

Strategy Role of Mycorrhiza Inoculation on Osmotic Pressure, Chemical Constituents and Growth Yield of Maize Plant Grown under Drought Stress

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Abstract

The present work was carried out to investigate the role of mycorrhiza inoculation at two harvesting stages (90-days and 30-days) of maize plants grown in pot experiment with different moisture content levels 100%, 70%, 50% and 20%. Drought stress tolerant in maize plant was varied in different organs of the same plants and also varied among different stage of plant development. The sensitivity of maize plants was related with reduction of root soluble sugar, shoot and root soluble protein at 30-days of plant harvesting, and soluble sugar and soluble protein in both organs of both harvesting stages. This related with reduction in OP and lowering of water uptake which induced a marked decrease in fresh and dry matter production in shoot and root of both harvesting stages. AM inoculation increase maize tolerant to drought stress presented in increasing growth parameters, chemical constituents and minerals contents compared with untreated plants. Proline content with AM inoculation was more or less unchanged in shoot of plant harvesting at 30-days and in root of plant harvesting at 90-days. However, a marked increase was induced in plant harvesting at 30-days and in shoot of plant harvesting at 90-days. Mycorrhiza inoculation induced a significant increase in OP value either compared with corresponding level or compared with control value 100% as in plant 30-days of harvesting or compared with control only as in plant harvesting after 90-days. AM infection with different moisture content levels measured by N-acetyl glucosamine content were not affected by drought stress. Results showed also that control roots contained N-acetyl glucosamine would be attributed to mycorrhiza and other fungi naturally present in soil.

Keywords

Moisture Content, Mycorrhiza Inoculation, Maize Plant

1. Introduction

Drought is one of the most adverse abiotic stresses on plant growth and productivity, it induced morphological, physiological and biochemical effects, reduced CO₂ assimilation, leaf area, photosynthetic pigment content, stem growth, root proliferations disturbs water use efficiency. The role of chemical substances accumulation in drought plants has been researched to understand plant tolerance to water deficit [1] [2] [3] [4]. Numerous researchers have studied plant growth promoting fungi (PGPF) attributes of rhizospheric fungi [5] [6]. Among the PGPF, species of *Phoma*, *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma*, and arbuscular mycorrhizal fungus (AMF) have gained important due to their biotic role in plant growth promotes under drought stress conditions. Among mechanisms, stimulating plant growth by PGPF, production of phytohormones [7], decomposing organic matter [8], solubilization of unavailable soil bound nutrient elements [9], and protection of plants from biotic and abiotic stresses [7]. Indirect growth activators by plant growth promoter's fungi occur via niche exclusion, antibiosis, predation and mycoparasitism [10] [11]. Sometimes more than one mechanism is used to enhance growth [10]. Arbuscular mycorrhiza forming fungi (AMF) are obligate biotrophs that require the host plant to complete their life cycle. The fungus colonizes the root cortex and forms intracellular structures called arbuscules where the exchange of nutrients between the partners takes place. The extracellular hyphae spread widely into the surrounding soil, thereby reaching nutrient and improving plant growth. The role of AMF in growth promotion and stress suppression in plants is reported since the very old times [12]. The ability of AMF to promote plant growth is due to nutrient uptake, particularly phosphorus (P) [13] [14], AMF-colonized crop shows increased growth and yield [15] [16]. Xu *et al.* (2018) [17] showed that maize plants appeared to have high dependency on AMF which improved physiological mechanisms by raising growth, chlorophyll content, gas exchange and rubisco activity under salinity stress. Mathur *et al.* (2018) [18] showed that AFM plants increased relative water content both of plants leaf and soil indicating that AMF hyphae penetrated deep into soil and provided moisture to the plants. Thus this work was conducting to study the drought tolerance of maize plant at two different harvesting stages (30- and 90-days) and the combined action with mycorrhiza inoculation at these plant growth stages.

2. Materials and Methods

2.1. Experimental Sites and Drought Stress Treatments

Maize seeds (*Zea mays* L. cv. 215) were obtained from Agronomy Department, Faculty of Agricultural, Minia University, El-Minia, Egypt. Maize crop is one of the food crops that have several uses, whether as a food for man or as animal feed, due to its high nutrition value. Also, maize enters in the process of manufacturing some important products such as corn oil, fructose and starch [19]. Maize seeds were surface sterilized by immersion in a mixture of ethanol 96%

and H_2O_2 (1:1) for 3 minutes, followed by several washings with sterile distilled water, seeds were grown in 1 kg pots in Botany and Microbiology Department garden. Five seeds were sown in each pot and clay soil was brought to field capacity. The clay soil comprise four components minerals and soil organic matter make up the solid fraction, whereas air and water comprise the pore space fraction. A typical agricultural soil is usually around 50% solid particles and 50% pores (Adapted from Brady and Weil, 2002 [20]). Soil particle of clay is <0.002 invisible to naked eye. Considerations of working in controlled environments were followed by Tibbitts & Langhans (1993) [21].

