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Growth and Carbohydrate Compositions of Three *Gossypium* Species Inoculated with *Rhizophagus intraradices* under Salinity Stress

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ABSTRACT

Expansion of salt stress in cultivable fields prevents plant physiological functions and reduces crop yield. *Arbuscular Mycorrhizal Fungi* (AMF), as a bio-amelioration of salt stress, protects cellular osmosis via disaccharide and polysaccharide metabolism changes. In this study, three *Gossypium* species (i.e., *G. hirsutum*, *G. barbadense*, and *G. herbaceum*) colonized with *Rhizophagus intraradices* [with AMF and without AMF] were cultivated under saline irrigation treatments (ECe< 4 Ds m^{-1}= S0, 8-9= S1, and 12-13= S2) as a factorial experiment. Salinity treatments were initiated at flowering. Generally, according to physiological traits, [+AMF] colonized with *G. barbadense* was more tolerant in exposure to 12-13 dS m^{-1} salinity, while *G. hirsutum* with [+AMF] was just tolerant until 8-9 dS m^{-1}. This is because, the highest and the least leaf area were observed in *G. barbadense* [+AMF] under 8-9 and 12-13 dS m^{-1}, respectively. In 12-13 dS m^{-1}, the highest root volume, root dry weight, seed weight, and fiber weight were obtained in *G. barbadense* [+AMF]. Moreover, the highest sugar content in root and leaves and the highest starch content of root, leaves, and seed cotyledon were observed in *G. barbadense* [+AMF] under 12-13 dS m^{-1} treatment. Under 8-9 dS.m^{-1} salinity, the highest starch, Sucrose Phosphate Synthase (SPS) and Sucrose Phosphatase (SP) enzyme activities were in roots of *G. barbadense* [+AMF]. The present study suggests that despite dramatic physiological alterations under high-salinity in comparison with mild-salinity, AMF and *G. barbadense* showed the best symbiotic performance under 12-13 dS m^{-1}.

Keywords: Cotton, Bio-amelioration, Mycorrhiza, Symbiotic performance

INTRODUCTION

Soil salinization is known as a fundamental environmental concern. More than 800 million hectares of land, equivalent to over 6% of the world's total land surface, are affected by salinity (Guo *et al.*, 2019). Salt stress is one of the major limitations to crop growth and productivity (Arzani and Ashraf, 2016; Wei *et al.*, 2017). Irrigation in arid and semi-arid regions contributes to the accumulation of soluble salts and of exchangeable sodium in the soil where the roots grow (Arzani and Ashraf, 2016). Cotton is one of the most salt-tolerant crop species with a threshold electrical conductivity (EC) of 7.7 dS m^{-1} (Maas, 1990). So, the global area of cotton cultivation is predicted to range from 77 million acres during 2008-2009 to 85 million acres by 2018-2019 (Hudson *et al.*, 2009). The genus *Gossypium* includes more than 50 species (Gallagher *et al.*, 2017), among which *G. hirsutum* and *G. barbadense* are allotetraploid cultivated species (Ulloa *et al.*, 2017). It is controversial that in saline-soils, with EC of 10 and 20 dS m^{-1}, cotton yield declined to about

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84 and 54%, respectively (Qadir and Shams, 2008).

Generally, plant responses to salinity stress have been classified into two categories: primary "osmotic" and "ionic-specific", and secondary oxidative stresses (Arzani and Ashraf, 2016). The adjustment of osmotic potential assists to maintain pressure potential in plant cell, which is essential for its normal function and growth under abiotic stress conditions (Arzani, 2008). Among the organic compatible solutes, soluble sugar content has a crucial role in plant osmotic adjustment under salinity stress (Akrami and Arzani, 2018). Hence, the yield loss is considerably related to carbohydrate metabolism in various species of cottonseed inoculated with AMF under salinity stress (El-Shourbagy and Kishk, 1975). Under salinity stress, polysaccharides with low osmotic activity including starch or high soluble carbohydrate such as sucrose are accumulated in plants (Whittaker *et al.*, 2007). Sucrose was produced through a two-step process catalyzed by Sucrose Phosphate Synthase (SPS) and Sucrose Phosphatase (SP). SPS is activated by the synthesis of Sucrose-6-Phosphate (SP) from Fructose-6-Phosphate (Fru-6-P) and UDP-glucose (Stitt *et al.*, 1988). Invertase enzymes play an important role in the inversion of sucrose to glucose and fructose (Kulshrestha *et al.*, 2013). Also, SPS and SP play an important role in the partitioning of carbon between starch production and carbohydrate storage in photosynthetic and non-photosynthetic tissues during physiological and developmental processes (Chen *et al.*, 2005). *Arbuscular Mycorrhizal Fungi* (AMF) enhances Invertase-enzyme activity in plants under salinity (Zhang *et al.*, 2011) to hydrolyze sucrose for supplying hexoses-sugars, including glucose and fructose for fungi (Bücking *et al.*, 2012). For instance, Garg and Bharti (2018) found that *Cicer* [+AMF] increased salinity tolerance using starch hydrolysis to glucose. Casey *et al.* (2013) suggested that cell growth and glucose consumption efficiency of microorganisms would decline under salinity. Additionally, as AMF demands more carbohydrate, accumulation of carbohydrate concentrations in the host root increased. It also prompts potential tolerance to salinity in plants [+AMF] (Feng *et al.*, 2002). Several reports have illustrated that the increased sugar accumulation may also be due to the hydrolysis of starch to saccharides in mycorrhizal seedlings (Zhu *et al.*, 2017). AMF improved the root system via exploiting in soil pores. This process causes increase in water absorption and improves growth in cotton (Moreira Salgado *et al.*, 2017). Therefore, because of enhancing water absorption through AMF-spore germination and hyphal branching, Root Dry Weight (RDW) and Root Volume (RV) were accelerated (Asmelash *et al.*, 2016). Gutjahr (2014) reported that the formation of AMF colonization and the extent of root colonization depended on plant species and growing conditions. Furthermore, Yfoulis and Fasoulas (1973) declared that salinity would influence fiber production and seed weight by reducing photosynthesis products, such as carbohydrate concentration. Under saline soil conditions, these cotton species colonize with AMF to compensate salinity damage, therefore, it is necessary to evaluate their physiological mechanisms.

