasing water stress level. These changes were accompanied with an increment in proline and amino acids contents of L. sativum seeds. AMF inoculation clearly increased protein and mineral ions contents compared with non-AMF plants. Moreover, the composition of amino acids was changed by AMF inoculation, where their contents were mostly decreased specially after phosphate addition. It seems that the AMF symbiosis enhanced drought tolerance mechanisms of L. sativum plants.

INTRODUCTION

Garden cress (*Lepidium sativum* L.) locally known as Haloon belonging to family Brassicaceae which is native to Southwest Asia and spread to Western Europe. Garden cress is a fast-growing, green, cool-season perennial plant used as a leafy vegetable. Undisturbed, the plant can grow to a height of two feet with minimal maintenance. When mature, garden cress produces white or light-pink flowers, and small seed pods. It has long leaves at the bottom of the stem and small, bright-green, feather-like leaves arranged on opposite sides of its stalks at the top (Boulos, 1995). This herb is the best source of iron and is recommended in the treatment of iron-deficiency anemia. Garden cress seeds being the richest source of non-haeme iron [iron found in haemoglobin which is an easily absorbed dietary iron] help to increase the haemoglobin levels. When taken regularly, it helps to alleviate anemia (Chiej, 1984). It is advisable to have vitamin C half an hour after consumption of these seeds as it enhances iron absorption. It is also rich in foliate, calcium, ascorbic acid, tocopherol, and beta-carotene. Garden cress seeds are loaded with not just protein, but also linoleic and arachidic fatty acids. Since they contain phytochemicals that mimic estrogen to some extent, intake of these seeds is known to regulate menstruation and stimulate milk production in lactating mothers. That is precisely why women are given foods containing garden cress following childbirth because of its high iron and protein content (Shehzad *et al*., 2011). The blood-purifying as well as antioxidant properties of this amazing plant are well documented. Hence, its regular consumption can greatly help to boost one's immunity and prevent a gamut of diseases (EL-Darier and Youssef, 2000). The whole plant, along with its seeds, is said to be good for the eyes too. It is advisable to add it raw to salads, sandwiches, and chutneys, or to simply use it as a garnish along with coriander leaves for any food item. Since it is goitrogenic in nature, it may not

be suitable for patients suffering from hypothyroidism (Kobasi, 1993). Garden cress is genetically related to watercress and mustard, sharing their peppery, tangy flavor and aroma. Garden cress helps purify blood and stimulate appetite (Hajar *et al*., 1996). It is used during constipation as a laxative and a purgative. Paste made of the seeds can be taken internally with honey to treat amoebic dysentery. Garden cress crushed and drunk with hot water is beneficial to treat colic especially in infants. Garden cress seeds are good expectorants and when chewed they treat sore throat, cough, asthma and headache. The aerial parts are used in the treatment of asthma and cough (Zidan, 1991). The leaves, roots, as well as seeds of this plant are used in cooking as they are extremely nutritious (Khalil and Yousef, 2014) and also it contains very high amounts of BITC (benzyl isothiocyanate) which has emerged as a powerful anti-cancer compound. BITC was seen to kill 97% of ER- breast cancer cells (MDA-MB-231) (Rehman *et al*., 2011).

Water is the major component of the plant body. It's constituent about 80 to 90 % of fresh weight of most herbaceous plant organs and over 50 % of the fresh weight of woody parts. Water affects markedly, either directly or indirectly the most plant physiological and biochemical processes. Dehydration of plant tissues below some critical level is accompanied by irreversible changes in structure and ultimately by plant death. The importance of water in living organisms results from its unique physical and chemical properties, which also determine its functions in plant physiology, water is a major constituent of the protoplasm, it acts as a solvent for many solid and gaseous substances, forming a continuous liquid phase throughout the plant, it takes part in many important physiological reactions, it maintains cell turgor, which exerts an impact on many physiological processes (Khalil *et al.,* 2012). Water stress is a major factor limiting crop productivity world-wide (Jones and Corlett, 1992). Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. Drought stress tolerance is seen in almost all plants but its extent varies from species to species and even within the same species. Tolerance to abiotic stresses is very complex, due to the interaction between stress factors and various molecular, biochemical and physiological phenomenon affecting plant growth and development (Razmjoo *et al*., 2008). Water stress in general reduces nutrient uptake by roots and transport from root to shoots because of restricted transpiration rates and impaired active transport and membrane permeability effects, resulting in a reduced root-absorbing power of crop plants. Moreover, the decline in soil moisture is associated with a decrease in the diffusion rate of nutrients from the soil matrix to the absorbing root surface (Khalil and Yousef, 2014). Ahmad *et al.,* (2013) reported also that drought stress induced several biochemical alterations in plant tissue. Water stress favors the accumulation of products of primary metabolism as osmolytes, amino acids and reducing sugars (Wu, 2009). Drought conditions bring about quantitative and qualitative changes in plant proteins. In general, proteins in the plant leave decrease during water deficiency due to the suppressed synthesis (Dubey, 1999). Water stress alters gene expression and consequently, the synthesis of new proteins and mRNAs (Khalil and Yousef, 2014). Exposure of plants to drought conditions led to increases in free proline, soluble sugar, peroxidate (POD) activities and malondialdehyde (MDA) concentration, and inhibitions of protein synthesis have been proved in many literatures (Dhindsa, 1991, Zhang and Kirkham, 1994, Arji and Arzani, 2008). Also, Moinuddin and Chopra (2004) reported that osmotic adjustment involves an active accumulation of cellular solutes such as soluble proteins and certain ions like K+ within the plant in response to lowering of the cellular water potential and reducing the harmful effects of water deficit (Beck *et al.,* 2007). Osmotic adjustment has been reported by Gunes *et al.* (2008) in chickpea under water deficit conditions, Jiang and Huang (2002) on fescue plants, Kshamata *et al.* (2005) on cotton (Gossypium hirsutum), Valentovic *et al.,* (2006) on maize and Najaphy *et al*., (2010) on chickpea plants.