2.2. Drought Stress and Treatments with Mycorrhiza Colonization

The seedlings were left to grow under the desired soil moisture content levels (100%, 70%, 50% and 20%) in the first group of experiment, this considered untreated with inoculum (–AM). Soil moisture content was measured by calculate the soil field capacity, this consider as 100% moisture content and so could be determine the other lower soil moisture content. Three replicate from each treatment was prepared. The previous treatment was repeated as a second group of experiment but inoculated with mycorrhiza (+AM). Soil was either treated (+AM) or untreated (–AM) with AM spores and the entire soil was P-fertilized at the level 10 mg/Kg as KH_2PO_4 powder mixed thoroughly with the soil before sowing. The inoculum consisted of spores and hyphae of *Glomus mosseae*, 50 g was added per pot. The inoculum was collected from Egyptian Central of Agricultural Research. A week after sowing plants was thinned down to 2 per pot.

2.3. Laboratory Analysis for Metabolites

At the end of the experimental period (30-days and 90-days of harvesting) plant fresh and dry matter yield of the different organs (shoot and root) were determined. Determination of the dry matter involved harvesting and careful separation of fresh organs. Fresh organs were then dried in an oven at 80°C . Dry matter was determined after drying plants in an aerated oven at 70°C to constant mass. Soluble sugar was determined by the anthrone-sulfuric acids method [22]. Soluble protein was measured according to Lowry *et al.* (1951) [23]. Amino acids and proline were measured by Moore and Stein (1948) [24] and Bates *et al.*, (1973) [25]. Potassium was determined by flame photometric method (Williams and Twine 1960) [26], and calcium and magnesium by the versene titration method [27]. Phosphorus was determined by methods of Woods and Mellon, (1985) [28].

2.4. Mycorrhiza Colonization Measurement

After 90-days from sowing, roots were carefully washed from adhering using tap water. A sample of approximately 0.5 g fresh roots from each pot was removed to estimate the degree of infection of AM using direct measurement of the amount of the fungal hyphae by measuring the total chitin after conversion to

glucosamine [29]. Chitin was hydrolyzed by using *Trichoderma harzianum* crude filtrate. Fresh root (1 g) was incubated for 2 h at 37°C with 3 ml of 0.1 phosphate buffer (pH 5.8) and 1 ml crude filtrate. N-acetyl glucosamine was assayed photometrically according to Reissig *et al.* (1959) [30].

2.5. Statistical Analysis

The experimental data were subjected to the one way analysis of variances (ANOVA test) using the SPSS version 11.0 to quantify and evaluate the source of variation and the means were separated by the least significant differences, L. S. D. at P level of 0.05% (Steel and Torrie, 1960). Experimental data were subjected to one way analysis of variance and the means were separated by the least significant differences, L. S. D. [31]. Correlation coefficients were calculated using statgraphics 5.0 software.

3. Results

Fresh and dry matter were decreased as decreasing moisture content in shoot and root at both 30 and 90 days of plant harvesting especially at lower moisture content levels (30% M. C.) (Table 1). This decreasing effect was more prominent

Table 1. Interaction effect of AM inoculation and different moisture content levels (M. C.) on fresh and dry matter (g.plant⁻¹) grown at 30- and 90-days of maize plant harvesting.

| Treat. M. C. | | Shoot | | | | Root | | |
|----------------|--------|-------|---------|-------|--------|-------|---------|-------|
| Harv. (30-d) | f. m. | % | d. m. | % | f. m. | % | d. m. | % |
| 100% | 4.92 | 100 | 0.353 | 100 | 1.33 | 114.3 | 0.203 | 100 |
| 70% | 4.01** | 82.1 | 0.525** | 98.1 | 1.52** | 114.3 | 0.216** | 101.4 |
| 50% | 3.59** | 72.9 | 0.458** | 85.6 | 1.29** | 96.9 | 0.196** | 96.6 |
| 20% | 2.27** | 46.1 | 0.291** | 54.2 | 1.11** | 83.5 | 0.169** | 83.3 |
| 100% + AM | 5.93** | 120.5 | 0.657** | 186.1 | 1.86** | 139.2 | 0.232** | 114.3 |
| 70% + AM | 5.02** | 102.0 | 0.539** | 152.7 | 1.99** | 149.6 | 0.285** | 140.4 |
| 50% + AM | 3.90** | 79.3 | 0.434** | 122.9 | 1.3** | 97.7 | 0.210** | 103.4 |
| 20% + AM | 3.27** | 66.5 | 0.304** | 86.1 | 1.29** | 96.9 | 0.191** | 94.1 |
| L. S. D. 0.05% | 0.08 | | 0.02 | | 0.01 | | 0.008 | |
| Harv. (90-d) | | | | | | | | |
| 100% | 9.02 | 100 | 1.9 | 100 | 2.8 | 100 | 1.2 | 100 |
| 70% | 9.5 | 105.3 | 2.6** | 136.8 | 2.6* | 92.9 | 0.74** | 61.7 |
| 50% | 6.0** | 66.5 | 1.8* | 94.7 | 2.4** | 85.7 | 0.64** | 53.3 |
| 20% | 4.7** | 52.1 | 0.94** | 49.5 | 0.65** | 23.3 | 0.19* | 15.8 |
| 100% + AM | 10.3** | 114.2 | 2.5** | 131.6 | 2.9 | 103.6 | 1.3* | 108.3 |
| 70% + AM | 10.1** | 124.6 | 2.4** | 126.3 | 4.8 | 171.4 | 1.1* | 91.7 |
| 50% + AM | 10.0* | 110.8 | 2.1** | 110.5 | 3.2** | 114.3 | 0.89** | 74.2 |
| 20% + AM | 5.1** | 56.5 | 1.1** | 57.9 | 0.97** | 34.6 | 0.32** | 26.7 |
| L. S. D. 0.05% | 0.82 | | 0.13 | | 0.31 | | 0.11 | |