Considering the literature reviewed above, in the present study, the effect of *R. intraradices* symbiosis on the yield of three cotton species was investigated under salinity stress.

MATERIALS AND METHODS

This experiment was conducted in Cotton Research Institute of Gorgan- Iran, during 2019- 2021. Three selected species of *Gossypium* were *Gossypium hirsutum*: Golestan (G1), *Gossypium barbadens* (Termez14) (G2), and *Gossypium herbaceum* (Mehriz) (G3). Three levels of saline irrigation treatments including low-salinity [ECe< 4 dS m^{-1}= S_0], mild-salinity [8-9= S_1], and high-salinity [12-1 = S_2] and two inoculation treatments including inoculation with *R. intraradices* [+AMF] or not [-AMF] were used. To conduct the study, a factorial experiment arranged in a

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randomized complete block design with three replications was used.

Soil and Biological Material

Soil and roots of *Zea mays* were chopped and mixed with cotton seed (25 infectious propagules per cm^3 in average) (Gaur *et al.*, 2000). The soil of the seedbed was sandy-clay-loam in texture consisting of sand (22%), clay (66%), and loam (12%). Cottonseeds were inoculated with 25 g of mycorrhizae inoculum consisting of soil, spores, mycelia, and infected root fragments. These native AMF were isolated from the rhizosphere of maize (*Zea mays* L.) grown under shelter.

Growth Conditions

Cotton seed species were sown in pots under greenhouse (60 cm wide×40 cm long). To disinfect seeds. Five g of Gaucho (insecticide) and 5 g of Carboxin–Thiram (fungicide) were used. Trimming was carried out, leaving one or two plants per hole. The salinity irrigation treatments (NaCl) were applied at the flowering stage (when > 50% of the plants reached the flowering stage). Salinity water for each treatment was measured using EC-meter. After that, cottonseed leaves were cut from upper-most fully expanded leaves that were randomly selected from 5 plants per replicate. After ripping boll, seeds were harvested manually from each pot. Mature cotton seeds were harvested from each pot and then analyzed. Totally, all treatments with three replications were 54 pots.

Saccharides and Polysaccharides

Saccharides were determined in root, leaves, and seeds (Moing *et al.*, 2004) by a High-Performance Liquid Chromatography (HPLC) system (Waters Assoc., Milford, MA, USA), with the following parameters: 300×7.8 mm (Aminex) HPX 42C column (Bio Rad), Eluent: water, temperature: 80°C, flow rate: 0.5 mL min^{-1}, and Injection Volume: 20 µL. In summary, dry lyophilized tissues were extracted using ethanol at 80°C in three steps, each step lasted for 20 minutes (step 1: 0.75 mL of 80% ethanol; steps 2 and 3: 0.75 mL of 50% ethanol). After centrifugation, the homogenate was deionized. The ethanol was evaporated from the extracts using the SpeedVac and dry extracts were solubilized in 1 mL of double-distilled water. Finally, concentrations of saccharide were calculated using mannitol as an internal standard (Weiß and Alt, 2017).

To evaluate the starch according to phenol-sulfuric acid method (Rao and Pattabiraman, 1989), 10 mL of distilled water was added to the dried pellet. After mixing with $Ba(OH)_2$ (0.3N) and $ZnS0_4$(5%), the samples were centrifuged (3,000 rpm,10 minutes). One mL of phenol (5%) and 5 mL of sulfuric acid (98%) in 2 mL were added to the supernatant. Then, the absorbance of the extract was determined at a wavelength of 485 nm to detect starch. Finally, amounts of soluble carbohydrates and starch were reported as mg g^{-1} DW (Rao and Pattabiraman, 1989).

Sucrose Phosphate Synthase (SPS)

Frozen tissue (1 g) was ground in 3 mL buffer [Hepes-NaOH (pH 7.5), Na-EDTA, DTT, $MgCl_2$, BSA and Triton X-100] (Hubbard *et al.*, 1989). Then 70 µL of extraction buffer was added to 40 µL (Hepes-NaOH, $MgCl_2$, Fru6p, Glu6p and UDPG). After adding 70 µL KOH, SPS were read at 620 nm.

Sucrose Synthase

Sucrose synthase was done according to Anthron method of Van Handel (1968), and 25 Mm Fru was used instead of Fru-6p. In addition, this experiment was conducted without using Glu-6p.

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Invertase

The seeds of the fresh sample were homogenized with extraction buffers; [Hepes-NaOH (pH7.5), Na-EDTA, DTT, Bovine Serum Albumin (BSA) and insoluble Polyvinylpyrrolidone (PVP)] for Invertase enzyme activity using Miron and Schaffer (1991). After centrifugation at 18,000×g for 30 minutes, supernatant was dialyzed by 25 mM Hepes-NaOH (pH7.5) and 0.25 mM Na-EDTA. Then, it was used as the crude soluble enzyme extract. Pellet was homogenized in extraction buffer. Solubilization was done with NaCl (0.2-1M). Finally, Invertase activity was assayed in KH_2PO_4 (0.1M), citrate buffer (pH7.5), sucrose (0.1M) and enzyme extraction. The reaction was stopped and reducing sugar was measured using Dinitro-salicilic acid. After 30 minutes incubation for blank control, enzymatic extraction was added. Invertase activity enzyme was recorded at 540 nm.

Growth Traits

Leaf Area

The leaf area was evaluated using Image J software (LOCI, University of Wisconsin).

Root Dry Weight and Root Volume

Root Dry Weight (RDW) and Root Volume (RV) were estimated at harvest (Schuurman, 1971). Shoots were removed, roots were separated from soil using sieve number 2 and taproots were calculated for each pot. Also, the roots were rinsed free of medium under running water. To determine root system volume, the roots were submerged until the surface of the water was 2 mm (0.08 in) above the upper most lateral root. So, immersion of the root parts was measured; the new balance reading was recorded and used as an estimate of plant part volume. The washed root systems were blotted dry in a forced aeration oven at 65°C for 48 hours and then were weighed.

Total Seed Weight and Fiber Weight in Each Plant

Plants in each pot were harvested and all bolls on single plants were gathered. After ginning of cotton bolls, cottonseeds and fiber were collected and weighed separately.

