



## GROWTH RESPONSE AND WATER USE EFFICIENCY IN MAIZE (*ZEA MAYS L*) INOCULATED WITH DIFFERENT MYCORRHIZAL INOCULUMS

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### Abstract

Using mycorrhizal inoculum in sustainable agriculture attracted immense attention in recent years for improving plant growth, and water uptake. A plastic pot experiment was carried out at the College of Agricultural Engineering Sciences, University of Sulaimani, during June to July 2020 to study the effect of different mycorrhizal inoculum (*Glomus mosseae*, *Glomus geosporum* and a commercial inoculum INOQ Agri) on growth responses and water use efficiency (WUE) of two maize varieties (GLORIA and SY Miami). The root colonization percentage for GLORIA and SY Miami were (5, 83.33, 88.33, 88.33) % (3.33, 86.67, 78.33, 85) % for control, *G. mosseae*, *G. geosporum*, and INOQ Agri, respectively. Inoculated plants showed significantly better growth and water use efficiency compared to non-mycorrhizal plants. The best plant heights were in INOQ Agri plants (91.33, 90.67) cm in both GLORIA and SY Miami respectively. Highest shoot biomass (49.83, 44.50) g pot<sup>-1</sup> and root biomass (16.17, 15.67) g pot<sup>-1</sup> were found in INOQ Agri inoculant in both GLORIA and SY Miami respectively. The heights concentration of N, P and K were in INOQ Agri (1.53%, 0.61% and 0.14%) for GLORIA and (1.48%, 0.56% and 0.14%) for SY Miami respectively, Mycorrhizal inoculation also improved (WUE) in maize plants the average increasing percentage were (34.92%, 19.3%) for GLORIA and SY Miami cultivars respectively.

**Keywords:** Arbuscular Mycorrhiza, Water Use Efficiency, Maize, Biofertilizers.

## استجابة نمو وكفاءة استعمال المياه في نبات الذرة (*Zea mays L.*) الملقحة بلقاحات المايكورايزية المختلفة

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### الخلاصة

اجتذب استخدام لقاح فطر الميكوريزا في الزراعة المستدامة اهتمامًا واسعًا في السنوات الأخيرة لتحسين نمو النبات وامتصاص المياه. أجريت هذه الدراسة في البيت بلاستيكي في كلية علوم الهندسة الزراعية، جامعة السليمانية، خلال المدة من حزيران إلى تموز 2020 لدراسة تأثير ثلاثة لقاحات فطر مايكوريزا الشجيرية *Glomus*, *Glomas geosporum mosseae* ولقاح تجاري INOG Agri على تحسين استجابة نمو وكفاءة استعمال المياه لنوعين من الذرة SY Miami و GLORIA. ان نسبة الاصابة للجذور لكلا صنفين من نباتات الذرة SY Miami و GLORIA كانت 5%، 83.33%، 88.33% و 33.88% و 3.33%، 86.67%، 78.33% و 85% *G. geosporum*, *G. mosseae*, control و INOQ Agri على التعاقب، أظهرت المعاملات الملقحة تأثيراً معنوياً على النمو وكفاءة استعمال المياه مقارنة بالنباتات غير الملقحة وأعلى ارتفاعات نباتية كانت في النباتات الملقحة باللقاح INOG Agri 90.67، 91.33 سم للنوعين من الذرة SY و GLORIA و SY Miami على التعاقب. كانت أعلى النتائج للوزن الجاف الخضري 49.83، 44.50 غم وعاء<sup>-1</sup> والوزن الجاف للجذور 16.17، 15.67 غم وعاء<sup>-1</sup> في النباتات الملقحة بلقاح INOQ Agri في SY Miami و GLORIA على التعاقب. حقق التلقيح بالمايكوريزا إلى تحسين تركيز النيتروجين والفوسفور والبوتاسيوم في نباتات الذرة، وأعلى تركيز للعناصر N و P و K في النباتات الملقحة INOQ Agri، 1.53%، 0.61%، و 0.14% لصنف GLORIA و 1.48%، 0.56% و 0.14% في SY Miami على التعاقب. التلقيح بالمايكوريزا أدى إلى تحسين كفاءة استعمال المياه لنباتات الذرة وكان أعلى متوسط للنسبة المئوية 34.92%، 19.3% لكلا الصنفين SY و GLORIA على التعاقب.