*Significant differences and **highly significant differences as compared to the absolute control.

in shoot organ than in root at 30-days of plant harvesting, while this effect was more lighted in root than in shoot at 90-days of maize harvesting. The percent of reduction of fresh and dry matter at 20% M. C. level was 53.9%, 45%, 3.1%, 16.7% below control value 100%. At 30-days of maize harvesting in shoot and root respectively (**Table 1**). In maize plant harvesting at 90-days the percent of reduction at that level was 47.9%, 50.5%, 76.8% and 84.25% as compared with control plants. Decreasing in moisture content was lowered water content in shoot and root of both harvesting stage (**Figure 1**). The percent of decrease at 20% M. C. level was 43.2%, 86.2% in shoot and root at 30-harvesting while at 90-days of plant harvesting it was 52.8%, 28.8%, *i.e.* the reduction in water content at youngest plant resulted in shoot that was lower in water content while at oldest plant root was lower than shoot in water content. In non-mycorrhization maize plant the soluble sugar in shoot was significantly accumulated in shoot of plant harvesting at 30-days, the percent of increase at 20% M. C. was 46% over the control value 100% (**Table 2**). Whereas in root it slightly decreased

Table 2. Interaction effect of AM inoculation and different moisture content levels (M. C.) on soluble sugar and soluble protein ($\text{mg}\cdot\text{g}^{-1}\cdot\text{d.m.}$) grown at 30 and 90-days of maize plant harvesting.

| Treat. M. C. | Soluble sugar | | | | Soluble protein | | | |
|----------------|---------------|-------|--------|-------|-----------------|-------|--------|-------|
| Harv. (30-d) | Shoot | % | Root | % | Shoot | % | Root | % |
| 100% | 54.3 | 100 | 65.6 | 100 | 58.2 | 100 | 48.6 | 100 |
| 70% | 69.2** | 127.4 | 69.8** | 106.4 | 51.1** | 87.8 | 49.2** | 101.2 |
| 50% | 71.9** | 132.4 | 61.8** | 94.2 | 48.7** | 83.7 | 45.2** | 93.0 |
| 20% | 79.3** | 146.0 | 63.3** | 96.4 | 37.7** | 64.8 | 36.9** | 78.0 |
| 100% + AM | 73.0** | 134.4 | 83.3** | 126.9 | 75.2** | 56.2 | 56.2** | 115.6 |
| 70% + AM | 77.5** | 142.7 | 91.9** | 140.1 | 76.6** | 56.9 | 56.9** | 117.1 |
| 50% + AM | 77.5** | 142.7 | 94** | 143.3 | 75.3** | 50.9 | 50.9** | 104.7 |
| 20% + AM | 71.1** | 130.9 | 88.9** | 135.5 | 54.3** | 55 | 55** | 113.2 |
| L. S. D. 0.05% | 0.6 | | 0.7 | | 0.25 | | 0.21 | |
| Harv. (90-d) | | | | | | | | |
| 100% | 64.8 | 100 | 58.5 | 100 | 81.4 | 100 | 55.1 | 100 |
| 70% | 52.4** | 80.9 | 52.8** | 90.2 | 66.4** | 81.6 | 46.6** | 84.6 |
| 50% | 61.2** | 94.4 | 48.5** | 82.9 | 56.3** | 69.2 | 42.9** | 77.9 |
| 20% | 46.8** | 72.2 | 41.5** | 70.9 | 50.8** | 62.4 | 37.7** | 68.4 |
| 100% + AM | 80.2** | 123.8 | 67.0** | 114.5 | 84.4** | 103.7 | 58.9** | 106.9 |
| 70% + AM | 63.2* | 97.5 | 53.8** | 91.9 | 72.1** | 88.6 | 57.5** | 104.4 |
| 50% + AM | 66.2** | 102.2 | 64.3** | 109.8 | 60.3** | 74.1 | 57.3** | 103.9 |
| 20% + AM | 47.0** | 72.5 | 56.5** | 96.6 | 55.8** | 68.6 | 53.4** | 96.9 |
| L. S. D. 0.05% | 0.82 | | 0.13 | | 0.31 | | 0.11 | |

*Significant differences and **highly significant differences as compared to the absolute control.