Statistical Analyses

Statistical analyses were performed using the SAS software (9; SAS Institute, Cary, NC, USA). Means were compared by Least Significant Difference (LSD) at $P< 0.05$. To evaluate concentrations, a standard curve was generated and the best equation was selected based on linear correlation (r) and regression analysis using SPSS software version 16. Correlation coefficients were determined by SPSS software, using the Pearson's correlation coefficient method.

RESULTS

Saccharide

Results demonstrated that interaction of cotton species*salinity*AMF in saccharides of root, leaf and seed were significant ($P\leq 0.01$) (Table 1). Results (Table 2) showed that roots of *G. herbaceum* [+AMF] under low, mild, and high-salinity had the maximum saccharide content. Moreover, the highest saccharide content was observed in the seed coats of *G. barbadens* [-AMF] in low-saline soil, whereas under high-salinity, *G. barbadense* [+AMF] had more saccharide in comparison to *G. barbadens* [-AMF] (Table1). The leaves of *G. hirsutum* and *G. barbadense* [+AMF] under mild-salinity had more saccharide than [-AMF], while in high-salinity, the saccharide content of leaves in *G. barbadense* and *G. herbaceum* [+AMF] were more than that in [-AMF]. Saccharide

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Table 1. Two-way ANOVA analysis of saccharides and polysaccharides in leaf, root and seed coat. S (Salinity); G (cotton species); M (*Arbuscular Mycorrhizal Fungi*). [a]

Source	DF	Saccharides			Polysaccharides		
		Leaf	Root	SeedSC	Leaf	Root	Seed SC
Rep	2	0.56	0.61	0.69	0.0002	0.000003	0.000003
S	2	16.59 **	0.13 ns	14.25 **	0.0010 **	0.0043 **	0.0014 **
G	2	35.50 **	0.87 **	4.99 **	0.0028 **	0.0004 **	0.0021 **
M	1	0.007 ns	0.31 ns	1.37 ns	0.00002 ns	0.04 **	0.0001 ns
S*G	4	25.32 **	0.26 ns	15.96 **	0.0027 **	0.006 **	000007 **
G*M	2	4.66 *	4.31 **	2.67 *	0.0008 **	0.0012 **	0.0015 **
S*M	2	17.73 **	0.94 **	9.56 **	0.002 ns	0.0025 **	0.0004 **
S*G*M	4	43.42 **	0.54 **	7.92 **	0.0027**	0.0087 **	0.0005 **
Error	34	0.90	0.12	0.64	0.00008	0.00007	0.00005
CV		4.18	0.54	11.02	1.75	0.91	1.68

[a] Treatments at P≤ 0.05, derived from LSD. (ns: Not significant, * and **: Represent significance at P≤ 0.05 and P≤ 0.01, respectively.

Table 2. Average mean of mycorrhiza symbiosis, +AMF [M_0] and –AMF [M_1], different cotton species [*G. hirsutum*: (G1), *G. barbadens* and (G2*) G. herbaceum* (G3)] and salinity water treatments [low-Salinity (S_0), mild-Salinity (S_1) and high-Salinity (S_2)] on Saccharide and Polysaccharide.[a]

Treatment	Saccharide			Polysaccharide		
	Leaf	Root	Seed cotyledon	Leaf	Root	Seed cotyledon
$S_0G_1M_0$	22.40±0.23c-g	63.39±0.06cdef	31.12±0.70bcd	38.16±0.46g	67.03±0.55g	39.36±0.27cd
$S_0G_2M_0$	25.79±0.55abc	62.73±0.25gh	19.02±0.29g	38.72±0.20efg	74.14±0.70cd	41.43±0.34bcd
$S_0G_3M_0$	25.99±0.25ab	63.08±0.19efg	32.01±0.17bc	41.01±0.27c	69.66±0.40e	42.42±0.61bcd
$S_0G_1M_1$	22.03±0.05d-g	63.46±0.10cdef	34.11±0.85b	39.55±0.29cde	76.15±0.21a	38.99±0.33d
$S_0G_2M_1$	23.96±0.17a-e	64.03±0.18bc	33.79±0.24b	39.90±0.32cde	74.66±0.46c	43.81±0.50bc
$S_0G_3M_1$	20.98±0.05efg	63.92±0.13bc	30.08±0.83cde	40.30±0.55cd	73.51±0.29d	40.50±0.39bcd
$S_1G_1M_0$	21.31±1.15efg	64.52±0.13ab	28.15±0.12de	40.19±0.45cd	74.71±0.52c	43.95±0.51bc
$S_1G_2M_0$	23.19±0.65b-f	63.05±0.26fg	40.37±7.81a	38.69±0.76efg	75.05±0.06c	40.37±0.37bcd
$S_1G_3M_0$	19.52±0.42g	63.23±0.10defg	27.94±0.32de	40.41±0.30cd	64.28±0.16h	43.70±0.14bc
$S_1G_1M_1$	25.40±0.81a-d	62.20±0.19h	29.13±0.46cde	44.86±0.19a	74.17±0.26cd	40.49±0.52bcd
$S_1G_2M_1$	23.80±0.10b-f	63.83±0.12cd	30.57±0.27cde	39.88±0.34cde	79.19±0.05a	50.32±0.60a
$S_1G_3M_1$	15.31±0.79h	65.06±0.64a	27.82±0.45e	36.40±0.38h	68.87±0.23ef	39.07±0.24d
$S_2G_1M_0$	25.27±0.59a-d	63.66±0.26cdef	23.97±0.69f	43.45±0.22b	75.82±0.66ab	42.57±0.39bcd
$S_2G_2M_0$	24.36±0.76a-e	63.49±0.14cdef	14.68±0.39h	38.49±0.33fg	75.83±0.12ab	44.18±0.72b
$S_2G_3M_0$	19.83±0.69fg	63.58±0.10cdef	24.28±0.80f	40.04±0.46cd	74.50±0.13cd	40.09±0.23bcd
$S_2G_1M_1$	18.99±0.09g	62.25±0.16h	20.13±1.32g	40.30±0.30cd	68.48±0.48f	39.38±0.47cd
$S_2G_2M_1$	26.78±0.29a	63.73±0.12cde	20.42±0.86g	41.11±0.48c	76.06±0.08ab	48.98±0.41a
$S_2G_3M_1$	25.01±0.57a-d	63.63±0.18cdef	18.00±0.74g	40.97±0.33c	75.89±0.03ab	44.22±0.11b

[a] Values represent means of three replicates±SE. Different letters within the column represent significant difference among the treatments at P≤ 0.05, derived from LSD. ns: Not significant, * and **: Represent significance at P≤ 0.05 and P≤ 0.01, respectively, derived from two-way ANOVA. S: Salinity, G: Cotton species, M: *Arbuscular Mycorrhizal Fungi* (AMF).