كلمات مفتاحية: المايكورايزا الشجيرية، كفاءة استعمال المياه، الذرة، الأسمدة الحيوية.

### Introduction

Climate change and its consequence of water shortage is prominent challenges that face sustainable crop production in many agricultural regions around the world, these challenges will become more catastrophic with the predicted expansion 2.96% of the arid region by 2039 (16). Plants are continually exposed to biotic and abiotic environmental stress factors. One of the most serious abiotic stresses threatening

agricultural growth and output around the world is a lack of water (22). Despite the fact that crop traits were linked to enhanced drought resistance, allowing plants to absorb more water, lower water loss rates, and sustain higher physiological activity (34). On the other hand, Plants are commonly connected with microorganisms, this can influence plant responses to water constraint in the wild. Arbuscular mycorrhizal fungi (AMF) are one of the most widely distributed beneficial microorganisms, colonizing the majority of agricultural plant roots (22). Nearly 80 % of all terrestrial plant species may develop mutualistic symbiotic relationships with arbuscular mycorrhizal fungus (AMF) (26). The arbuscular mycorrhizal (AM) symbiosis typically promotes plant growth by allowing nutrients to be absorbed (18), direct water absorption and delivery to the host plants through fungal hyphae that improve performance and increase plant growth (32). Water-use efficiency is an important subject in agriculture in semiarid regions, because of the increasing areas under irrigation and the high water requirements of crops (4). AMF can enhance the tolerance of their host plants to water deficiency stress, according to a growing body of research (13). Maize (*Zea mays* L.), is one of the recognized widely produced and consumed cereal crops worldwide, with the record global production forecast of 112 million tonnes in 2022(8). Besides its primary use 61% as livestock feed, 13% consumed by human and it plays a vital role as staple foods for millions of people (9). Maize plants is a good host for mycorrhizal fungi and derive significant benefits from the mycorrhizal symbiotic relationship (17). Kurdistan region in north Iraq is situated in semi-arid region and drought has severely affected Iraq in recent decades which was characterized by a large drop in rainfall with a harsh impact on the vegetative cover (1). In addition, soils in the Kurdistan region area have a calcareous nature that suffers from severe phosphorus deficiency (14, 23). Overcoming these challenges with biofertilizer is of great significance. The objectives of this research were therefore to evaluate the effects of a different mycorrhizal inoculant on maize growth and water use efficiency.

### Material and Methods

Experimental conditions: A pot experiment was conducted from June to July 2020 in the plastic house belong to the department of horticulture, College of Agricultural Engineering Sciences, University of Sulaimani to study the effect of different mycorrhizal inoculation on growth response and water use efficiency in two commercial varieties of Maize (*Zea mays* L). The plants were grown in plastic pots holding 4 kg of soil that was collected at 0-30 cm depth from the agricultural farm of the College of Agricultural Engineering Sciences, University of Sulaimani. The soil samples were air-dried at room temperature and crushed thoroughly to pass through a 4-mm sieve. The soil has been classified as Vertisols based on the US soil taxonomy with silty clay loam texture (11.2% sand, 51.7% Silt and 37.1% clay). The soil had a pH of 7.7 (1:2.5, Soil: water suspension), 361 g kg<sup>-1</sup> of CaCO<sub>3</sub>, 6.1 C g kg<sup>-1</sup> of OC, 0.57 g N kg<sup>-1</sup> of total N, C/N ratio of 10.7:1, 461 mg P kg<sup>-1</sup> of total P, 4.5 mg P kg<sup>-1</sup> of available P, 0.11 g kg<sup>-1</sup> of available K and EC (1:2.5, soil: water) 0.76 dS m<sup>-1</sup>. Two different varieties of Maize (*Zea mays* L) SY Miami (syngenta) and GLORIA cultivars were used. With three different arbuscular mycorrhizal fungus (AMF) inoculants; *Glomus mosseae*, *G. geosporum* and INOQ Agri (INOQ GmbH, Germany). The inoculum consists of rhizosphere soil holding spores, hyphae and colonized root fragments of maize plants. Twenty grams of each mycorrhizal

