

Full Length Research Paper

Improving effects of mycorrhizal symbiosis on sorghum bicolor under four levels of drought stress

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This study was conducted to determine the symbiotic effects of arbuscular mycorrhizal (AM) fungi on the shoot and root characteristics and mycorrhiza influence on relationships between minerals (phosphorus and Nitrogen) acquisition and yield components under drought stress in *Sorghum bicolor*. A pot experiment was carried out in Shiraz, Iran in 2010 growing season. The experiment was conducted using split-plot arrangements in a randomized complete block design (RCBD) with three replications. Treatments were based on drought stress in four levels and mycorrhiza infection stages consisted of mycorrhiza inoculated (M1) and non-mycorrhiza (M0). The results showed that the drought stress had significant influences on root colonization percent, shoot dry matter weight, plant height, leaf area, root dry matter weight, root/shoot ratio, root length and root wet weight. It seems to be that the mycorrhiza had visibly increased the biomass of sorghum by impacts on the root characteristics, such as: root weight, root length, and root/shoot ratio. The cluster analysis indicated that shoot dry matter weight was correlated with root dry matter weight in non-mycorrhizal condition. Plant height and root length traits were also correlated with leaf area and root wet weight. While, these relations changed in mycorrhizal condition and plant height, leaf area, root wet weight and root length were correlated with root colonization percent. Also cluster analysis indicated the great improvement in relations between morphological traits and nutrients absorption and yield components by effects of mycorrhizal inoculation.

Key words: Mycorrhiza, drought stress, sorghum bicolor.

INTRODUCTION

Water shortages and soil water losses due to environmental change (Al-karaki and Clark, 1998) challenges to sorghum production. Also drought is a problem seriously influencing production and quality in arid ecosystems. Among the diverse consequences of a drought effect on plant development in these ecosystems, restricted nutrient and water acquisition are commonly recognized (Agnew and Waren, 1996). It is well known that a considerable number of bacterial and fungal species possess and constitute some functional relationships with plants, and undoubtedly they are able to exert beneficially effects on plant growth (Vessey,

2003).

Considerably, there are significant evidences which express these beneficial microbial effects. These microbes or fungi can enhance the plant resistance to different kinds of environmental stresses, e.g. water and nutrient deficiency or even contamination by heavy metals (Shen et al., 1997). Inoculation of plant roots with arbuscular mycorrhizal (AM) fungi may be effective in improving crop production under drought conditions. Colonization of roots by AM fungi has been shown to improve productivity of numerous crop plants in soils under drought stress (Alizadeh et al., 2011; Al-Karaki and

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Al-Raddad, 1997; Al-Karaki and Clark, 1998; Sylvia et al., 1993). Also, it was reported by many authors that, the mycorrhizal colonization had results in the enhancement of root surface which can lead to an optimal nutrient absorbance; correlatively the fungal hyphae may extend into the soil for several centimeters and consequently lead to a large absorbance amount of nutrients for the host root (Khan et al., 2000). Improved productivity of AM plants was attributed to enhanced uptake of some macronutrients such as phosphorus, nitrogen and potassium (Mehdizadeh et al., 2010) and some micro-elements such as zinc and copper (Alizadeh and Namazi, 2011). In addition, other factors associated with AM fungal colonization may influence plant resistance to drought. These include changes in leaf elasticity (Auge et al., 1987a), improved leaf water and turgor potentials, maintenance of stomata opening and transpiration (Auge et al., 1987b), increased root length and depth, and development of external hyphae (Davies et al., 1992).

Mycorrhizal symbiosis can act as a connector of root and the surrounding soil microhabitats by linking the biotic and geochemical portions of the ecosystem (Toro et al., 1997). Considerably, factors such as: condition of soil, interactions between roots and shoots, the related functional and signal aspects, turgor pressure, yield threshold will lead to a root growth and root elongation (Munns and Cramer, 1996). It was shown that, the presence of AMF causes the host plant to grow more efficiently through a series of complex communications under the biotic and abiotic stressful conditions, such as: drought (Subramanian and Charest, 1997; Porcel et al., 2003), salinity, heavy metal contamination (Rivera-Becerril et al., 2002), suboptimal root zone temperature (Liu et al., 2004), or even soil compaction (Miransari et al., 2007). The extended network of AMF enables the fungi to increase the nutrient uptake in plants by improving the soil structure directly (Harrier, 2001), or indirectly (Rillig and Mummey, 2006).