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was shown to be less abundant in seed coat (between 4.2 and 11.3 mg g^{-1} DW) under low-salinity. AMF would not be able to influence saccharide content in cottonseed, while saccharide content was higher than [-AMF] in the seed coat of *G. hirsutum* and *G. herbacea* [+AMF] by 52% and 36% (8-9 dS m^{-1}) and 10 and 23% (high-salinity), respectively (Table 2).

Starch

Analysis variance (Table 1) showed that interaction of cotton species×salinity×AMF treatments in polysaccharides of root, leaf, and seed were significant (P≤0.01). According to Table 2, starch storage increased more in the roots of *G. barbadens* under low-salinity compared to the others. In addition, in high-salinity, *G. barbadens* [+AMF] and *G. hirsutum* [-AMF] had similarly the highest starch content in root (1 mg DW^{-1}) (Table 1). Although the roots of *G. barbadens* [+AMF] had 0.3% more starch under high-salinity than other species [-AMF], the highest starch content was observed in this species, under mild-saline water compared to [-AMF]. Under mild and high-salinity, *G. barbadens* [+AMF] had more starch than [-AMF] plants. It was observed that the *G. hirsutum* leaves had the highest starch content. Also, *G. herbacea* [+AMF] had the highest concentration under high-salinity (Table 2). Starch content was lower in the seed cotyledon [+AMF] under salinity than the [-AMF] species (Table 2).

SPS and SP-activity

As Table 3 shows, interaction of cotton species×salinity×AMF in SPS and SP-activity of seeds were significant (P≤ 0.01). *G. hirsutum* and *G. herbaceum* [+AMF] under high-salinity had the most and least SPS-activity, respectively (Figure 1). Results represented that in all salinity treatments *G. hirsutum* [+AMF] had lower SPS in comparison to [-AMF] plants (Figure 1).

In low-salinity, *G. herbaceum* [+AMF] had the most SP-activity. Under mild-salinity, *G. herbaceum* [-AMF], SP-activity with 1.326 µmol min^{-1} g^{-1} M was the lowest, while SP-activity was more in *G. herbaceum* [+AMF] with 16.254 µmol min^{-1} g^{-1} M than in [-AMF] (Figure 2).

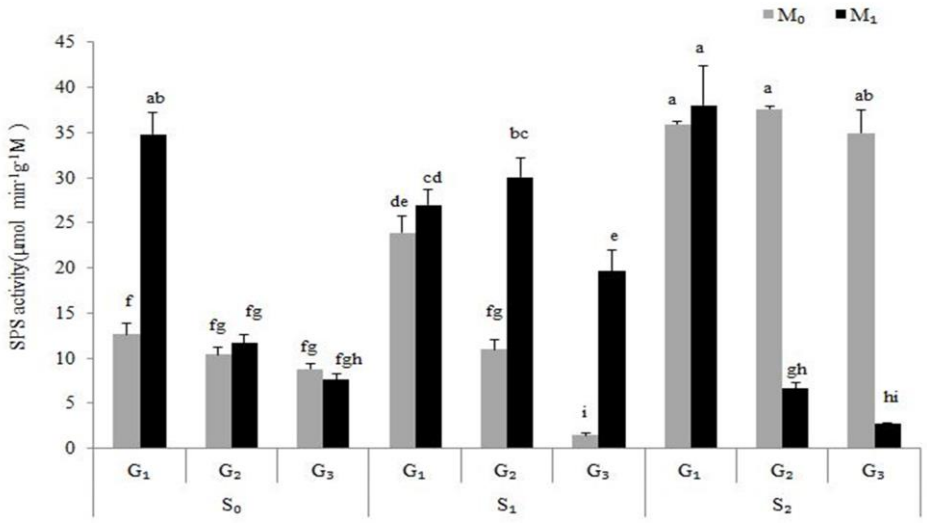

Figure 1. Effects of saline water conditions (low-salinity [S_0], mild-salinity[S_1] and high-salinity [S_2]) on three cotton species (*G. hirsutum* [G1], *G. barbadens* [G2] and *G. herbacea* [G3]) and -AMF[M_0], +AMF[M_1] on SPS-activity (µmol $min^{-1}g^{-1}M$).

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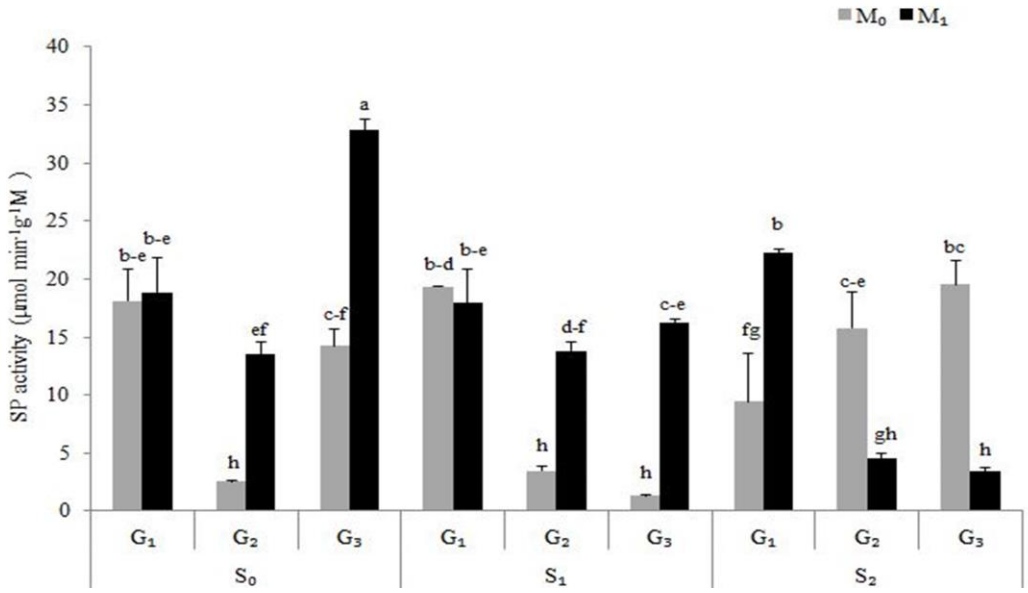

Figure 2. Effects of saline water conditions on SP-activity (µmol min^{-1} g^{-1} M). Symbols S and G are defined under Figure 1.