inoculum were placed 2 cm just below the seeding for mycorrhizal treatments. Four seeds were sown in each pot and thinned to two after 14 days. Plants were irrigated to maintain 70% of field capacity by regular weighting of pots during the growing season whenever needed and recorded. The experiment was set in a factorial experiment with completed randomized design (C.R.D) with three replications for each treatment. The maize variety was main factor and the subfactors were the mycorrhizal inoculum and its interaction.

**Plant sampling and analysis:** The Plant height (cm) was measured after 60 days of sowing and the plants were harvested. The Shoot and root dry weighted were measured (g) after oven drying at 65°C for 72 hours. For mineral analysis, Nitrogen (N) was determined in a wet-digestion by the Kjeldahl method, and phosphorus (P) was determined by spectrophotometer as described in (19). Potassium (K) was measured by using Flame-photometer (27). The percentage of roots infection were determined following (20). Method, the roots were thoroughly washed with tap water to remove the soil, then for each replicates ten randomly root segment (1cm) were cleared in 10% KOH overnight. For neutralizing and removing KOH, segments were placed in 10% HCl for 10 minutes, washed with distilled water, followed by staining in 0.01% Acid fusion and finally microscopically examined for mycorrhizal infection. The mycorrhizal dependency (MD) was calculated using the following equation (30).  

$$MD (\%) = \frac{\text{Dry weight of mycorrhizal plants} - \text{Dry weight of non-mycorrhizal plants}}{\text{Dry weight of mycorrhizal plants}} * 100$$

**Water-use efficiency:** Water use efficiency (WUE) was calculated by dividing the dry weight of vegetative biomass (DW) (kg. pot<sup>-1</sup>) by the amount of applied water (IW) (m<sup>3</sup> pot<sup>-1</sup>) for each treatment (6).