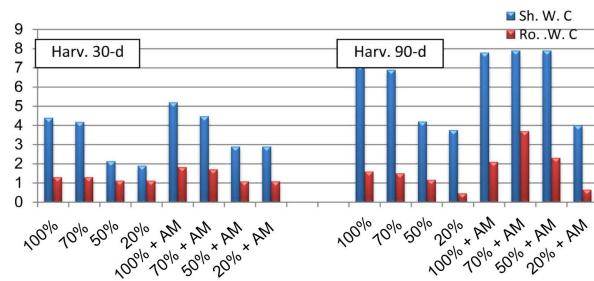


Figure 1. Interaction effect of AM inoculation and different moisture content levels (M. C.) on water content (g.plant⁻¹) grown at 30 and 90-days of maize plant harvesting.

as decreasing M. C. However, the plant harvesting after 90-days soluble sugar showed irregular decreasing effect as decreasing M. C. levels, the percent of decrease was 27.8% and 29.1% in shoot and root respectively compared with control plants (**Table 2**). Soluble protein was markedly lowered in shoot and root at both harvesting stages (30 and 90-days). The percent of reduction was 35.2%, 22%, 37.6%, 31.6% in shoot and root at both 30 and 90-days of plant harvesting respectively (**Table 2**). Total sugar showed a marked reduction in shoot and root of both harvesting stages reached a low level at 20% M. C. level (**Figure 2(a)**). Exit from previous trend total sugar in shoot of plant harvesting after 30-days a significantly accumulated as decreasing M. C. level (**Figure 2(a)**). Total protein was markedly increased in shoot of plant harvesting at 30-days while run around control value in root (**Figure 2(b)**). Whereas total protein tended to decrease in both shoot and root of plant harvesting at 90-days. Amino acids content run around control value 100% in shoot and root of maize plants harvesting at 30-days, while a smooth reduction was exhibited in plants harvesting at 90-days (**Table 3**). Decreasing moisture content induced in most cases unchanged effect in proline content in shoot at both tested harvesting stages (**Table 3**). In root decreasing M. C. exhibited a marked increase in proline content at both plant harvesting stages (**Table 3**). Potassium content was mostly increased in both tested organs of maize plants at both stages of plant harvesting (30 and 90-days) (**Figure 3(a)**). This increasing effect was highly recorded in shoot than in root. The percent of increasing was 128.9%, 175.9%, 115.8%, 137.2% in shoot and root at 20% M. C. level at both harvesting stages, *i.e.* root is higher in increasing K⁺ content than shoot organ. Ca²⁺ and Mg²⁺ content were significantly increased in shoot and root at 30 and 90-days harvesting stages (**Figure 3(b)**, **Figure 3(c)**). The percent of increasing at 20% M. C. level was 114.3%, 106.3%, 128.6%, 107.7% in case of Ca²⁺ in shoot and root of two harvesting stages. Also, the percent of enhancement in Mg²⁺ content at that level was 109.1%, 164.3%, 200%, 133.3% in shoot and root at both tested plant growth stages, *i.e.* the percent of activation was highly effective in root than in shoot at 30-harvesting stage while this effect was recorded in shoot than in root at 90-days of plant growth (**Figure 3(b)**). Osmotic pressure in maize plant harvesting after 30-days was markedly increased up to 50% M. C. level, after that a reduction was exhibited in shoot organ while in root organ it run at irregular

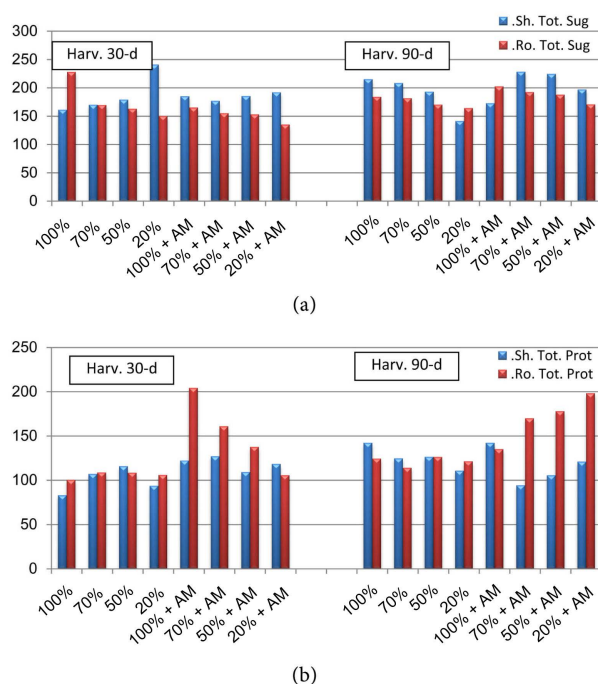


Figure 2. Interaction effect of AM inoculation and different moisture content levels (M. C.) on total sugar ($\text{mg}\cdot\text{g}^{-1}\cdot\text{d.m.}$) (a) and total protein (b) ($\text{mg}\cdot\text{g}^{-1}\cdot\text{d.m.}$) content at 30 and 90-days of maize plant harvesting.