Table 3. Two-way ANOVA analysis Enzyme activity, root volume, leaf area and yield parameters. [a]

Source	DF	Enzyme activity			Root and leaf		Yield (Per plant)	
		SP	SPS	Invertase	Root volume	Leaf area	Total seed weight	Fiber weight
Rep	2	1.44	0.01	0.006	3.41	0.104	2.4	3.09
S	2	234.02 **	812.94 **	0.652 **	46.99**	0.957 **	321.9**	50.07 **
G	2	79.63 **	413.54 **	0.083 **	60.08 **	1.30 **	38.5 **	25.34 **
M	1	175.07 **	0.33 ns	0.021**	6.55 ns	0.141 ns	806.1 **	144.1 **
S×G	4	91.27 **	30.39 **	0.065 **	24.20 *	0.503 *	187. 1 **	49.2 *
G×M	2	4.58 ns	182.96 **	0.049 **	10.31 ns	0.238 ns	46.7 **	15.3 **
S×M	2	196.33 **	974.79 **	0.027 **	11.98ns	0.354 ns	118.9 **	7.32 **
S×G×M	4	193.21 **	221.86 **	0.137 **	30.23 *	0.689 *	83.1 **	11.1 **
Error	34	6.98	6.02	0.002	8.74	0.180	4.7	34.6
CV		19.24	12.44	0.44	26.54	12.90	21.9	19.02

Invertase Activity

As Table 3 shows, interaction of cotton species×salinity×AMF in Invertase-activity of seeds were significant (P≤0.01). Under low-salinity and 8-9 dS m^{-1}, *G. hirsutum* had the most Invertase-activity (Figure 3). In high-salinity, *G. hirsutum* [+AMF], 11.07 µmol min^{-1} g^{-1} M, had the most Invertase-activity. As can be seen, Figure 3 shows that Invertase-activity was more in *G. herbaceum* [+AMF] than [-AMF] under mild and high-salinity.

Growth Traits

Leaf Area

Table 3 shows that interaction of cotton species, AMF and salinity significantly affected LA (P≤ 0.05). The highest LA was observed in *G. herbaceum* [+AMF] under

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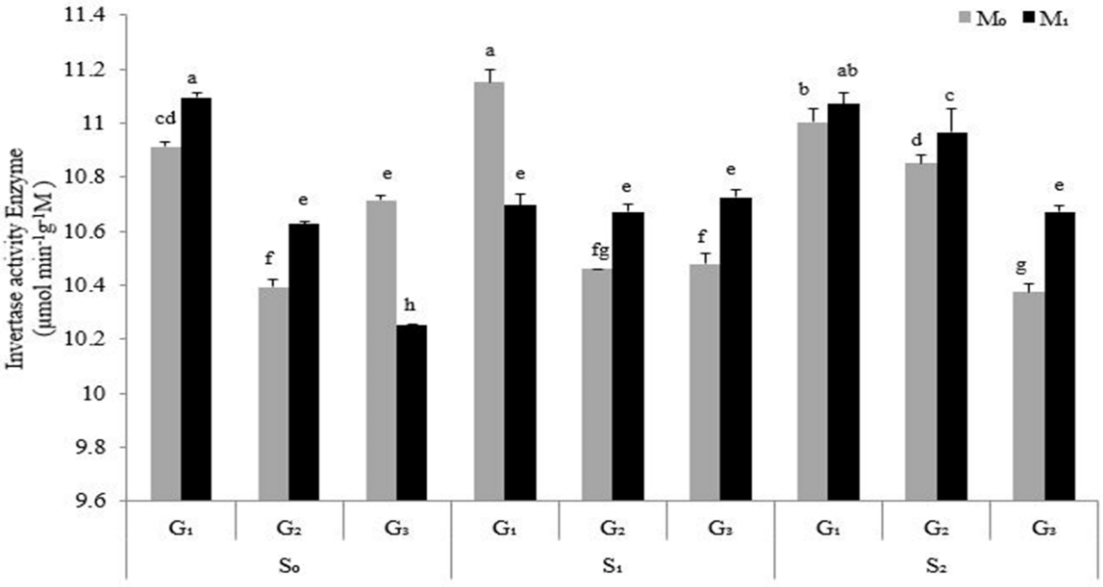

Figure 3. Effects of saline water conditions on Invertase-activity (µmol min^{-1} g^{-1} M). Symbols S and G are defined under Figure 1.

low-saline condition (Table 2). The least LA was obtained in *G. hirsutum* [-AMF] under high-salinity (Table 4). Under mild-salinity stress, *G. barbadens* [+AMF] had the greatest LA (Table 4).

Root Dry Weight and Root Volume

As results indicate, interaction of salinity with [+AMF] or [-AMF] in RDW and RV of different cotton species were significant (Table 3). According to Table 4, *G. barbadens* [+AMF] under low-salinity had the highest RDW and RV compared to the other species. Under high-salinity, [+AMF] symbiosis induced noticeable variation in RDW of *G. herbaceum*. The present work revealed that under low-salinity, RDW of both *G. barbadens* and *G. herbaceum* [+AMF] were more than the RDW of these species [-AMF] (Table 4). Therefore, the comparison of RDW and RV in cotton species [+AMF] and [-AMF] under high-salinity indicates that [+AMF] would be more efficient just with *G. barbadens* than with other cotton species. Also, expanded RDW and RV of *G. hirsutum* [+AMF] were the least in comparison to the other cotton species. *G. barbadens* was able to colonize with [+AMF] by extending RV, especially under high-saline irrigation, whereas the RV of *G. herbacea* [+AMF] was lower than *G. herbacea* [-AMF] under high-salinity and low-salinity (Table 4).