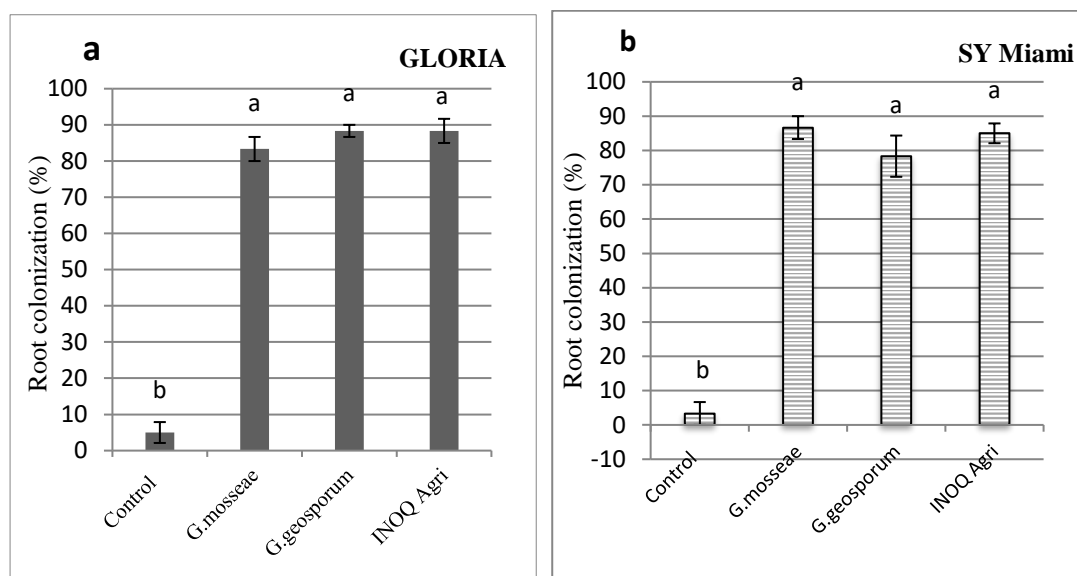
$$WUE = \frac{DW}{IW}$$

**Statistical analysis:** Data were Statistically analyzed using XL-STAT 2019 biostatistics software (Addinsoft, Paris, France). Analysis of variance (ANOVA) was performed to find the significant differences among treatments using Duncan's multiple range tests, at  $P = 0.05$ .

## Results and Discussion

**Root Colonization Percentage:** Mycorrhizal inoculation significantly affected root colonization percentage compared to non-inoculated plants but no significant differences were observed between different mycorrhizal inoculums in both SY Miami and GLORIA cultivars (Figure.1 a and b). The root colonization percentage for GLORIA and SY Miami were (5, 83.33, 88.33, 88.33) % (3.33, 86.67, 78.33, 85) % for control, *G. mosseae*, *G. geosporum*, and INOQ Agri, respectively. Minor infection of roots of uninoculated treatments with mycorrhizal fungi was observed in the roots of the two maize varieties that were not inoculated with AM fungus. This is because the used soil in this experiment where not sterilized and mycorrhizal fungi naturally persist in most agricultural soils, however, the low infection rate in non-inoculated treatment might be because agricultural tillage practices damage and reduce AM

fungal abilities to interact with the plant host. The increases in AM fungal root colonization from inoculums indicate the success of introduced mycorrhizal fungi in outcompeting the existing natural AM fungal community and the efficiency of the used inoculum (12, 15).



**Figure 1, a and b Percentage of root colonization in two maize varieties (a: GLORIA and b: SY Miami) inoculated with three different AM inoculum (*G. mosseae*, *G. geosporum*, and INOQ Agri).**

Treatments with the same letter above the bar indicate that values do not significantly different ( $P \leq 0.05$  according to Duncan's multiple range tests).

Plant Growth: Mycorrhizal inoculants (*G. mosseae*, *G. geosporum*, and INOQ Agri) significantly influenced plant height compared to non-inoculated plants (table 1). The highest values were found in INOQ Agri inoculant 91.33, 90.67 cm and the lowest were found in control plants 80.67, 80.31 cm in both GLORIA and SY Miami cultivars respectively. The total root biomass was significantly greater in both cultivars, plants inoculated with *G. mosseae*, *G. geosporum* and INOQ Agri compared to non-inoculated plants, the highest root biomass were recorded in INOQ Agri inoculant 16.17, 15.67 g pot<sup>-1</sup> and the lowest were 10.43, 10.73 g pot<sup>-1</sup> in both GLORIA and SY Miami cultivars, as well shoot biomass in the GLORIA cultivar had significantly affected by all mycorrhiza inoculants. The highest values was found in INOQ Agri inoculant 49.83 g pot<sup>-1</sup> and the lowest was found in control plants 39.90 g pot<sup>-1</sup>, but in SY Miami cultivar shoot biomass not significantly affected by Mycorrhizal inoculants, and the highest shoot biomass recorded in INOQ Agri inoculant 44.50 g pot<sup>-1</sup> and the lowest was found in control plants 39.00 g pot<sup>-1</sup>, (Table1). However, no significant differences were observed within mycorrhizal inoculants (Plant height, Shoot and root biomass) in both GLORIA and SY Miami cultivars (Table1). Addition of mycorrhizal inoculums improved plant growth and the mycorrhizal dependency ranged from 19.79% to 23.19% in GLORIA variety, and 12.95% to 17.30% in SY Miami, and the best results were from INOQ Agri inoculums for both maize varieties. Other researchers have proved the role of arbuscular mycorrhizal symbiosis to plant growth such.as (12, 33). Increased plant