The isolation of AMF may differ in their ability to influence on the stability of soil aggregates (Piotrowski et al., 2004; Enkhtuya and Vosatka, 2005). AMF produces a glycoprotein called glomalin, which cause the AMF to form a stable structure of aggregates with the host plant (Wright and Upadhyaya, 1996; Rillig, 2004). Under the stress free conditions AMF are able to act synergistically and enhance the plant growth by a series of mechanisms such as: enhance the fungal germination and growth (Carpenter-Boggs et al., 1995) or enhance the permeability cells of root, resulting an increase in water absorption and nutrient uptake (Artursson et al., 2006).

MATERIALS AND METHODS

This experiment was carried out in pot condition in natural farm environment in 2010 growing season. The experiment was carried out in a factorial based on randomized complete block design (RCBD) in three replications using sorghum cv. Atlas in Shiraz, Iran. In each pot, three seeds of sorghum were planted. Each pot was

consisted of 6.5 kg sterile soil with sandy- loom texture. The soil for the plots were chosen from a normal agricultural field from the depth between 0 to 20 cm and consequently were sterilized under pressure of 15 psi at 121.5°C for 2 h to be cleaned from other microorganisms. The seeds which were germinated in germinator were inoculated by *Glomus intraradices*, an Arbuscular Mycorrhizal Fungus.

Consequently, treatments were conducted base on drought stress in four levels: T0: re-irrigation when 75% of plant-available water was existed. T1: re-irrigation when 55% of plant-available water was existed. T2: re-irrigation when 35% of plant-available water was existed. T3: re-irrigation when 15% of plant available water was existed. To conduct the treatments for water stress, first the Bulk Density (Pb) were determined, and under the category of Field Capacity (FC) and Permanent Wilt Point (PWP) the "Available" Soil Water (ASW) were analyzed by following formula $ASW = FC - PWP$. Notably, the available water at FC point was supposed as 100% and the other treatments calculated based on it.

In each pot the amount of N and P were calculated by sampling from leaf in silk growth level. Plant samples were sent to the laboratory immediately. They were cleaned thoroughly after being washed with ordinary and distilled water in the laboratory. Then they were dried in oven for 72 h and in 70 centigrade degree and grinded afterwards. To measure nitrogen, 0.3 g of the plant sample were digested using sulfuric acid, salicylic acid and distilled water and then, its amount was specified with kjeldahl nitrogen procedure. To measure other elements, 1 gram of samples put in the electrical kiln in the temperature of 550 centigrade degree for 5 h to become ash, then, it was digested with chloridric acid 2 normal. The elements were measured as follows: potassium, using the film photometer machine (Fateh electric, model 405- made in Iran) and phosphor, using the method of calorimetric with spectrophotometer machine, wavelength of 880(nm) (ERMA PHOTIC 100-made in Japan).

Finally, root colonization percent, shoot dry matter weight, plant height, leaf area, root dry matter weight, root/shoot ratio, root length and root wet weight were measured. The root length was calculated as follow formula: $RL = 11/14 \cdot ND$ Where, N: number of roots from the horizontal and vertical lines; D: the sideward length of square. The response of treatments to mycorrhiza inoculation was measured by following formula: $RTM = [(Mycorrhiza \text{ dry matter} - \text{Non-mycorrhiza dry matter}) / \text{Non-mycorrhiza dry matter}] \cdot 100$. Finally, the obtained data were analyzed by SAS software ver. 9.1 (2001).

RESULTS AND DISCUSSION

Drought stress effects on mycorrhizal colonization

Fungal colonization significantly influenced by drought stress by the analysis of variance (Table 1). The difference between colonization in all levels of irrigation showed that the drought clearly decreased mycorrhiza activity. Drought effects in the vegetative developmental stage of sorghum on the root colonization by hyphae, vesicles and arbuscules depended on the fungal species (Auge et al., 1995). When the fungus was *Glomus intraradices*, hyphae and arbuscules colonization reduced under drought when compare with higher soil water contents (Busso et al., 2008) Also in *Azadirachta indica* with increased stresses such as salinity level, there was a decrease in percent of root infection by AMF (Pande and Tarafadar, 2002).

Table 1. Analysis of variance for studied traits of *Sorghum bicolor* under effect of mycorrhiza inoculation and drought stress.