Seed Weight and Fiber Weight

Table 3 shows that cotton species, AMF, and salinity significantly affected ($P \leq 0.01$) seed weight and fiber weight. The findings showed that *G. barbadens* [+AMF] had the highest total seed per boll under non-salinity (Figure 4). Under mild saline irrigation, total seed weight increased by 22.85 and 16.78 g/boll in *G. barbadens* and *G. herbacea* [+AMF], respectively (Figure 4). Based on results, under high salinity, *G. barbadens* [+AMF] had the highest total seed weight/boll (9.36 g/plant). It was noteworthy that seed weight was the same in *G. herbacea* and *G. hirsutum* under high-salinity, and was lower than the seed weight in *G. barbadens*. In addition, the highest fiber weight was obtained from *G.*

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Table 4. Average mean of mycorrhiza symbiosis, +AMF [M_0] and –AMF [M_1], different cotton species [*G. hirsutum*: (G1), *G. barbadens* and (G2*) G. herbaceum* (G3)] and salinity water treatments [low-Salinity (S_0]), mild-Salinity (S_1) and high-Salinity (S_2)] on root dry weight, root volume and leaf area.[a]

Treatment	Root dry weight (g per plant)	Root volume (cm^3)	Leaf area (cm^3)
$S_0G_1M_0$	1.66 ± 0.22 d	52.67 ± 6.49 fg	222230 ± 43850 a
$S_0G_2M_0$	7.44 ± 2.55 a	253.67 ± 75.76 b	136754 ± 31998 a-c
$S_0G_3M_0$	5.89 ±1.03 ab	125.00 ± 20.23 c-e	6679 ± 3988 d
$S_0G_1M_1$	3.19 ± 1.06 cd	221.50 ± 6.06 b	162457 ± 37084 a-c
$S_0G_2M_1$	7.71 ± 0.08 a	357.50 ± 53.46 a	53303 ± 21118 cd
$S_0G_3M_1$	2.97 ± 0.95 cd	62.50 ± 1.44 e-g	249075 ± 99073 a
$S_1G_1M_0$	2.32 ± 0.88 cd	76.50 ± 9.53 d-g	5724 ± 2589 d
$S_1G_2M_0$	3.80 ± 1.32 b-d	251.50 ± 23.41 b	12216 ± 3105 d
$S_1G_3M_0$	2.89 ± 0.22 cd	91.00 ± 5.20 d-g	8438 ± 2695 d
$S_1G_1M_1$	2.19 ±0.11 cd	79.67 ± 5.24 d-g	162748 ± 43285 a-c
$S_1G_2M_1$	3.91 ± 0.75 b-d	192.50± 10.69 bc	170857 ± 61249 ab
$S_1G_3M_1$	4.30 ± 0.57 b-d	142.00 ± 6.93 cd	153821 ± 36946 a-c
$S_2G_1M_0$	4.35 ± 0.82 bc	101.00 ± 0.57 d-g	939 ± 90 d
$S_2G_2M_0$	2.78 ± 0.15 cd	69.67 ± 12.36 e-g	9754 ± 4936 d
$S_2G_3M_0$	2.54 ± 0.03 cd	110.00 ± 3.84 d-f	6776 ± 1268 d
$S_2G_1M_1$	1.89± 0.59 cd	37.00 ± 8.09 g	107020 ± 71025 b-d
$S_2G_2M_1$	2.91 ± 0.39 cd	146.33 ± 5.20 cd	9489 ± 6851 d
$S_2G_3M_1$	1.98 ± 0.36 cd	42.50 ± 6.06 fg	94791 ± 41137 b-d

[a] Values represent means of three replicates±SE. Different letters within the column represent significant difference among the treatments at P≤ 0.05, derived from LSD. ns: Not significant; * and **: Represent significance at P≤ 0.05 and P≤ 0.01, respectively, derived from two-way ANOVA. S: Salinity, G: Cotton species, M: *Arbuscular Mycorrhizal Fungi* (AMF).

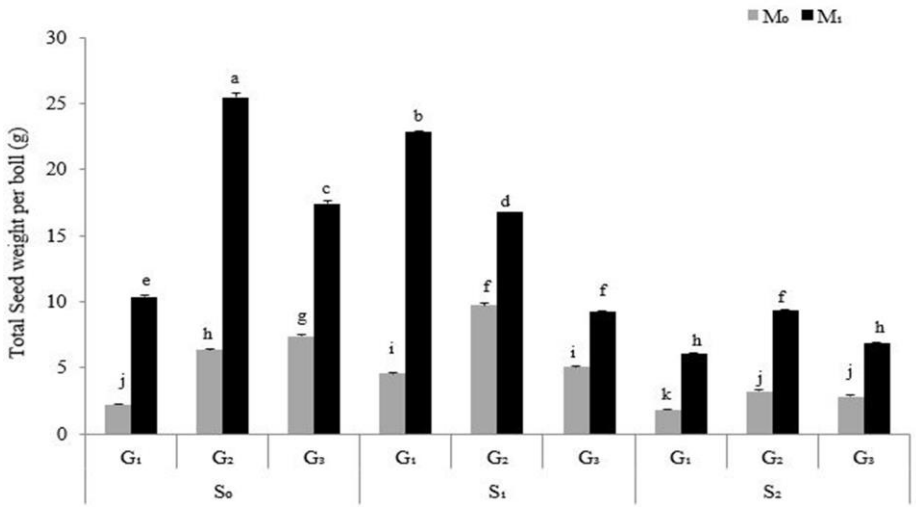

Figure 4. Effects of saline water conditions on total seed weight (g). Symbols S and G are defined under Figure 1.