In addition to intrinsic protective systems of plants against stress, plants grow in association with a number of soil microorganisms that can alleviate the stress symptoms. Arbuscular mycorrhizal (AM) fungi that associated with the roots of most plants not only stimulate the growth of plants but also contribute in enhancing plant tolerance to abiotic stress such as salinity, temperature stress and drought (Navarro *et al.,* 2011). Previous studies have indicated that inoculation with AM fungi appeared to improve drought tolerance of host plants (Cuenca *et al*., 1997 and Khalil and Yousef, 2014). The role of AMF in plant growth and nutrition has been related to the uptake of immobile ions of plant nutrients, such as phosphate. It had been indicated that AM fungi improved phosphorus nutrition which considered as crucial factor for increased drought tolerance of mycorrhizal plants (Azcón-Aquilar *et al.,* 1993). However, research on the role of AMF in plant N nutrition has gradually been accumulating and it has been suggested that AMF can also enhance plant N acquisition (Ames *et al.,* 1984). Furthermore, Prabhu *et al.,* (2013) revealed that, there was an increase in protein expression pattern in AM fungi infected soil samples compared to non-infected soil samples. However, there is very little research on improving drought tolerance of medicinal plants using AMF associations.

Therefore, the present investigation was planned to explore the ability of *Lepidium sativum* L. plants of tolerating various degrees of water stress, and possible alleviating of the harmful effects of water stress by studying

NPK, protein contents and the compounds generated (amino acids) by plants as a result of defense mechanisms which permit understanding of the molecular mechanism involved in their medicinal properties.

Materials and Methods

The experimental site:

An experiment was conducted during two winter seasons of 2012/2013 and 2013/2014 at the green house of the National Research Center, Dokki, Cairo, Egypt.

Growth Conditions:

Seeds of Garden cress (*Lepidium sativum* L.) provided from the Egyptian Agricultural Research Center and were thoroughly washed with distilled water then the seeds were sown at a rate of three seeds per hole on the 1st of October in earthenware pots 40 diameter and 40 cm height with perforated bottoms, and were filed with 10 kg of clay loam soil (as shown in Table 1). All pots were weighted every 1to 3 days on abeam balance. The pots were then irrigated to restore the soil to the appropriate moisture regime by adding a calculating amount of water. The general principal stated by Boutraa and Sanders (2001) was used for the water treatments application. All pots received recommended doses of N and K fertilizers, N 40–50 kg/ha, P 50–60 kg/ha and K 25–30 kg/ha. P fertilizer was added at four rates, P0 (zero phosphorus), P1 (25% recommended), P2 (50% recommended), P3 (100% recommended). Also, K was applied as potassium sulphate (48% K2O) which was added immediately after thinning, N was added as ammonium nitrate (33.5% N) which was divided into three equal portions the first immediately after sowing, the second after thinning and the third after two weeks from the second, according to the recommendations of Agriculture Ministry.

Table (1): Mechanical and chemical analyses of the soil used during the experiment.

Season	1st season	2nd season
sand %	26	25.7
silt %	36	35.4
clay %	38	37
Texture	clay loam	clay loam
F.C.%	30.9	31.2
W.P.%	16.2	14.3
CaCO3 %	4.5	4.3
OM %	1.3	1.1
PH	7.7	7.7
EC	0.6	0.55
Na^{+}	2.1	1.90
Mg^{++}	0.82	0.80
Ca^{++}	1.00	0.97
K^{+}	1.11	0.94
$HCO3^{-}$	1.14	1.02
Cl^{-}	0.75	0.64
$CO3^{--}$	2.00	1.99
$SO4^{--}$	1.65	1.44

Experimental design and treatments:

The experiment included 15 treatments which were the combination between three soil moisture levels (W1=85 % depletion of the available soil water, W2=55% depletion of the available soil water and W3= 25% depletion of the available soil water) and five treatments of arbuscular Mycorrhizal (AM) fungi which were (control without mycorrhiza and phosphorus, zero phosphorus (P0) + mycorrhiza, 25% recommended phosphorus + mycorrhiza (P1), 50% recommended phosphorus + mycorrhiza (P2) and 100% recommended phosphorus + mycorrhiza (P3)), treatments were arranged in a randomized complete blocks design with five replicates. The different soil moisture treatments were assigned at random in the main plots, while sub-plots were devoted to the

different arbuscular mycorrhiza inoculum treatments and phosphate treatments. Due to involvement of heavy cost for the purchase of chemicals in amino acid analysis the following treatments only were chosen in this part of the experiment for amino acids analysis only (control= without AMF and phosphate treatments, P0+AMF and P3+AMF for each soil moisture level used in the experiment i.e. W1, W2 and W3).

Preparation of inculum:

The arbuscular mycorrhiza inoculum was purchased from Agricultural Microbiology Department National Research Centre, Cairo, Egypt. The inoculum was mixture of Glomus spp. (G. mosseae and G. fasciculatum) originating from the rhizosphere soil of maize and alfalfa. The inoculum was multiplied in pot cultures contains peat: vermiculite: perlite mix 1:1:1 by volume with maize and alfalfa (Badr El-Din *et al.,* 1999) and consisted of a mixture of spores, root fragments, hypha and growth medium. The inoculum material contained 275 spores and 240 propagules gm^{-1} oven dry biases using the most probable number test (Mahaveer *et al*., 2000). The non -inoculated control was without mycorrhizal. Miorrhizal inoculation was done by planting the seed over a thin layer of the mycorrhizal inoculum at the time of sowing at a rate of 10 gm/pot. The inoculum material contained 275 spores gm^{-1} on oven dry bases, whereas 10g of autoclaved soil was used for the non-mycorrhizal treatments. Phosphate treatments were given after 40 days of AMF inoculation.