growth due to AM symbiosis is usually associated with increased absorption of hyphal nutrients from the soil. The formation of mycelial networks by AMF with plant roots significantly improves root access to large soil surfaces and improves plant growth (5). Mycorrhiza fungi improve plant nutrition by increasing the mobility and availability of various nutrients (24). And greater rate of photosynthesis which increase more plant growth and raised plant height (26). It also improve the quality of soil by influencing its texture as well as the structure and consequently plant health (28).

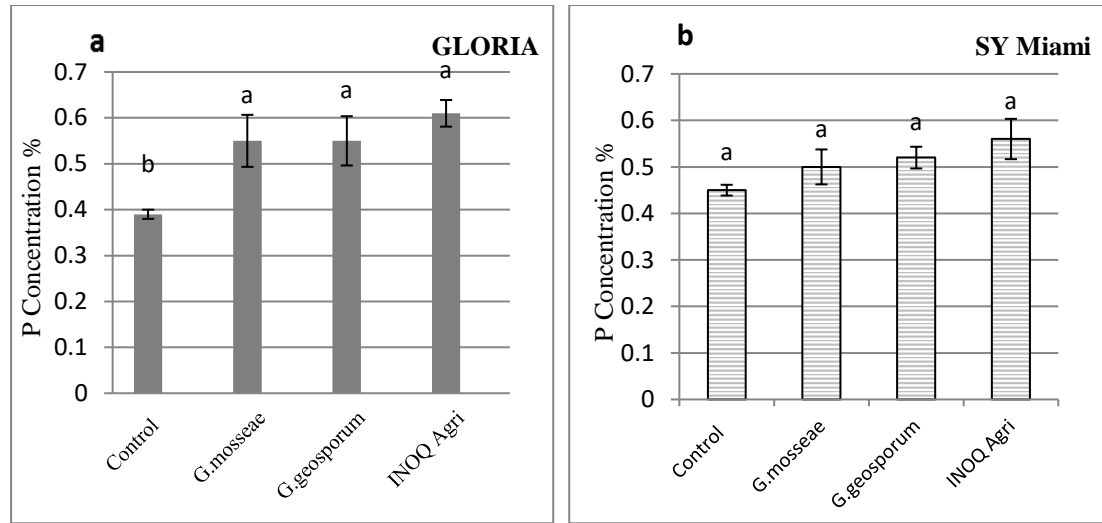
**Table 1 Plant height, biomass (shoot and root) and Mycorrhizal dependency of two maize varieties inoculated with three different AM inoculum (*G. mosseae*, *G. geosporum*, and INOQ Agri).**

Maize Cultivars	Mycorrhiza inoculums	Plant height (cm)	Biomass (g pot <sup>-1</sup> )		Mycorrhizal Dependency (MD %)
			Shoot biomass	Root biomass	
<b>GLORIA</b>	Control	80.67 ±(0.33)b*	39.90 ± (0.47) b	10.43 ± (0.3) b	
	<i>G. mosseae</i>	90.00 ± (0.76) a	49.50 ± (1.5) a	15.00 ± (0.76) a	21.94± (0.62)
	<i>G. geosporum</i>	89.50 ± (2.78) a	48.00 ± (1.73) a	14.93 ± (0.32) a	19.79± (3.43)
	INOQ Agri	91.33 ± (1.17) a	49.83 ± (3.53) a	16.17 ± (1.17) a	23.19± (4.27)
<b>SY Miami</b>	Control	80.31 ± (0.46) b	39.00 ± (0.68) ab	10.73 ± (0.22) b	
	<i>G. mosseae</i>	90.87 ± (2.3) a	43.40 ± (0.97) a	14.00 ± (1.44) a	12.95± (4.94)
	<i>G. geosporum</i>	89.83 ± (2.59) a	43.33 ± (0.73) a	14.70 ± (1.04) a	14.08± (3.79)
	INOQ Agri	90.67 ± (3.22) a	44.50 ± (1.32) a	15.67 ± (0.17) a	17.30± (0.94)

The columns with the common letter are not significantly different at  $P \leq 0.05$  according to Duncan's multiple range tests. Values are means ± standard errors from three replicates.