S.O.V.	df	Mean of squares										
		Root colonization percent	Shoot dry matter weight	Plant height	Leaf area	Root dry matter weight	Root/Shoot	Root length	Root wet weight	P Uptake	N Uptake	Grain yield
R	2	3.0 ^{ns}	59.5**	8.97 ^{ns}	2692781**	27.984*	0.0002171*	6698 ^{ns}	1672 ^{ns}	0.113 ^{ns}	0.149 ^{ns}	0.105 ^{ns}
M	1	21702.0**	3220.2**	988.17**	40809984**	439.990**	0.0048170**	21169895**	479685**	0.111**	0.69*	56.12**
T	3	453.01**	2020.5**	421.39**	52149911**	24.461*	0.0025171**	1399877**	417710**	0.487**	4.36**	29.45**
M×T	3	452.94**	448.3**	20.50**	1388553**	10.587 ^{ns}	0.0000939 ^{ns}	261002**	33231**	0.142**	0.16 ^{ns}	0.255*
E	14	3.4	5.4	0.02	122983	7.354	0.0000360	3202	3554	0.456	0.42	0.057
%CV		-	4.28	1.37	9.44	13.07	8.33	3.79	2.35	5.44	4.71	5.06

R: Replication, M: Mycorrhiza, S: Stress, E: Error, CV: Coefficient of variation, ns, * and **: Not significant, significant at the 5 and 1% levels of probability, respectively.

Mycorrhiza and drought stress effects on root and shoot traits

The analyses of variance among the treatments (Table 1) showed; mycorrhiza had significant influences on roots and shoots dry weight and correlatively, the highest shoots dry weight was achieved by M1T0 treatment (194.3 g) despite the fact that there was not any significant difference between the T0 and T1 but it significantly decreased by drought stress in T3 and T4 (Table 2). As the results, Mycorrhiza totally increased shoot dry matter by 14.9% and root dry matter by 32.6%, the root dry weight had no significant difference between T0, T1, T2 but the decrease was significant in the high water deficiency in T3.

Root/Shoot

The individual effects of mycorrhiza and drought stress on root/shoot ratio was significant by the analysis of variance but the interaction of drought stress and mycorrhiza on root/shoot ratio was not significant (Table 1). Also in mean comparisons we saw that the root/shoot ratios were raised

significantly by water deficiency tension, but it had the higher value in mycorrhiza treatments. It seems, in drought stress condition the root of plant had supported with the mycorrhiza and it caused a growth in root/shoot ratio whereas the highest amount of root/shoot ratio was achieved in the highest drought tension treatment, T3 (0.245) even though the significant difference was between (T0, T1) and (T2, T3) treatments and we had a 15.4% increasing in root/shoot ratio in mycorrhiza inoculated treatments instead of non-mycorrhizas.

Shoot growth is usually more affected than root growth in a water stress condition (Munns and Cramer, 1996; Pardo et al., 2000). But in water stress nutrient absorbance will decrease when nutrients (especially Nitrogen uptake) reduce, consequently, less cytokinin will be produced in the roots and it will be sent to the shoots. Considerably, the lower amounts of cytokinin result in the reduction of cell division in the shoots while in the roots this may lead to neutralizing the suppressing effect of cytokinin on cell growth and cell development. Also transfer of more sucrose to the roots enhances root to growth under these conditions (Van der Werf and Nagel, 1996).

Root Length

The analysis of variance also showed that mycorrhiza and drought stress had significant affection on root length (Table 1). The means comparison showed that, the enhancement of drought stress had caused a decrease in root length. On the contrary, in treatments which had been inoculated by mycorrhiza, the root length had achieved the higher value. The root extension by mycorrhiza was evident in those treatments. Undoubtedly, the root extension helps the plant to be more connected with soil and uptake more nutrients which consequently will lead to biomass synthesis and growth. Considerably, by the drought stress affecting, the highest root length was achieved in T0 treatment (8161.7 cm) and the lowest length was achieved in T3 treatment (7071.7 cm). To sum it up, mycorrhiza improved root elongation by 28% when drought reduced it by 12.7% in our highest stress treatment (T3).

Leaf area

Leaves considerably affected by the treatments.

Table 2. Effect of Mycorrhiza and Drought stress on traits of *Sorghum bicolor*.