Sampling and measurements:

When plants reached suitable maturity, Seeds were then collected from each treatment to determine the total nitrogen which was determined by using semi-micro Kjeldahl method described by Bremner and Mulvaney (1982), Total phosphorus which was determined by using Spectrophotometer according to Olsen and Sommers (1982). K content was determined photometrically using a flame photometer according to the method of Jackson (1970).Crude protein percentage was determined in seeds using the equation of Alsmeyer *et al.,* (1974).

Quantitative determination of amino acids contents: determined by the method of Pellet and Young (1980). Amino acids contents of sserc nedrag seeds were accomplished by using amino acid analyzer (Beak man system 7300 high performance analyzer). The seeds powder (0.1gm) was dissolved in 10ml of 6N HCl in a sealing tube. The mixture was hydrolyzed at 110oC for 24 hours, filtered and the hydrolyzed protein-amino acids were obtained by evaporation of the hydrolysate to dryness. The residue was washed with distilled water; the volume of the filtrate was adjusted to 100ml, using distilled water. Content of different amino acids are expressed in terms of µg/100 mg dry weight of plant sample.

Measurement of Proline: Proline content was estimated following the method of Bates *et al.* (1973).

Extraction: Five hundred milligram of plant material was taken in a pestle and mortar and homogenized with 10 ml of 3 percent aqueous sulfosalicylic acid. Then the homogenate was filtered through whatman No. 2 filter paper. The residue was re-extracted two times with 3 per cent sulfosalicylic acid and pooled. The filtrates were made up to 20 ml with 3% sulfosalicylic acid and used for the estimation of proline.

Estimation: Two milliliter of extract was taken in a test tube and 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added to it. The mixture was incubated for one hour at 100oC in a water bath. The tubes were transferred to an ice bath to terminate the reaction. Then to each test tube 4 ml of toluene was added and mixed vigorously using a test tube stirred for 10-20 secs. The toluene containing the chromophore was separated from the aqueous phase with the help of separating funnel and the absorbance was measured at 520 nm in a spectrophotometer using an appropriate blank. The proline content was determined from a standard curve prepared with proline and the results were expressed in µg /100g dry weight.

Statistical analysis:

The collected data of the two seasons were subjected to statistical analysis of variance using the normal (F) test; the means were evaluated by using Least Significant Difference (LSD) test at 5% probability level according to Snedecor and Cochran (1980).

Results and Discussion

Mineral ions content:

Data presented in Table (2) illustrated the effect of different depletion levels and different AMF treatments as well as their interaction on N, P and K accumulation of garden cress seeds.

1. Effect of water stress:

The obtained results pointed out in both growing seasons that there was a significant difference observed between different depletions levels in the mean values of their seeds mineral content. Increasing soil moisture level caused significant increase in the mineral content of garden cress seeds, where the maximum means of N, P and K % were observed under the lowest depletion level W3. While, the minimum means of N, P and K % were obtained under the highest depletion level W1 compared with the other water treatments. The relationship between nutrient uptake and drought stress is complex. Where water stress in general reduces nutrient uptake by roots and transport from roots to shoots because of restricted transpiration rates and impaired active transport and membrane permeability effects, resulting in a reduced root-absorbing power of crop plants. Moreover, the decline in soil moisture was associated with a decrease in the diffusion rate of nutrients from the soil matrix to the absorbing root surface (Khalil and Yousef, 2014). The decreased levels of each of N, P and K in response to stress were ascertained by the work of each of Razi and Sen (1996), Schier and McQuattie (2000), Bie *et al.* (2004), Koyro (2006), Wu and Xia (2006), and Khalil1 and Yousef (2014). Such reductions in the contents of these elements in different tissues were attributed primarily to soil water deficiency which markedly reduces the flow rates of elements in soil, their absorption by stressed root cells and also its ability to translocate through the different organs and tissues. This situation resulted in an interruption in the various metabolic pathways carried out by plants i.e. respiration, photosynthesis, biosynthesis of phospholipids, nucleic acids, plastids, enzymes...etc., disorders in both plasma membrane permeability and stomatal osmotic regulations, thus plants seized growth and eventually died (Khalil and Abdel-Kader, 2011).

2-Effect of AM fungi and different phosphate levels:

Data presented in (Table 2) had clearly shown also that N, P and K concentrations were positively responded to arbuscular mycorrhizal fungi (AMF) treatment even before phosphate addition compared with control plants. Moreover, adding and increasing phosphate concentrations showed gradual and significant increases in N and P contents of garden cress seeds compared to control plants. Where, the maximum increases in their contents were observed under P3XAMF treatment compared with control plants in both seasons. As for K% increasing phosphate concentrations revealed insignificant increase in K content compared with control plants. Where the highest means observed under P0XAMF treatment compared with control one. Furthermore, all phosphate concentrations showed significant decrease in K content of garden cress seeds compared with P0XAMF treatment in both seasons. There were many evidences that mycorrhizal plants could absorb more P if lower P concentration exists in the soil solution. This probably resulted from a greater absorption surface area provided by extensive fungal hyphae (Raju *et al.,* 1990) and enhanced root growth. Similar results had been reported by (Raju *et al.,* 1990, Al-Karaki and Al-Raddad, 1997b and Navarro *et al.,* 2011). The extra P in Microhizal plants could be due either to an indirect mycorrhizal effect on the root structure or physiology or to direct uptake by hyphae with subsequent transfer to the root or both (Sander and Tinker, 1973). A large fraction of phosphate in the soil was in the form of organic compounds, AM fungi secrete phosphatase to help hydrolyze phosphate from such compounds. Many researches approach provided clear evidence that the external mycelium of the fungus played a direct role in the transport of the N. The present results corroborate other reports (Frey and Schüpp, 1993, Tobar *et al.,* 1994 and Abbaspour *et al.,* 2011) indicating that the external mycelium of AM fungi actively assists host plants to enhance N uptake. As a result, mycorrhizal plants could take up more nutrients via extraradical hyphae, which provides larger surface areas than the roots alone and reduce the distance for diffusion, thereby enhancing the absorption of immobile nutrients (Jaleel *et al.,* 2007). The higher density of extra radical hyphae in soil, the higher absorption surface, and the more effectively mycorrhizal plants will absorb these nutrients (Azevedo-Neto *et al.,* 2006).

3- Effect of interaction between water stress, AM fungi and different phosphate levels:

The data of interaction between different depletion levels and different AMF treatments showed that, W3X P3XAMF treatment proved to be the most effective interaction in increasing N and P content of garden cress seeds in both seasons compared with the other treatments and with significant differences (Table 2). Furthermore, the data of interaction revealed that W3XP0XAMF treatment showed the highest significant records for K content compared with the other treatments under different depletion levels in both seasons. Accumulating evidence suggested that mineral nutritional status of plants greatly affected their ability to adapt to adverse environmental conditions and in particular to abiotic stress factors. Impairment of the mineral nutrition status of plants exacerbates the adverse

effects of abiotic and could alleviate the adverse effects of stress on plant growth. The protection of Microhizal plants against water stress was partially related to K uptake. Potassium played an important role in the drought

Table (2): Effects of different soil moisture levels, arbuscular mycorrhizal fungi, three phosphate levels and their interactions on mineral ions content and crude protein % of *Lepidium sativum* L. in the two seasons of 2012/2013 and 2013/2014 (combined analysis of two seasons).

Treatments		N %	P %	K %	Crude protein %
Effect of different soil moisture levels					
W1		1.39	0.41	0.93	8.667
W2		1.50	0.46	0.99	9.342
W3		1.72	0.54	1.721	10.738
$LSD_{0.05}$		0.13	0.03	0.11	0.37
Effect of AM fungi and three phosphate levels					
Cont.		1.46	0.41	0.96	9.10
P0XAMF		1.50	0.45	2.06	9.36
P1XAMF		1.52	0.47	0.99	9.53
P2XAMF		1.57	0.49	1.01	9.80
P3XAMF		1.62	0.51	1.03	10.12
$LSD_{0.05}$		0.18	0.06	0.09	0.44
Interaction effect of different soil moisture levelsX AM fungi					
W1	Cont.	1.31	0.35	0.90	8.21
	P0XAMF	1.36	0.40	0.90	8.48
	P1XAMF	1.39	0.41	0.93	8.71
	P2XAMF	1.42	0.43	0.95	8.88
	P3XAMF	1.45	0.46	0.95	9.06
W2	Cont.	1.43	0.40	0.93	8.94
	P0XAMF	1.45	0.43	0.95	9.04
	P1XAMF	1.47	0.45	0.97	9.17
	P2XAMF	1.53	0.48	1.02	9.58
	P3XAMF	1.60	0.51	1.05	9.98
W3	Cont.	1.63	0.49	1.04	10.17
	P0XAMF	1.69	0.53	4.33	10.56
	P1XAMF	1.71	0.55	1.07	10.71
	P2XAMF	1.75	0.56	1.07	10.94
	P3XAMF	1.81	0.57	1.10	11.31
$LSD_{0.05}$		0.07	0.03	0.05	0.21

Cont.= zero phosphorus and zero AMF. W1=85% depletion W2=55% depletion W3=25%depletion
P0=zero phosphorus P1=25% phosphorus P2= 50% phosphorus P3= 100% phosphorus.

tolerance of plants and was related to stomatal movement in responses to the changes produced in leaf water status (Premachandra *et al*., 1993; Ruiz-Lozeno *et al.*, 1995).Or may be due to the enhanced acquisition of P, Zn, Cu, and Fe by mycorrhizal plant which had been reported by (Araujo-Alves *et al.*, 2000, Kafkas and Ortas, 2009, Mohammadi and Banihashemi, 2010a, Navarro *et al*., 2011). Mycorrhizal colonization improved nutritional status in garden cress plants exposed to drought stress. The presented results corroborate other reports by (Frey and Schüpp, 1993; Tobar *et al.*, 1994).

Protein content:

1. Effect of water stress:

Examination of the collected data in both seasons revealed that there was an increasing trend of reduction in seeds protein (%) with increasing water stress level, the percentage of reduction reached to 19.3 and 13% for W1and W2 respectively compared to W3 (Table 2). Water stress has a profound effect upon plant metabolism and results in a reduction in protein synthesis. Water stress conditions bring about quantitative and qualitative changes in plant proteins. There are many groups of proteins that increased under stress condition; they are called

dehydrinproteins. They have similar role with proline in resisting drought and adjusting osmosis (Mohammadkhani and Heidari, 2008). It was reported decrease in protein solution in shoot organs synchronized with increasing stress level in some plants such as onion (Arvin and Kazemipour, 2001), Legume (Ashraf and Foolad, 2005) and rice (Sikuku *et al*., 2010). Several proteins were reduced by stress in maize mesocotyls (Valentovic *et al.,* 2006). It was expressed that drought caused disorder in proteins synthesis of weed plants (Creelman *et al.,* 1990). Moreover, Niakan and Ghorbanali (2006) observed that increasing drought level caused decrease in the amount of protein solution in shoot organs of soybean leaves. This process accompanied by increase in proline concentration due to protein destruction and its synthesis reduction (Rahdari *et al.,* 2012). Water stress reduced protein content due to the hydrolysis and destruction of leaf proteins and as a result of accumulation of free amino acids for maintaining and adjusting osmotic pressure in plant cells. This case was reported in soybean, corn and sunflower (Izzo *et al.,* 1990). In general, proteins in most plants decreased during water deficiency due to the suppressed synthesis (Dubey, 1999). Protein degradation might be also the result of increased activity of protease or other catabolic enzymes, which were activated under drought stress, or due to fragmentation of proteins due to toxic effects of reactive oxygen species resulting in reduced protein content (Ahmad *et al.,* 2013). Moreover, Khalil (2006) suggested that under stress conditions proteins were converted into free amino acids; the reduction in protein content under higher stress levels may be due to the disturbance in nitrogen metabolism, or due to the inhibition of nitrate absorption. Moreover, Kshamata *et al.* (2005) indicated on cotton (Gossypium hirsutum) that LEA proteins might act as a novel form of molecular chaperone, or 'molecular shield', to help in preventing the formation of damaging protein aggregates during water stress. At last, water stress alters gene expression and consequently, the synthesis of new proteins and mRNAs (Hass, 1975).

2-Effect of AM fungi and different phosphate levels:

Pots treated with AM Fungi showed greater protein (%) than untreated pots. Phosphate addition revealed progressive increase in protein % compared with control plants in both seasons, thus the greatest significant means for protein% were obtained as response to P3XAMF treatment, while the lowest means obtained for control treatment. The percentage of increase became 2.85%, 4.7%, 7.69% and 11.2% respectively for P0, P1, P2 and P3 compared with control plants (Table 2). There were many researches revealed that the application of mycorrhizal inocula increased the total content of proteins per plant including Zhang and He, 2007, Baozhong et al., 2010, Harish *et al.,* 2010, and Abbaspour *et al.,* 2011. This increase in protein content have been attributed to that AM fungi enhance the activity of antioxidant enzymes in host plants or may due to that the fungus induced plant metabolism (Wu and Xia, 2004; Wu et al., 2007b, Mohammadi and Banihashemi, 2010a).

3- Effect of interaction between water stress, AM fungi and different phosphate levels:

By increasing the severity of drought from 25 to 85% depletion of the available soil water, the protein content of *Lepidium sativum* treated plants (with AM fungi) were significantly higher than the untreated ones, where the highest significant values for protein % recorded under the combined effect of 25% depletion of the available soil water and the 100% phosphate level in inoculated plants (W3XP3XAMF) compared with the other treatments in both seasons. While the lowest means observed under control treatment of the most stressed level W1. Such increase in protein% may be due to that AM Fungi improved water relations of the host plants resulting from enhanced P nutrition (Safir *et al.,* 1972; Davies *et al.,* 1992, Ruiz-Lozano and Azcón, 1995) and hyphal uptake of water (Faber *et al.,* 1991; Bethlenfalvay *et al.,* 1998).

Amino Acids content:

Data concerning the effect of Arbuscular Mycorrhizal Fungi (AMF) and the highest phosphate level on the concentration (µg /100g dry weight) of free amino acids of *Lepidium sativum* L. seeds grown under different soil moisture levels were presented in Table 3.

1. Effect of water stress:

Nitrogen metabolism in plant tissues has received great attention under water stress conditions mainly through its relation to protein and amino acids metabolism. Seventeen different amino acids were estimated in Lepidium sativum L. seeds; which were Aspartic, Threonine, Serine, Glutamic acid, Glycine, Alanine, Valine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Histidine, Lysine, NH4, Arginine and Proline. The results pointed out that each of Aspartic, Glutamic acid, proline, Glycine, Alanine, phenylalanine, Histidine, Lysine, and

NH4, increased with increasing water stress level, where the highest records for these amino acids were obtained under the lowest soil moisture level W1. While, each of Threonine, Serine, Methionine, Isoleucine, Leucine, Tyrosine, Valine and Arginine revealed marked increase under moderate soil moisture level and reached their maximum values under W2 treatment, increasing soil moisture content above this value led to gradual decrease in their concentrations. The data illustrated also that the greatest beaks in amino acids in response to water stress were observed for Glutamic acid, proline, and NH4 followed by Alanine, Aspartic, phenylalanine, leucine and Histidine (Table 3 and Fig. 1).

Table (3): Effects of different soil moisture levels, arbuscular mycorrhizal fungi and the highest phosphate level on amino acids content of *Lepidium sativum* L. seeds.