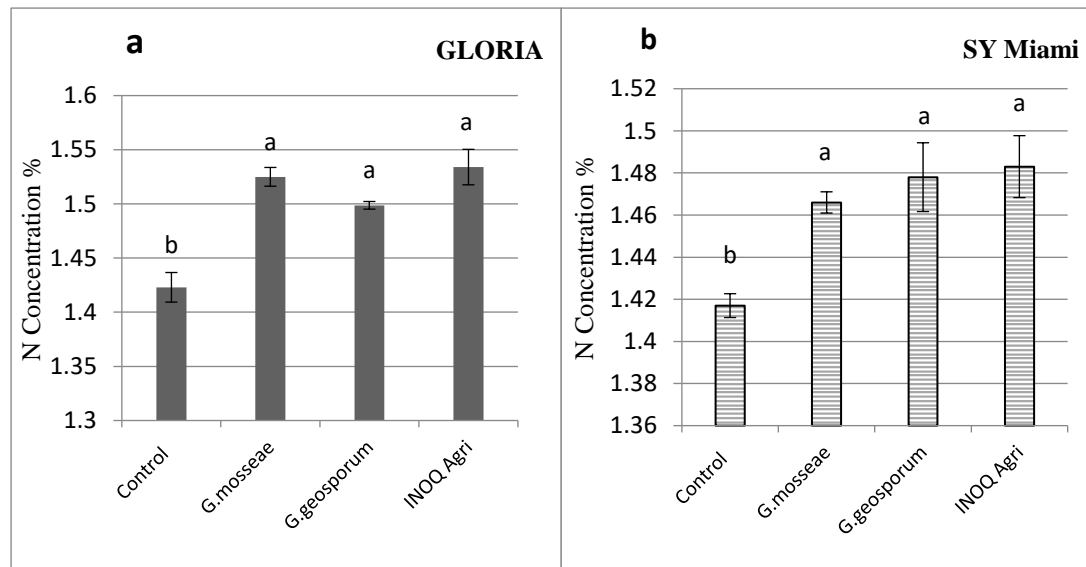
Nutrient content: The results in presented figures (2-4a, 2-4b) revealed that mycorrhiza inoculation improved nutrient status in the shoot of the maize plants compared to the non-inoculated plants, particularly in GLORIA cultivars. The best results were in INOQ Agri 1.53%, 0.61% and 0.144% for GLORIA variety and 1.48%, 0.56% and 0.14% for SY Miami for N, P, and K respectively. Moreover, the average increasing percentage mycorrhizal inoculants 6.75, 46.15, 6.02 % for GLORIA variety and 4.16, 17.77, 3.75 % for SY Miami for N, P and K respectively. (fig.2, 3, 4(a and b)). As in previously studied characteristics, no significant differences were observed between mycorrhiza inoculants *G. mosseae*, *G. geosporum* and INOQ Agri in both GLORIA and SY Miami varieties (fig. 2,3,4(a and b)). Some researchers had recorded the role of AMF in the uptake of soil nutrients, particularly nitrogen and phosphorus, which can effectively promote the growth of host plants (25, 29). Mycorrhizal inoculation increases the nutrient concentration in plants by increasing the root surface area for nutrient and water absorption. It increases plant roots branching allows mycorrhizal hyphae to extend from the root to the soil and allows plant roots to interact with the larger surface area of the soil (11).