Table 3. Interaction effect of AM inoculation and different moisture content levels (M. C.) on amino acids and proline content ($\text{mg}\cdot\text{g}^{-1}\cdot\text{d.m.}$) grown at 30- and 90-days of maize plant harvesting.

| Treat. M. C. | Amino acids | | | | Proline | | | |
|----------------|-------------|-------|--------|-------|---------|-------|---------|-------|
| Harv. (30-d) | Shoot | % | Root | % | Shoot | % | Root | % |
| 100% | 5.33 | 100 | 4.22 | 100 | 0.727 | 100 | 0.474 | 100 |
| 70% | 5.69** | 106.8 | 4.52** | 107.1 | 0.695** | 95.6 | 0.582 | 123.6 |
| 50% | 4.28** | 80.3 | 4.38** | 103.8 | 0.682** | 93.8 | 0.490 | 103.4 |
| 20% | 5.51* | 103.4 | 4.22 | 100 | 0.924** | 129.6 | 0.597* | 126.8 |
| 100% + AM | 6.59* | 123.6 | 8.43** | 199.8 | 0.700** | 96.3 | 0.546 | 115.2 |
| 70% + AM | 9.16** | 180.3 | 8.19** | 194.1 | 0.703** | 96.7 | 0.586** | 122.4 |
| 50% + AM | 6.79** | 127.4 | 7.64** | 181.0 | 0.725 | 99.7 | 0.993** | 209.5 |
| 20% + AM | 7.22** | 135. | 5.11** | 121.1 | 0.865** | 118.9 | 0.860** | 181.4 |
| L. S. D. 0.05% | 0.2 | | 0.19 | | 0.11 | | 0.2 | |
| Harv. (90-d) | | | | | | | | |
| 100% | 5.39 | 100 | 5.97 | 100 | 0.546 | 100 | 0.431 | 100 |
| 70% | 5.30 | 98.3 | 5.47 | 91.6 | 0.532 | 97.4 | 0.461 | 106.9 |
| 50% | 5.30 | 98.3 | 5.44 | 91.1 | 0.547 | 100.2 | 0.53 | 122.9 |
| 20% | 5.55 | 102.9 | 5.21 | 87.3 | 0.561 | 102.7 | 0.644 | 149.4 |
| 100% + AM | 7.11** | 131.9 | 6.24** | 104.5 | 0.675** | 123.6 | 0.494 | 114.6 |
| 70% + AM | 7.21** | 133.8 | 6.35** | 106.4 | 0.748** | 136.9 | 0.434 | 100.7 |
| 50% + AM | 8.21** | 152.3 | 5.18** | 68.8 | 0.686** | 125.6 | 0.446 | 103.5 |
| 20% + AM | 9.00** | 166.9 | 4.91** | 82.2 | 0.664** | 121.6 | 0.461 | 106.9 |
| L. S. D. 0.05% | 0.2 | | 0.18 | | 0.14 | | 0.13 | |

*Significant differences and **highly significant differences as compared to the absolute control.