treatments	W1			W2			W3		
	control	cont+AMF	AMF+P3	control	cont+AMF	AMF+P3	control	cont+AMF	AMF+P3
Aspartic acid	26.624	22.11	22.1088	21.17	19.7568	19.32	20.06	19.553	19.049
Threonine	6.9204	6.162	5.71	7.9	7.488	6.0192	5.57	5.2235	5.035
Serine	11.3412	9.228	8.3	11.9008	10.36	8.7776	5.78	4.7039	4.6722
Glutamic acid	53.55	47.8232	42.8896	45.38	34.831	34.0336	25.39	16.978	16.4043
Proline	47.56	38.6964	37.2882	42.26	30.5614	30.2011	40.88	24.10122	24.0168
Glycine	8.21	7.128	6.9744	7.27	6.8672	6.56	5.94	5.04	5.02
Alanine	25.19	24.07.4	22.5792	24.723	22.84	22.216	21.11	20.5752	20.57
Valine	6.19	7.06	5.3052	6.6384	8.3296	6.176	6.09	5.6453	5.4649
Methionine	6.37	4.7904	4.9312	7.79	6.5664	6.1644	5.84	4.3818	4.2783
Isoleucine	5.25	4.4064	4.9968	6.59	6.0768	5.5616	4.82	4.586	4.2872
Leucine	20.34	19.1412	14.6848	31.8	24.47	19.14	23.83	13.35	10.99
Tyrosine	11.5632	8.86	7.8128	13.8192	12.65	11.1084	9.06	8.6151	8.7517
Phenylalanine	32.24	25.67	25.672	31.02	24.47	19.014	17.90	15.52	12.6269
Histidine	24.46	21.43	19.1536	22.704	20.0304	17.7204	13.8	10.879	10.4914
Lysine	17.91	16.9	15.7776	17.7616	15.3312	14.6928	8.67	8.0436	8.0938
NH4+	36.62	29.5392	30.2052	31.83	28.992	28.4288	20.67	18.908	18.3646
Arginine	8.55	7.668	7.516	9.66	8.4704	8.064	7.36	6.422	6.0316

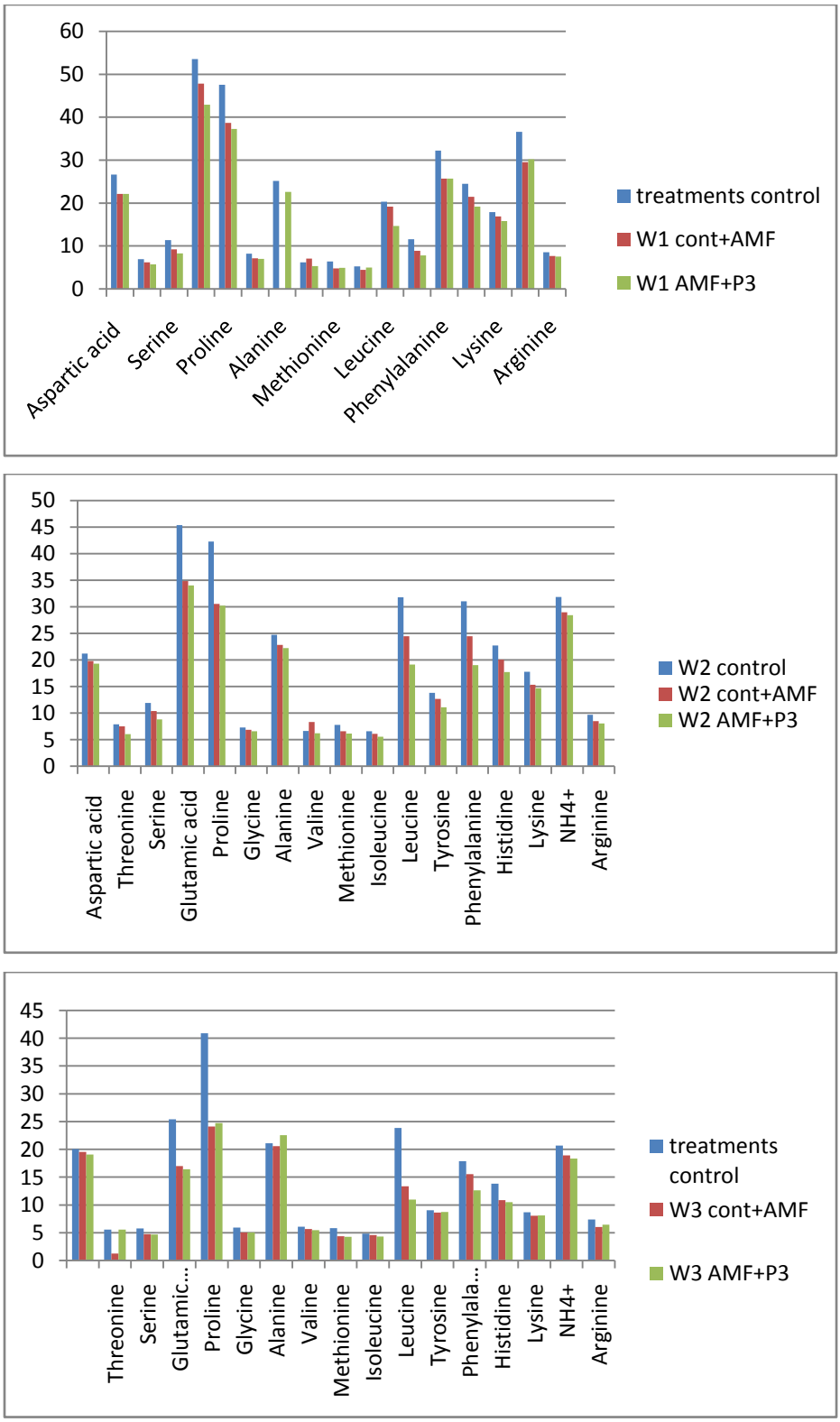

Fig 1: Effect of different soil moisture levels, AMF and the highest phosphate level on amino acids content of *Lepidium sativum* L. seed

It was found that Aspartic acid function is essential for purine, pyrimidine, asparagine, Therionine, Methionine, Lysine and inositol synthesis. Glutamic acid function is essential for Argenine, Glutamine and Proline synthesis. Proline is a key osmoregulatory element in plants experiencing conditions of water stress. Glutamic acid and glycine participate in the synthesis of glutathione increasing the antioxidant capacity of the plant. Serine is also important for cysteine and Glycine synthesis. Valine maintains the balance of branched chain amino acids, whereas Leucine regulates the protein turnover (mTOR signaling) and gene expression (Wu, 2009) (Fig.2).