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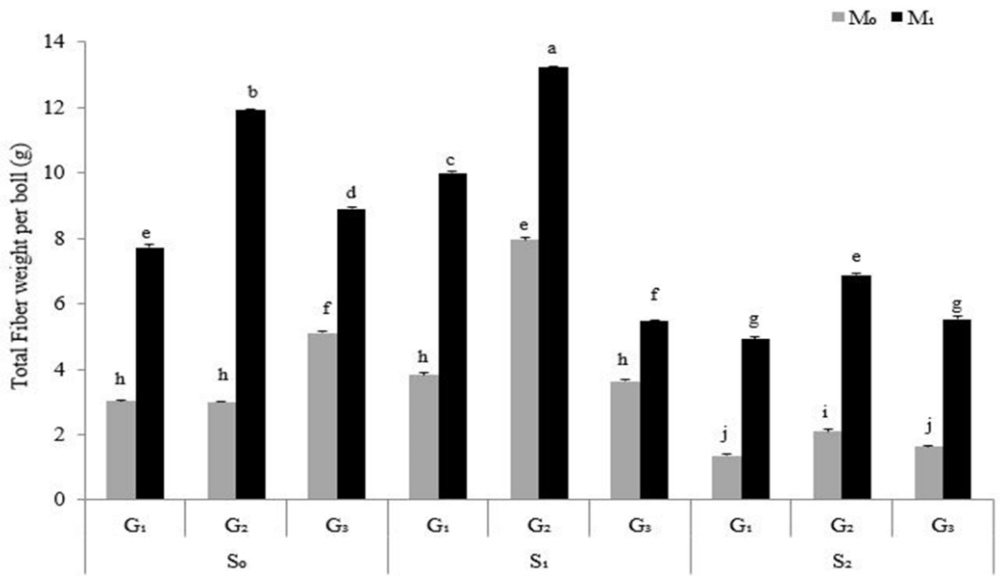

Figure 5. Effects of saline water conditions on total fiber weight (g). Symbols S and G are defined under Figure 1.

barbadens [+AMF] and *G. hirsutum* [+AMF] under mild-salinity condition with 13.2 g/plant, the latter of which had a high fibre weight (Figure 5). Under high-salinity, *G. hirsutum* [+AMF] had significantly the highest fiber weight (Figure 5).

DISCUSSION

Saccharide

In our study, root colonization by AMF had significantly more saccharide content compared to leaf and cottonseed. As stated by Lu *et al.* (2015), saccharide acts as a signaling molecule under salinity stress. Moreover, carbon is produced from carbohydrate accumulation in roots transfers to AMF (Shi *et al.*, 2014). Porcel and Ruiz-Lozano (2004) observed that under salinity stress, elevated carbohydrate content in soybean roots *R. intraradice related to* They also revealed that saccharide, as the major carbohydrate form, was absorbed by AMF. However, based on this result, lower saccharide content was obtained in leaves and not in cottonseed. Our results supported Pluskota *et al.* (2015) suggestion that high saccharide content in the seed, compared to the seed coat and leaf, be used as a substrate source for sucrose and starch syntheses. In orange under drought stress, Wu *et al.* (2017) concluded that carbohydrates were the important compatible solutes for Osmotic Adjustment (OA), and increased in leaves of plants colonized by *Funneliformis mosseae* and *Pyrodictium occultum*. The protective role of soluble sugars has also been outlined by the emphasis placed on OA in mediating the plant response to salinity stress (Akrami and Arzani, 2018).

Starch

According to these results, accumulation of starch in leaves and seed cotyledon of all studied species was the same, but more starch was stored in the root than in the other organs. In exposure to mild and high-salinity, *G. barbadens* symbiosis with [+AMF] balanced the amount of salt by accumulating starch in root and seed. Therefore, polysaccharide storage showed that exchangeable sugar was reduced in *G. barbadens* in mild and high-saline levels. However, in exposure to high-salinity stress

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with [+AMF], *G. hirsutum* showed more tolerance against 12-13 dS m^{-1} salinity stress by storing lower polysaccharide in comparison with others cotton species,. Interaction of root [+AMF] contributes to the exchange of carbohydrate and mineral nutrition between plants and [+AMF] (Feng *et al.*, 2002). Studies of cotton species with [+AMF] indicated that the degree of salinity stress plays an essential role in the amount of polysaccharides production, such as starch (Augé *et al.*, 2015). Interestingly, glucose and fructose had higher concentration than starch (Wang *et al.*, 2015). Starch serves as a temporal sink during the early stage of embryo growth with hexoses (Yang *et al.*, 2017). Bayani *et al.* (2016) showed that the starch and carbohydrate content increased in *Hordeum vulgare* L. under abiotic stress.

SPS and SP-activity

AMF caused higher sucrose-6-phosphate synthesis in comparison with starch, in *G. hirsutum* under high-salinity (Geigenberger *et al.*, 2004). Under low- and mild-salinity, significant changes in SPS-activity and SP-activity were obtained in the roots of *G. barbadens* and *G. herbaceum.* Therefore, sucrose-6-phosphate production was shown to be more than starch in low-salinity and mild-salinity. This increase in production has two main reasons. The presence of AMF in root stimulates SPS-activity in *G. barbadens* and *G. herbaceum*, and, as a result, SPS-activity is enhanced in the presence of SP (Chen *et al.*, 2005).

Under low- and mild-salinity, *G. barbadens* and *G. herbaceum* stimulated the increased SPS-activity in root via AMF symbiosis. In line with (Chen *et al.*, 2005) reports, it can be concluded that sucrose 6-phosphate production was more than starch. So, the enhancement of SPS-activity was obtained in the presence of SP. In *G. barbadens* and *G. herbaceum* [+AMF], Fru6P and UDP-Glu were used for SPS and SP-activity as substrates (Geigenberger *et al.*, 1999). According to Geigenberger *et al.* (2004), under mild-salinity, SPS has a critical role partitioned between sucrose and starch exchanges. Desingh and Kanagaraj (2007) reported that salinity caused a decline in SPS-activity in the roots of cotton under increasing salinity, as observed in *G. herbaceum* [-AMF] under high-salinity. AMF stimulated SPS-activity via breaking down SPS and SPP. Therefore, saccharide is directly used by AMF to induce sugar accumulation (Wu *et al.*, 2017). The aforementioned findings are parallel with the results of this research about SPS-activity in the roots of cotton [+AMF]. As Langenkämper *et al.* (2002) found, regulation of SPS-activity depends on environmental stress.