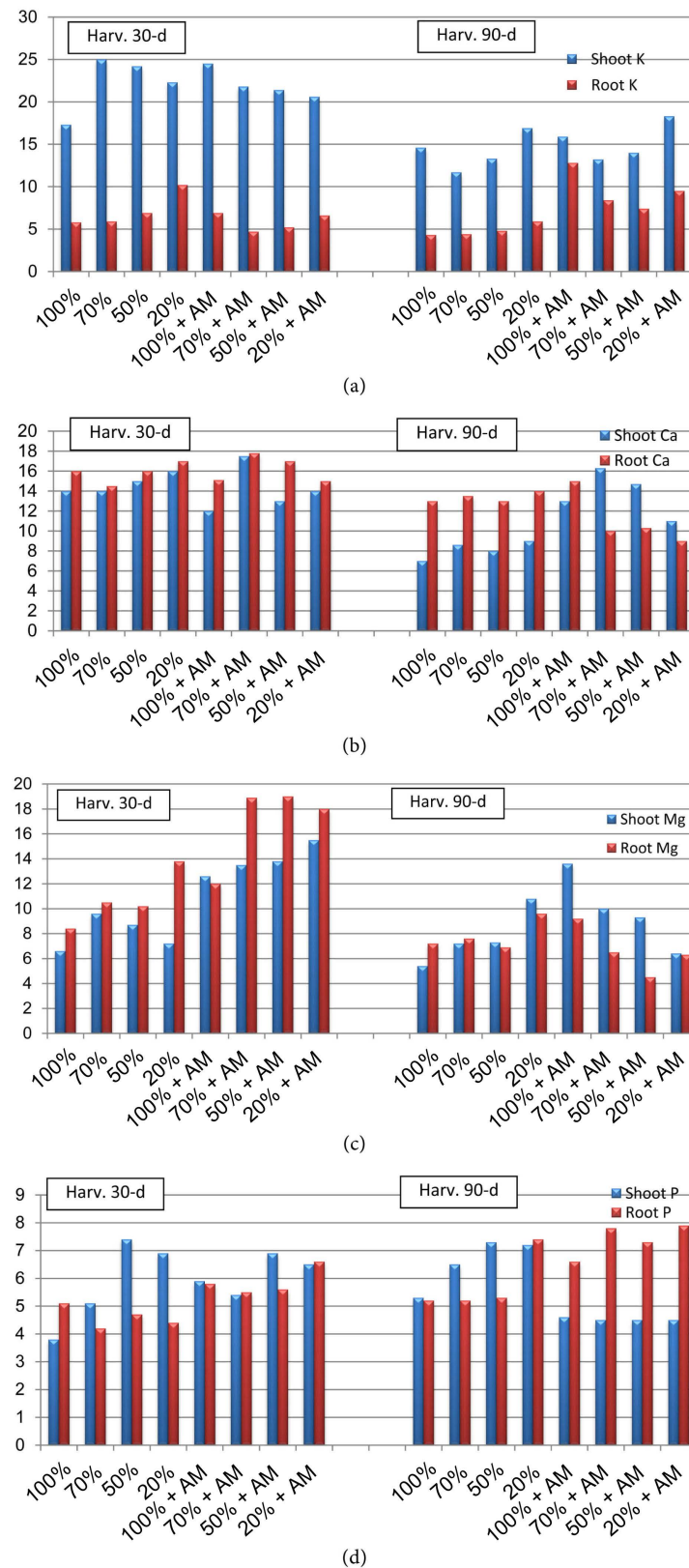


Figure 3. Interaction effect of AM inoculation and different moisture content levels (M. C.) on K⁺ (a); Ca²⁺ (b); Mg²⁺ (c) and P³⁺ (d) minerals content (mg·g⁻¹·d.m.) grown content at 30 and 90-days of maize plant harvesting.

trend (**Figure 4(b)**, **Figure 4(c)**). However osmotic pressure was significantly decreased as decreasing M. C. in shoot and root of plant harvesting at 90-days, the percent of reduction at 20% M. C. level was 76.6%, 81.2% in shoot and root respectively (**Figure 4(a)**, **Figure 4(b)**).

4. Mycorrhiza Inoculation

Plant mycorrhization has resulted a highly significant increase in fresh, dry matter and water content of shoot and root compared with un-inoculated plants at both harvesting stages (**Table 1**). Plant inoculated with mycorrhiza in most cases accumulated soluble sugar and soluble protein in both tested organs compared with either control value or with corresponding moisture content level, this activation was more obvious at 30-days harvesting plants (**Table 2**). AM inoculation was significantly increased amino acids in shoot and root in plant collected at both harvesting stages (30- and 90-days). This effect was highly effective at 30-days harvesting stage. Plant inoculated with AM induced no marked effect in proline content of shoot, in root accumulated a huge amount of proline content at 30-days of plant harvesting (**Table 3**). Whereas proline content in plant harvesting at 90-days run around control value in root, whereas a marked increase in shoot with AM inoculation under different levels of M. C. levels (**Table 3**). Inoculated maize plants with AM significantly increased potassium content in both tested organs at 90-days of plant harvesting while no significant change was exhibited at 30-plant growth stage (**Figure 2(a)**). Ca^{2+} and Mg^{2+} were mostly increased with mycorrhiza inoculation especially at 90-days of plant growth in shoot and root and mostly observed in Mg^{2+} content (**Figure 2(b)** & **Figure 2(c)**). Mycorrhization treatment resulted no marked change in P^{3+} content in root organ while showed slightly increase in shoot of plant grown for 30-days compared with drought plant only (**Figure 2(d)**). However plant harvesting at 90-days plant P^{3+} content was elevated with decreasing M. C. levels in shoot and root compared with un-inoculated plants (**Figure 2(d)**). Mycorrhizal inoculation of maize plant increased OP in shoot and root compared with either corresponding level or with control value 100% M. C. level in maize plant harvesting at 30-days (**Figure 2(a)** & **Figure 2(b)**). Whereas this activation in maize plant harvesting at 90-days was compared only with the corresponding level of M. C. **Table 4** represents the degree of root colonization by AM as measured by

Table 4. N-acetyl glucoseamine content ($\mu\text{g}\cdot\text{g}^{-1}$ fresh root) in maize roots as indication for mycorrhizal colonization.

| % of moisture content | Control | % | Mycorrhiza Inoculation | % |
|-----------------------|---------|------|------------------------|-------|
| 100 | 126.5 | 100 | 152 | 120.2 |
| 70 | 125 | 98.8 | 135.4 | 107.0 |
| 50 | 126 | 99.6 | 168.5 | 133.2 |
| 20 | 116 | 91.2 | 140.2 | 110.8 |

*Results are mean of three readings.