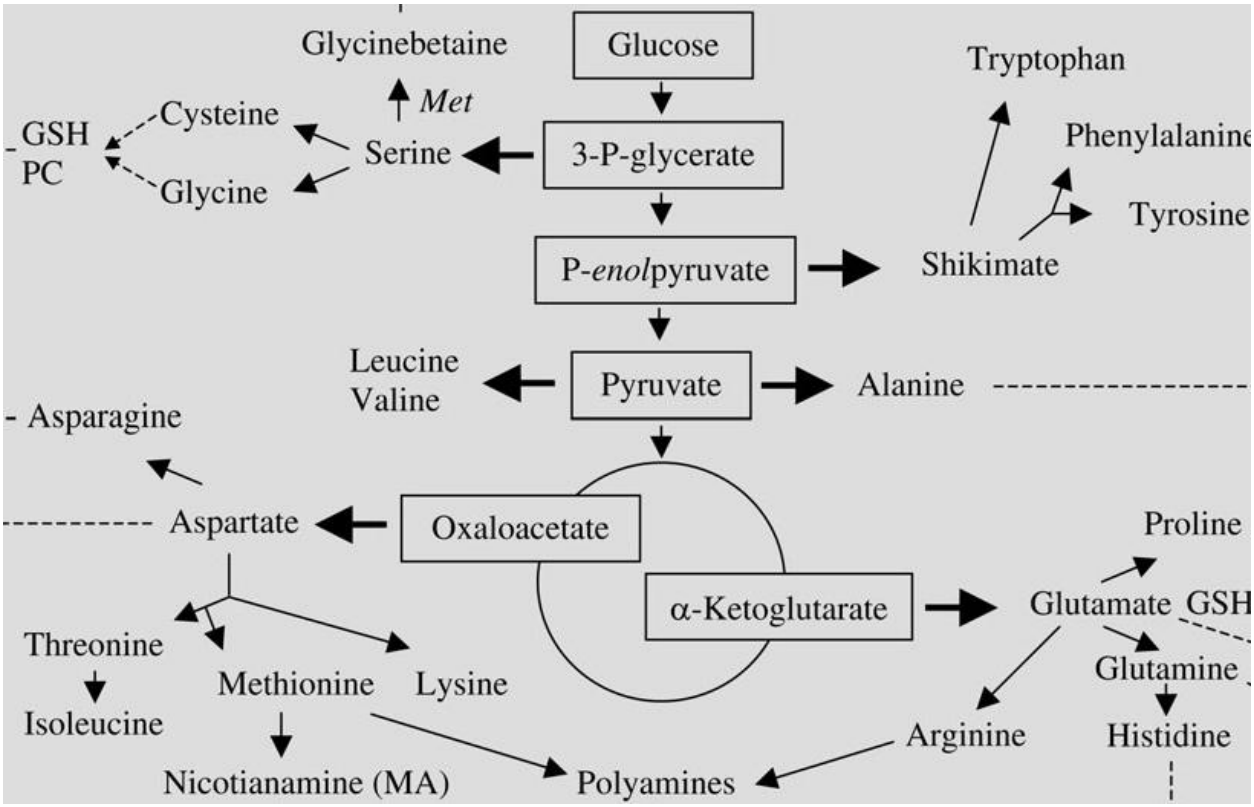

Fig. 2. Schematic depiction of synthetic pathways involved in the synthesis of amino acids and its relation to N-metabolites in plants. The inner area shows the central pathways of glycolysis and citric acid cycle and the linking metabolites to amino acid synthesis. Also shown is the branching to glutathione (GSH), phytochelatin (PC), polyamine, nicotianamine, and mugineic acid (MA) synthesis. Rahdari, et al., 2012.

Changes of amino acids and protein have been mentioned in many reports which have stated that water stress caused different responses depending on the level of stress and plant type. The accumulation of free amino acids in response to drought stress was well documented by Kasturi Bai and Rajagopal, 2000, Asha and Rao, 2002; Yadav et al., 2005, Tan et al., 2006, Sircelj et al., 2007, Maricle *et al.,* 2008 and Rahdari, *et al.,* 2012. Such increase in amino acids values in response to water stress treatments was considered as one of the defense mechanism which stressed plants can lead in order to control osmotic pressure of stressed cells and tissues so as to raise their ability of water and solute uptake from soil (Rodriguez *et al.,* 1996). Strogonov (1964) attributed the accumulations of amino acids to the hydrolysis of proteins. It may be further added that, these accumulations may occur in response to the change in osmotic adjustment in cellular contents (Greenway and Munns, 1980 and Shao *et al.,* 2007). Furthermore, Moinuddin and Chopra (2004) reported that osmotic adjustment involves an active accumulation of cellular solutes such as soluble proteins within the plant in response to lowering of the cellular water potential and reducing the harmful effects of water deficit. As a consequence of solutes accumulation the osmotic potential of the cell was lowered, which in turn attracted water into the cell and led to maintaining its turgor. The stress adaptation effectors like protective proteins or osmolytes like proline usually undergo metabolic turnover and therefore, were not present once and for all (Beck *et al.,* 2007). Jiang and Huang (2002) reported that the accumulation of the osmolytes can help the chickpea plants to maintain the cell turgor and the structural integrity of membranes (Najaphy *et al.,* 2010). Under water stress conditions plants synthesized alcohols, sugars, proline, glycine, betaine and putrescine and