		Mean										
Treatment		Root colonization percent	Shoot dry matter weight (g)	Plant height (cm)	Leaf area (cm ²)	Root dry matter weight (g)	Root/Shoot (ratio)	Root length (cm)	Root wet weight (g)	P Uptake (%)	N Uptake (%)	Grain yield (gr/pot)
Mycorrhizal inoculation	M ₀	0.00 ^b	155.33 ^b	175.1 ^b	11427 ^b	32.787 ^b	0.2110 ^b	7350.9 ^b	4263.5 ^b	0.243 ^b	0.969 ^b	12.70 ^b
	M ₁	60.83 ^a	179.02 ^a	187.9 ^a	14160 ^a	40.369 ^a	0.2389 ^a	8585.2 ^a	4546.3 ^a	0.356 ^a	1.822 ^a	15.76 ^a
Drought stress	T ₀	38.34 ^a	183.34 ^a	189.6 ^a	15841 ^a	36.536 ^{ab}	0.2008 ^d	8161.7 ^a	4743.1 ^a	0.365 ^a	1.692 ^a	16.43 ^a
	T ₁	36.34 ^a	177.34 ^b	187.6 ^b	14765 ^b	36.967 ^{ab}	0.2083 ^c	7906.7 ^b	4508.8 ^b	0.335 ^b	1.527 ^a	15.68 ^a
	T ₂	26.17 ^b	165.16 ^c	176.4 ^c	10431 ^c	37.809 ^a	0.2300 ^b	7393.5 ^c	4179.8 ^c	0.283 ^c	1.459 ^a	13.02 ^b
	T ₃	20.34 ^c	125.33 ^d	172.4 ^d	10137 ^c	35.001 ^b	0.2450 ^a	7121.6 ^d	4187.7 ^c	0.215 ^c	0.906 ^b	11.77 ^c
Interaction	M ₀ ×T ₀	0.00 ^e	175.01 ^b	185.0 ^a	15097 ^b	33.167 ^b	0.1900 ^a	7336.7 ^c	4543.3 ^b	0.296 ^{ab}	1.021 ^{ab}	15.13 ^a
	M ₀ ×T ₁	0.00 ^e	170.67 ^b	182.3 ^a	13651 ^c	33.567 ^b	0.1966 ^a	7120.2 ^c	4224.3 ^b	0.240 ^b	1.027 ^{ab}	14.26 ^b
	M ₀ ×T ₂	0.00 ^e	158.33 ^c	169.6 ^b	8573 ^e	33.615 ^b	0.2133 ^a	6409.7 ^d	4133.7 ^c	0.233 ^b	0.923 ^{ab}	11.20 ^c
	M ₀ ×T ₃	0.00 ^e	117.33 ^d	163.6 ^b	8388 ^e	30.800 ^c	0.2266 ^a	5960.0 ^d	4152.7 ^c	0.215 ^b	0.726 ^b	10.23 ^c
	M ₁ ×T ₀	76.67 ^a	191.67 ^a	194.3 ^a	16586 ^a	39.905 ^a	0.2100 ^a	8986.7 ^a	4943.0 ^a	0.430 ^a	2.183 ^a	17.83 ^a
	M ₁ ×T ₁	72.67 ^b	184.00 ^a	193.0 ^a	15879 ^b	40.367 ^a	0.2200 ^a	8693.3 ^a	4793.3 ^{ab}	0.396 ^a	2.027 ^a	17.10 ^a
	M ₁ ×T ₂	50.33 ^c	171.99 ^b	183.3 ^a	12289 ^d	42.002 ^a	0.2466 ^a	8377.3 ^b	4226. ^{0b}	0.340 ^a	1.993 ^a	14.33 ^b
	M ₁ ×T ₃	40.67 ^d	133.33 ^b	181.3 ^a	11887 ^d	39.201 ^a	0.2633 ^a	8283.3 ^b	4222.7 ^c	0.313 ^{ab}	1.087 ^{ab}	13.30 ^b

ns, * and **: Not significant, significant at the 5% and 1% levels of probability, respectively. Means in each column, followed by similar letter(s) are not significantly different at 5% probability level, using Duncan's Multiple Range Test.