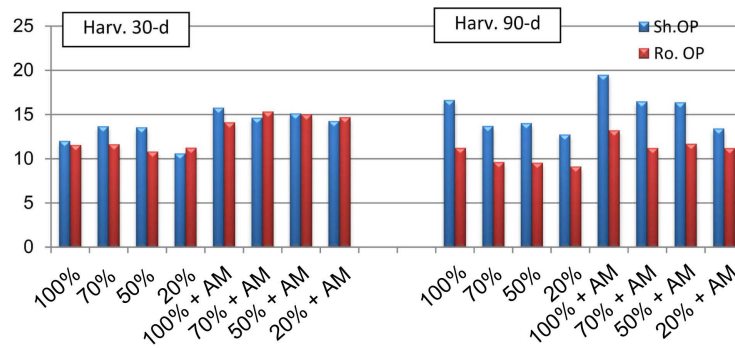


Figure 4. Interaction effect of AM inoculation and different moisture (M. C.) levels on osmotic pressure (mOsmo/H₂O) grown at 30- and 90-days of maize plant harvesting.

the amount of N-acetyl glucosamine per one gram of fresh root. Inoculated roots showed higher amounts of N-acetyl glucosamine than control. The data also showed that there was no direct relationship between moisture content and the degree of AM colonization.

5. Discussion

From previous data it can be demonstrated that drought stress tolerant varied in different organs of the same plants and also varied among different stages of plant development. While root organ tolerate drought stress up to 20% moisture content, shoot organ exhibited a degree of sensitivity especially at 50% and 20% moisture content levels in plant harvesting after 30-days. Whereas plant harvesting after 90-days tolerate up to 70% moisture content level, after that a dramatic sensitivity was recorded presented in production of fresh, dry matter and water content. The sensitivity of maize plants was related with reduction of root soluble sugar, shoot and root soluble protein at 30-days of plant harvesting stage, and soluble sugar and soluble protein in both organs at both harvesting stages. This related with reduction in OP and lowering of water uptake which induced a marked decrease in fresh and dry matter production in shoot and root at both harvesting stages. The soluble sugar in shoot of plant harvesting at 30-days concomitant with stable value of amino acids which concomitant with increasing effect in OP and this functioning in plant survival at this stage. Also, minerals has a role in previous trend increasing K⁺, Ca²⁺, Mg²⁺ in shoot and root in plant harvesting at 30-days and 90-days. These factors related with increasing OP value especially in shoot of both harvesting stages (30-days and 90-days). The inhibitory effect of drought on growth parameters could be attributed to the osmotic effect of water stress [32] [33]. Also, the reduction of yield may be ascribed to the harmful effect of soil moisture stress and nutrient balance disorder in root media [34], or reduced rate of new cell production may be make additional contribution to the inhibition of growth [35]. The reduction in growth criteria due to drought stress might be related to disturbance of water flow from root to shoot [36], decrease in water potential of cell sap [37], or inhibition of cell division [38]. One distinctive feature of most plants growing in stress environments

is the accumulation of proline [39] and it has been inferred that there may be a relationship between cellular proline level and cell turgidity via osmotic adjustment [40] [41]. Osmotic adjustment helps to maintain cell turgor, which can allow cell enlargement and plant growth during water stress; and it can allow stomata to remain at least partially open and CO₂ assimilation to continue at water potentials that would be otherwise inhibitory [42]. Supported the previous view that drought stress is among the factors most limiting to plant productivity [43] [44]. The plant tolerance at 70% M. C. level represented in production of fresh and dry matter at 30-days of plant harvesting was parallel with increasing trend in soluble sugar and amino acids in shoot and root and root protein, shoot K⁺, Mg²⁺ and P in shoot and root which resulted activation trend in OP in both organs at that level [45] [46] [47]. Proline content showed a variable trend while run around control value 100% in shoot, in root it was significantly increased as decreasing moisture content at both tested harvesting stages. This increasing effect of drought stress in proline content in root organ can be consider as a sign of tolerant as decreasing M. C. levels of plant harvesting at 30-days. While it consider as a sign of sensitivity in root of plant harvesting at 90-days. *i.e.* the causes of proline accumulation were varied at different stages of plant growth. Lutts *et al.*, (1999) [48] suggested that proline accumulation in rice under water deficit was most likely a symptom of injury rather than an indicator of increased tolerance. Proline content of salt-stressed plants was previously reported in different species [49] [50] [51] [52] However, Hamdia Abd El-Samad (2016) [41] indicated that there is a relation between Na⁺, proline and dry matter and salt tolerance of different crop plants, maize, wheat, broad bean, cotton and parsley plants and emphasized the protective role of proline under salinity. Mycorrhiza inoculation induced a significant increase in OP value either compared with corresponding level or compared with control value 100% as in plant 30-days of harvesting or compared with control only as in plant harvesting after 90-days. The percent of reduction in OP at 20% M. C. level was 85.8% and 97.2% at 30-days of harvesting and at 90-days, it was 76.6% and 81.2% in shoot and root. After AM inoculation the percent of increase in OP at 20% M. C. level was 11.87% and 27.1% over control plants 100% in shoot and root of plant of 30-days harvesting. While in plant harvesting after 90-days the percent was 80% and 100% in shoot and root compared with corresponding level of moisture content. This activation in OP with AM reflected in increasing water uptake under different level moisture content in shoot and root of both harvesting stages. Also enhancement effect on soluble sugar, soluble protein and amino acids was shared in OP effect. This induced an increasing effect on photosynthetic efficiency which in turn permits increasing effect in carbohydrate and nitrogen metabolism. These reflected on increasing maize drought tolerance as presented in growth yield [53]. Osmolytic accumulation in plant cells can act as a mechanism of osmotic adjustment for decreasing the cellular osmotic potential and thus for maintaining water absorption and turgor. Osmolytic accumulation can also protect cellular components, such as cell membranes and proteins, and sustain the