AM fungi also change the kinetic properties of the roots and increase their ability to absorb nutrients that improve crop productivity (26).



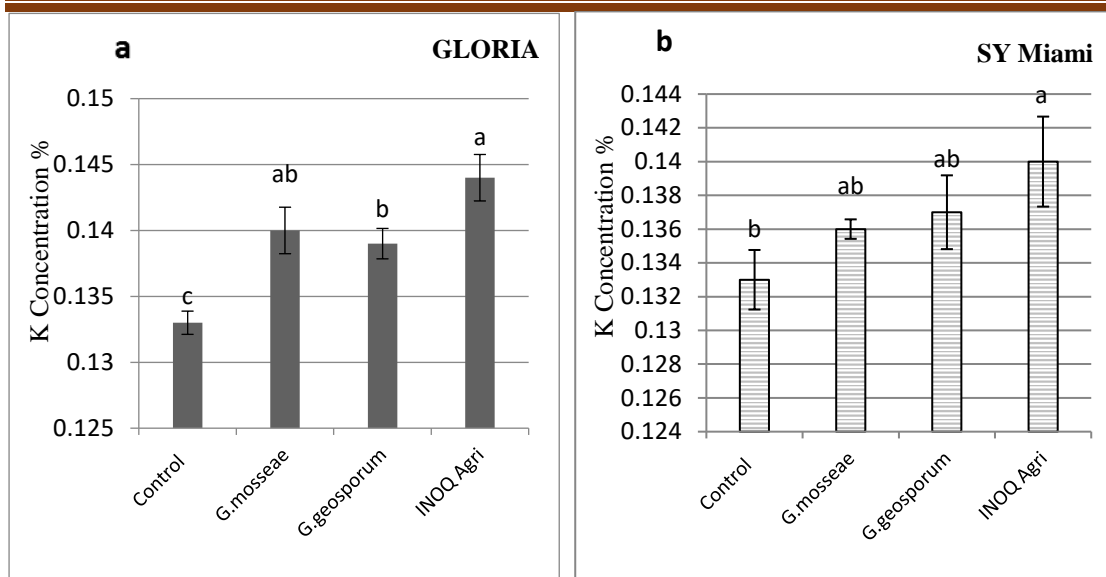
**Figure 2, a and b Phosphorus concentration of shoots in two maize varieties (a: GLORIA and b: SY Miami) inoculated with three different AM inoculum (*G. mosseae*, *G. geosporum*, and INOQ Agri).**

Treatments with the same letter above the bar indicate that values do not significantly differ ( $P \leq 0.05$  according to Duncan's multiple range tests).



**Figure 3, a and b Nitrogen concentration of shoots in two maize varieties (a: GLORIA and b: SY Miami) inoculated with three different AM inoculum (*G. mosseae*, *G. geosporum*, and INOQ Agri).**

Treatments with the same letter above the bar indicate that values do not significantly differ ( $P \leq 0.05$  according to Duncan's multiple range tests).