The variance analysis (Table 1) showed that the effect of mycorrhiza, drought stress and the Interaction of them on leaf area were significant at 1% of probability. Mycorrhizal plants showed greater LA than non-mycorrhizal plants, leaf area improvement by mycorrhiza treatment in this experiment was about 22.7%. In the well watered treatment LA in mycorrhizal plants was greater than non-mycorrhizal plants. In moderate stress and the severe stress conditions LA in mycorrhizal plants was greater than non-mycorrhizal plants

particularly in plants infected by mycorrhiza (Amerian and Stewart, 2001). Often mycorrhizal improvement of drought tolerance occurs via drought avoidance. It can be a function of the often observed improved acquisition of phosphorus, nitrogen and other growth promoting nutrients by AMF plants (Alizadeh et al., 2010; Augé, 2001). Also several mechanism have been proposed to explain the protection of AMF symbiosis, such as changes in plant hormones (Danneberg et al., 1992; Goicoechea et al., 1995),

increased leaf gas exchange and photosynthetic rate (Ruiz-Lozano et al., 1996a); direct hyphal water uptake from the soil and transfer to the host plant (Faber et al., 1991; Ruiz-Lozano and Ázcón, 1996), enhanced activity of enzymes involved in anti-oxidant defense (Ruiz-Lozano et al., 1996b), nitrate assimilation (Ruiz-Lozano and Ázcón, 1996), enhanced water uptake through improved hydraulic conductivity and increasing leaf conductance and photosynthetic activity (Dell-Amico et al., 2002), osmotic adjustment (Augé et al., 1986)

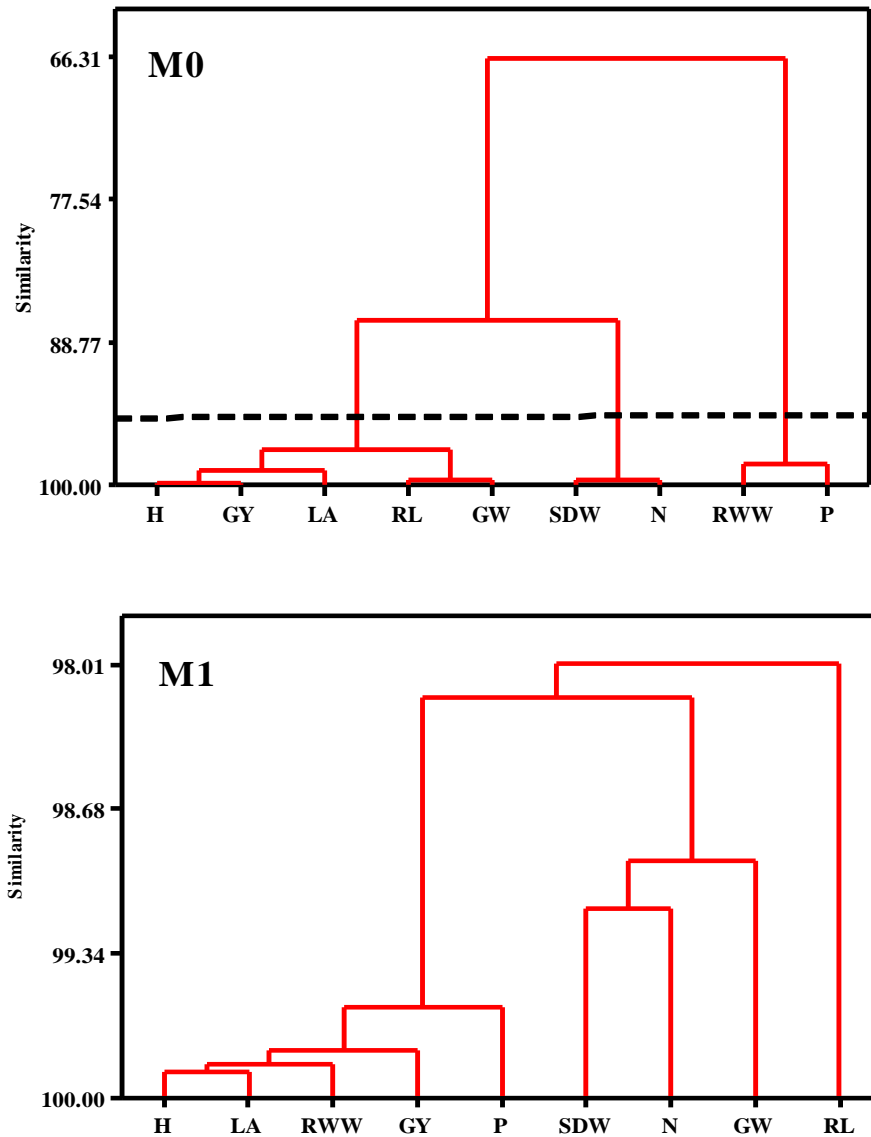


Figure 1. M0: Cluster analysis of studied traits in non-mycorrhizal condition using Ward method. M1: Cluster analysis of studied traits in mycorrhizal condition using Ward method. Plant height (H), leaf area (LA), shoot dry weight (SDW), root length (RL), Root wet weight (RWW), 1000-grain weight (GW), grain yield per pot (GY), absorbed nitrogen (N), absorbed phosphorus (P).

and changes in cell-wall elasticity (Augé et al., 1987; Sanchez-Diaz and Honrubia, 1994).