physiological activity of plants [54]. Proline content with mycorrhiza inoculation was more or less unchanged in shoot of plant harvesting at 30-days and in root of plant harvesting at 90-days. However, a marked increase was induced in plant harvesting at 30-days and in shoot of plant harvesting at 90-days. This indicated that proline was response varied in different organs and in different harvesting stages. It can be consider in these organs which accumulated as a sign of osmotic adjustment [41]. The colonization of roots by AM fungi in various plant species induces proline accumulation when water is limiting [55]. The enhanced accumulation of proline in these studies was linked to AM-induced drought resistance with proline acting as osmoprotectant. Conversely, in several studies, while proline content increased in response to water deficit, a lower accumulation of proline has been observed in mycorrhiza plants relative to nonmycorrhizal counterparts. The enhanced accumulation of proline in these studies was linked to AM-induced drought resistance with proline acting as osmoprotectant. Conversely, in several studies, while proline content increased in response to water deficit, a lower accumulation of proline has been observed in mycorrhiza plants relative to nonmycorrhizal counterparts [56] [57] [58] [59] [60]. Wu, *et al.*, (2017) [61] showed that lower proline accumulation in AMF plants under drought stress. Our results therefore suggest that AMF strongly altered leaf sucrose and proline metabolism through regulating sucrose- and proline-metabolized enzyme activities, which is important for osmotic adjustment of the host plant. Also AM significantly accumulated total sugar and total protein which served in increase plant efficiency to increase dry matter of both testing harvesting stages. Chun *et al.* (2018) [62] reviewed comprehensively compiles significant correlations and limitations associated with plant stress tolerance and evasion mechanisms. Proline has every possibility of consideration as an indicator and potential marker for possible injury by osmotic stress. Wu and Ning Zou (2017) [63] have studies indicated a quick response to, drought and salinity stresses involving several mechanisms, such as root morphological modification, reactive oxygen species change, osmotic adjustment, direct absorption of water by extra radical hyphae, up-regulated expression of relevant stressed genes, glomalin-related soil protein release, etc. The underlying complex, multi-dimensional strategy is involved in morphological, physiological, biochemical, and molecular processes. The AMF responses are often associated with homeostatic regulation of the internal and external environment, and are therefore critical for plant health, survival and restoration in native ecosystems and good soil structure. The present work showed that AM infection levels measured by N-acetyl glucosamine content were not affected by drought stress. However these results are consistent with those Simpson and Dafit (1990) [64] [65] and Smith and Read (2008) reported that AM infection levels of maize and sorghum were not affected by water stress. Results showed also that control roots contained N-acetyl glucosamine would be attributed to mycorrhiza and other fungi naturally present in soil. This study indicates that improvement of maize grown under different moisture content by mycorrhiza inoculation could be attributed to improved water and mineral up-

take especially K^+ , Ca^{2+} , Mg^{2+} rather than P. In contrast with some published studies [66] [67]. Mycorrhiza symbiosis mitigated the accumulation of total sugar and total protein which served in increasing dry matter of shoot and root of two tested harvesting stages.

6. Conclusions

From previous results, it can be concluded that:

- 1) Drought tolerance varied in different organs and also at different stages of harvesting.
- 2) Increasing soluble sugar, soluble protein, amino acids, K^+ , Ca^{2+} , Mg^{2+} and P with AM inoculation can be served in increasing OP values, increasing water uptake and hence growth yield.
- 3) AM application induced an accumulation of total sugar and total protein which served in increasing dry matter of shoot and root of two tested harvesting stages.
- 4) Proline can be record as a sign of osmotic stress injury as in root of plant harvesting at 30-days and 90-days or as contribution in osmotic adjustment as in shoot of both harvesting stages under decreasing moisture content.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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