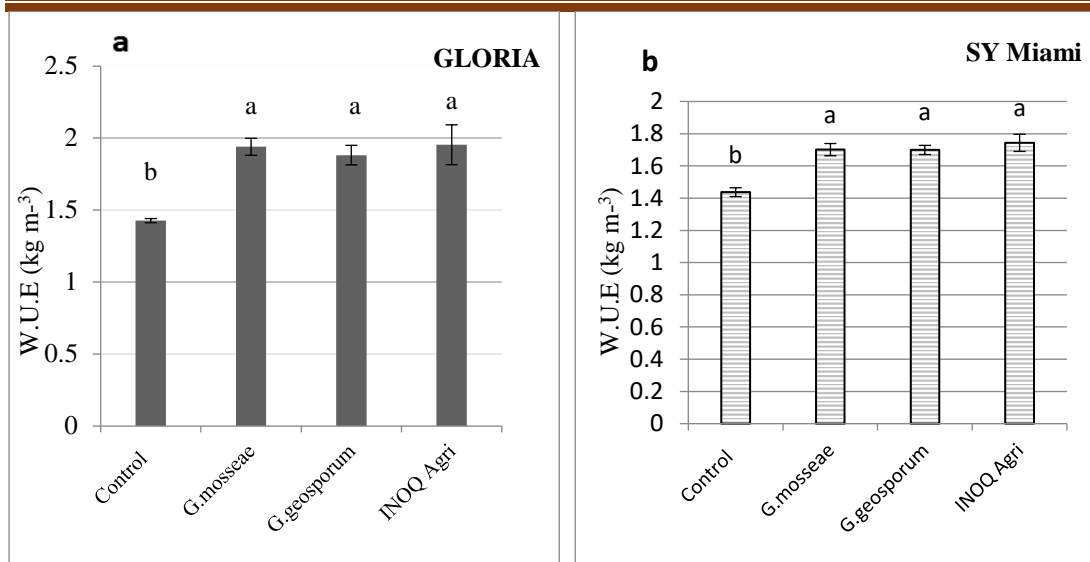


**Figure 4, a and b Potassium concentration of shoots in two maize varieties (a: GLORIA and b: SY Miami) inoculated with three different AM inoculum (*G. mosseae*, *G. geosporum*, and INOQ Agri).**

Treatments with the same letter above the bar indicate that values do not significantly differ ( $P \leq 0.05$  according to Duncan's multiple range tests).

Water use efficiency: Mycorrhizal biofertilizers significantly improved water use efficiency in maize plants compared to non-inoculated plants with an increased percentage amounting 35.95, 31.89, 36.93 % 18.37, 18.23, 21.29 % for each Mycorrhizal species *G. mosseae*, *G. geosporum* and INOQ Agri in both GLORIA and SY Miami cultivars respectively, and the highest increased percentage were found in INOQ Agri inoculant 36.93, 21.29 % in both GLORIA and SY Miami cultivars respectively (figure.5 a and b). There were no significant differences in terms of water use efficiency between Mycorrhizal species *G. mosseae*, *G. geosporum* and INOQ Agri in both maize varieties. The positive effect of mycorrhizal inoculation on WUE is perhaps because AMF can increase the water absorption capacity of the roots and maintains optimal moisture around the roots (10). Some studies have previously reported the mechanisms involved in WUE improvement in plants infected with AMF. The primary mechanism involved in WUE optimization has been traced back to osmotic tuning optimization (31), water and nutrient uptake from the soil by hyphae (24), improving the hydraulic conductivity of the root (3). And Regulation of stomatal conductance by hormonal changes (2). The result of this study agrees with the conclusions of other works (7, 21). In the beneficial effect of AMF inoculation in improving growth and water use efficiency.





**Figure 5, a and b Effect of a different AM inoculum (*G. mosseae*, *G. geosporum*, and INOQ Agri) on water use efficiency in two maize varieties (a: GLORIA and b: SY Miami).**

Treatments with the same letter above the bar indicates that values do not significantly different ( $P \leq 0.05$  according to Duncan's multiple range tests).

The successive use of mycorrhizal biofertilizer is depend on the compatibility between the fungus and host, in general, the results of this study showed that the two varieties of Maize (*Zea mays* L) GLORIA and SY Miami (syngenta) plants have benefited from mycorrhizal symbiosis. Growth and water use efficiency increased in mycorrhizal plants compared to non-mycorrhizal plants. The boost in water use efficiency of inoculated maize plants resulted in improving plant growth and absorption of nutrients, especially phosphorus. The beneficial gain differs from one to another variety; the beneficial advantage in the GLORIA cultivar was higher than SY Miami cultivar. The best results were found in INOQ Agri inoculant compared to control however, there were no differences among (AMF) inoculants (*Glomus mosseae*, *G. geosporum* and INOQ Agri) in the results.

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