accumulate that of those molecular weights were low (Chopra and Sinha, 1998). Several comprehensive studies using transgenic plants or mutants demonstrate that proline metabolism had a complex effect on development and stress responses. Very high accumulation of cellular proline (up to 80% of the amino acids pool under stress and 5% under normal conditions) due to increased degradation and decreased synthesis of proteins under a variety of stress conditions had been documented in many plant species. Under some conditions, various plants produce a large amount of proline to enhance osmosis and prevent dehydration (Jinying *et al.,* 2007). Thus, the accumulation of proline and different amino acids could have provided the root with an osmotic mechanism to maintain a favorable potential gradient for water entrance into the roots (Irigoyen *et al.,* 1992), leading, therefore, to a lower stress injury in the plant (Porcel and Ruiz-Lozano, 2004). Accumulation of free proline was a common response in plants exposed to drought stress and it could function as hydroxyl radical scavenger to protect against membrane damage and protein denaturation (Akram *et al.,* 2011). Moreover, Proline is a non-protein amino acid (Singh *et al.,* 2000), which acting as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger (Sharma and Dietz, 2006) and as a solute that protects macro-molecules against denaturation and as a means of reducing acidity in the cell (Kishor *et al.,* 2005). Proline has been proposed to act also as a compatible osmolyte and to be a way to store carbon and nitrogen. Several studies showed also that proline may have an antioxidant activity acting as a ROS scavenger. Proline may also function as molecular chaperones able to stabilize the structures of proteins and enhance the activity of different enzymes, and its accumulation play a role in maintenance of cytosolic pH and regulation of intracellular redox potential (Ahmad *et al.,* 2013). Phutela *et al.* (2000) on Brassica junce suggested that proline accumulated in tissues of stressed plants due to the increased rate of its synthesis by pyrroline-5- carboxylate synthetase and the decreased rate of its degradation by proline oxidase enzyme. Also, Abdalla and El-Khoshiban (2007) reported that there was a direct proportional relationship between the proline amounts in shoots and roots of two *Triticium aestivum* cultivars and the severity and duration of drought as compared to untreated Triticum varieties. In addition, resistant varieties contained more proline than sensitive ones. They reflected the increases in proline values with drought to the defense mechanism which stressed plants take so as to reduce cell osmotic potential, thus increasing cell water uptake with concomitant increases in both cell turgidity and its activity. In addition, Azarpanah *et al.* (2013) recorded increase in accumulation of proline and carbohydrates which considered as osmotic adjustment in response to drought stress condition in Zea mays L. These results are in accordance with the findings of Mohammadkhani and Heidari (2008), Tatar and Gevrek (2008) and Azarpanah *et al.* (2013) they all reported that proline content increased under drought stress conditions, they indicated also that proline accumulation occurs in all plants organs, accumulation of proline and other amino acids during drought stress induce water absorption in plant roots and results in growth continuity.

2-Effect of AM fungi and the highest phosphate level:

In order to tolerate drought stress, plants will accumulate high concentration of low molecular - mass organic solutes such as soluble sugars, proline or other amino acids to regulate the osmotic potential of cells aiming at improving absorption of water under drought stress (Zhang *et al.,* 2010). Our data indicated that the concentrations of soluble proteins and proline in garden cress seeds increased during water stress in both AMF and non-AMF plants. These results agree with previous reports by other worker (Porcel and Ruiz-Lozano, 2004, Wu *et al.,* 2007b,). Furthermore, AMF plants had lower amino acids and proline than non-AMF plants when exposed to drought stress conditions, which may be attributed to either greater drought resistance of AMF plants or less injury in AMF plants grown under drought stress conditions (Zhang *et al.,* 2010). The results also indicated that under all soil moisture levels, adding the highest phosphate level (P3) was mostly lowered the concentrations of the different amino acids than that which treated with AM fungi only (cont+AMF) and non-AMF plants. Such decrease in amino acids concentrations may be due to that AMF inoculation increase P uptake which used as P-mediated in N2 fixation and to enhance N uptake and then protein formation (Raei and Weisany, 2013), they also recorded enhancement of uptake of macronutrient such as K and S with AMF inoculation, which increased plant tolerance to water stress. These results agree with previous reports by other worker (Wu and Xia, 2006 and Wu *et al.,* 2007b). Recently, Govindarajulu *et al.* (2005) revealed that Arginine was synthesized from inorganic N absorbed by extra radical mycelium and translocate to the intra radical mycelium, which exist in host plant roots.

Water deficit in plants induces an increased concentration of free radicals in cells thus generating oxidative stress (Ruiz-Lozano, 2003). Plants possess a number of antioxidant mechanisms that protect them from the production of reactive oxygen species (ROS). The term ROS in generic, embracing not only free radicals such as superoxide and hydroxyl radicals, but also H2O2 and singlet oxygen (Urso and Clarkson, 2003). These free radicals are neutralized by an elaborate antioxidant defense system, including enzymatic and non-enzymatic antioxidant. In our study, it showed that AMF cress plants had a lower content of soluble proteins under drought stress, indicating that AMF infection might alleviate or decrease RNA disassembly and might enhance the ability of non- enzymatic

antioxidant defense system. This is consistent with our earlier finding (Baozhong *et al.,* 2010; Zhang and He, 2007). Moreover, Abbaspour *et al.* (2011) suggest that the presence AM fungi inside the roots of any plant increased plant drought tolerance, by means of drought avoidance and drought tolerance mechanisms. It seems that the AMF symbiosis enhanced antioxidant enzymes, adjustment osmotic and nutrient acquisition, under drought stress. These findings contribute to the advances in the knowledge of AMF-induced drought stress tolerance.

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