Mycorrhizal affection on relations between nutrients absorption, morphological traits and yield components

To show the effects of mycorrhiza on relationships between our studied traits and nutrients absorption and yield components, cluster analysis was usable. The dendrograms of cluster analysis using Ward method were

illustrated in Figures 1 and 2. In the dendrograms (Figure 1), traits are presented on the horizontal axis and the correlation coefficient distances on the vertical. In non-mycorrhizal dendrogram (M0) the studied traits were grouped into three clusters. Based on the results, 1000-grain weight and grain yield per pot was located in the first cluster, shoot dry weight and absorbed nitrogen were placed in the second cluster and root wet weight and absorbed phosphorus were located in the third cluster, indicating that in non-mycorrhizal condition the root and shoot matters and absorbed nitrogen and phosphorus had the lower relationship with grain yield compared with

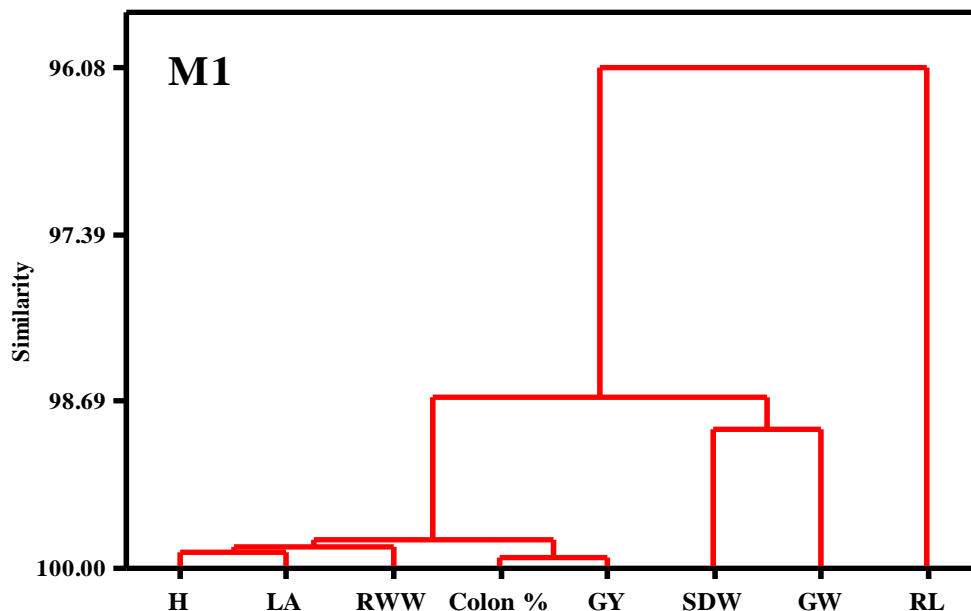


Figure 2. Cluster analysis of mycorrhizal colonization percent and studied traits in mycorrhiza condition using Ward method. Plant height (H), leaf area (LA), shoot dry weight (SDW), root length (RL), root wet weight (RWW), 1000-grain weight (GW), grain yield per pot (GY), mycorrhizal colonization percent (Colon %).

other traits (Figure 1- M0), but these relations changed in mycorrhizal condition (Figure 1- M1) and all the traits was located in a same cluster and they had highly great positive relationship in at least 98% of similarity. Also, Phosphorus and Nitrogen uptake optimization by mycorrhiza inoculation were reported by Mehdizadeh et al. (2010) and mycorrhizal grain yield improvement reported by Alizadeh et al. (2011).

In the dendrograms (Figure 2), traits are presented on the horizontal axis and the correlation coefficient distances on the vertical. Based on cluster analysis of mycorrhizal colonization percent and studied traits in mycorrhizal condition, all the studied traits had a high relationship with mycorrhizal colonization, in at least 96% of similarities, and the highest relationship was between mycorrhizal colonization and grain yield. So the fact that Mycorrhiza caused the positive relationship between our studied traits and the yield components were confirmed by cluster analysis, furthermore it showed that nitrogen and phosphorus mycorrhizal uptaking was highly related to this relationship (Figures 1 and 2).

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