Invertase Activity

According to our data, under high-salinity, Invertase-activity in the roots of *G. hirsutum* [+AMF] was the highest. This result can be well interpreted referring to Viola *et al.* (2001) findings that sucrose cleavage to glucose and fructose by cell wall invertase enzyme activity of root, and that AMF symbiosis with *G. hirsutum* stimulated this reaction. It was concluded that the reduction in Invertase and SP-activity in the root of *G. hirsutum* [+AMF] under mild-salinity was related to SPS improvement to the extent that sucrose accumulated. As mentioned by Geigenberger *et al.* (2004), reduction in the expression of Invertase-activity converts to lower energy consumption, sucrose degradation, for protecting oxygen. Therefore, AMF by sucrose degradation helped less carbon link to the cell wall.

Root Volume and Root Dry Weight

Results showed that, under high-salinity, not only did AMF assist in improving RV, but also it accumulated more dry matter in the roots of *G. barbadens*. However, under mild-salinity, the RV of *G. barbadens* [+AMF] was lower than [-AMF]. The decrease in the attraction of AMF towards the root resulted in

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depletion in AMF colonization (Gamalero *et al.*, 2009), which can be observed in AMF relationship with *G. barbadens*. Thus, special plant species with different AMF species have various reactions under salinity stress. Variation in RV was different in all cotton species [+AMF]. There is no standard principle that can be used to anticipate the outcome of this research on *R. intraradices* and saline irrigation (Ibrahim *et al.*, 2011). Abdel-Rahman *et al.* (2011) reported an improvement in AMF resistance to salinity in mycorrhizal plants while no improvement was observed in controlled situations. Although AMF survived when grown under saline conditions (Johnson-Green *et al.*, 1995), results demonstrated that it was not suitable for the symbiosis of *G. hirsutum* and *G. herbacea* roots, especially under high-salinity. Similar findings were reported by researchers revealing that root growth was reduced in plants [+AMF] under salinity stress (Porcel *et al.*, 2012).

Seed Weight and Fiber Weight

Interruption in dividing assimilates and nutrition under salinity stress prevents growth and yield traits, such as seed weight and fiber weight, and eventually reduces cotton yield (Dai *et al.*, 2014). Increased cotyledon weight and prolonged storage content promoted the embryo weight (Yang *et al.*, 2009). Fiber weight of *G. barbadens* with [+AMF] was the most. Furthermore, it appears that AMF assists fiber maturation, especially under salinity stress (Razzouk and Whittington, 1991). Van der Heijden *et al.* (1998) proved that different plants interacted with specific AMF species that could influence their growth, whereas other AMF species damaged the plant.

CONCLUSIONS

Generally, it is reported that *G. barbadens* has the highest ability to alleviate damaging effects of stress through *R. intraradices* colonization. This study indicated that cotton species in symbiosis with *R. intraradices* can have different results under salinity stress conditions. At high-salinity, *R. intraradices* did not establish appropriate symbiosis with the roots of *G. hirsutum* and *G. herbacea*.

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رشد و ترکیبات کربوهیدراتی سه گونه *Gossypium* تلقیح شده با *Rhizophagus intraradices* در تنش شوری

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چکیده

افزایش تنش شوری در مزارع قابل کشت مانع از عملکرد فیزیولوژیکی گیاه و باعث کاهش عملکرد محصول می شود. قارچ میکوریز آربوسکولار (AMF)، به عنوان تعدیل کننده تنش شوری زیستی، اسمز سلولی را از طریق تغییرات متابولیسم دی ساکارید و پلی ساکارید محافظت می‌کند. در این مطالعه، سه گونه Gossypium (*G. hirsutum*، *G. barbadense* و *G. herbaceum*) کلونیزه شده با *Rhizophagus intraradices* [با AMF و بدون AMF] با تیمارهای آبیاری شور (۴> ECe دسی زیمنس بر متر در متر) (S0)، ۸-۹ دسی زیمنس در متر (S1) و ۱۲-۱۳ دسی زیمنس متر در متر ۱ (S2)) به عنوان یک آزمایش فاکتوریل کشت شدند. تیمارهای آبیاری شور در مرحله گلدهی آغاز شدند.. به طور کلی، با توجه به ویژگی‌های فیزیولوژیکی، [AMF+] *G. barbadense* در مواجهه با شوری ۱۳-۱۲ دسی زیمنس بر متر تحمل بیشتری داشت، در حالی که *G. hirsutum* با [AMF+] فقط تا شوری ۹-۸ دسی زیمنس بر متر، متحمل بود. زیرا بیشترین و کمترین سطح برگ به ترتیب در [AMF+] *G. barbadense* تحت شرایط شوری ۹-۸ دسی زیمنس بر متر و ۱۳-۱۲ دسی زیمنس بر متر مشاهده شد. در مواجهه با شوری ۱۳-۱۲ دسی زیمنس بر متر، بیشترین حجم ریشه، وزن خشک ریشه، وزن دانه و وزن الیاف در [AMF+] *G. barbadense* به دست آمد. همچنین بیشترین میزان کربوهیدرات در ریشه و برگ و بیشترین میزان نشاسته ریشه، برگ و لپه بذر در تیمار [AMF+] *G. barbadense* تحت تیمار شوری ۱۳-۱۲ دسی زیمنس بر متر مشاهده شد. تحت شوری ۹-۸ دسی زیمنس بر متر، بیشترین فعالیت آنزیم ساکارز فسفات سنتاز (SPS) و ساکارز فسفاتاز (SP) در ریشه [AMF+] *G. barbadense* بود. تحت شوری ۹-۸ دسی زیمنس بر متر، بیشترین فعالیت آنزیم ساکارز فسفات سنتاز (SPS) و ساکارز فسفاتاز (SP) در ریشه [AMF+] *G. barbadense* بود. مطالعه حاضر نشان می دهد که علیرغم تغییرات فیزیولوژیکی چشمگیر در شرایط شوری بالا در مقایسه با شوری ملایم، AMF و *G. barbadense* بهترین کارایی همزیستی را در ۱۲-۱۳ دسی زیمنس بر متر از خود نشان دادند.

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