



## Effects of *Glomus intraradices* on the drought resistance and growth of corn plant (*Zea mays* L.)

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**Abstract:** A pot experiment was carried out at the greenhouse of Faculty of Agriculture (Saba bacha), Alexandria University. The experiment was conducted to investigate the role of *Glomus intraradices* fungi in the effects on the growth and water requirement of pot-grown corn (*Zea mays* L.). Four water regimes (20%, 30%, 40% and 50% of the available soil water content) were conducted. The Arbuscular Mycorrhizal Fungi AMF inoculation could significantly increase plant growth (including plant height, leaf area, and fresh and dry mass), enhance relative leaf water content, transpiration rates and stomatal conductance, and improve plant drought tolerance. The water consumption of the mycorrhizal plants producing 1 g of dry matter was 20%–35% of water content conditions. These findings indicate that the mycorrhizae enhanced the water utilization efficiency.

**Keywords:** *G. intraradices*, corn plant (*Zea mays* L.), drought resistance.

### INTRODUCTION

*Glomus* can be associated with the roots of most plants and can strongly impact water retention properties and the subsequent drought responses of its hosts. Previous work indicated that mycorrhizal associated citrus rootstocks exhibited higher root hydraulic conductivity than non-mycorrhizal plants, resulting in enhanced drought resistance ((Graham and Syvertsen 1984). The effects of The Arbuscular Mycorrhizal Fungi (AMF) on water absorption and utilization in apple, cherry and birch-leaf pear trees were studied under normal water status and drought-stressed conditions(Liu and Luo 1988, Liu 1989a, b, Wu *et al.*, 2006). Under normal water conditions, AMF enhanced the relative leaf water content and transpiration rates and decreased stomatal resistance in apple and cherry trees. Under drought conditions, AMF also enhanced stomatal conductance, transpiration rate and relative leaf water content, and decreased leaf water potential and the permanent wilting point.

When mycorrhizal and non-mycorrhizal plants were watered under continuous drought conditions, the pressure inflation of mycorrhizal plants recovered fast and the plants exhibited an enhanced growth compared with the nonmycorrhizal plants(Liu and Luo 1988, Liu 1989a, b, Kaya *et al.*, 2003, Piniol *et al.*, 2005, Wu *et al.*, 2006). Plant growth Promoting rhizobacteria (PGPR) may also contribute to drought amelioration(Rubin *et al.*, 2017). Corn (*Z. mays* L) is an important crop and one of two sources for cereal flour used in the world for making bread. For example in Egypt, the average area of corn in 1999 was 1.648 million feddan which produced 5.438 million tons. Although Corn is relatively drought-resistant (ACSRT,1999), regular access to water sources ensures adequate corn production. Previous research indicated that AMF could improve drought resistance in different plants. In the current study, Corn seedlings were used to study the mechanism underlying drought tolerance and water retention in seedlings inoculated with AMF under various soil water content

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conditions. The findings here provided a rationale cause for applying mycorrhizae to cereal flour.

## MATERIALS AND METHODS

The mycorrhizal inoculum, (*Glomus intraradiaces*) used as stock culture prepared from Faculty of Agriculture., Ain Shams University of Cairo. Seeds of corn (*Zea mays* L) were surface-sterilized for 3 min in 0.1% HgCl<sub>2</sub> solution, then washed three times in water and soaked for 24 h in water at 40°C. Seeds were subsequently germinated at 28°C in an incubator. Water tests were conducted in plastic test pots, each containing 3 kg of sandy loam and has the following general properties: pH, 8.00; organic matter, 2.34 g kg<sup>-1</sup>, the available nitrogen content was 45.22 mg kg<sup>-1</sup> available P, 3.5 mg kg<sup>-1</sup> soil. The procedures used for soil analysis were those described by (Page and Page 1982). In this soil, the maximum water holding capacity was 16%. All soil samples were steam-sterilized at 121°C for 2 h. Water control began after the seeds were sown. The eight treatment groups included inoculated or non-inoculated seeds maintained in soil with water content controlled at 20%, 30%, 40% or 50% of the available water. All experiments were conducted in a greenhouse in the specimen garden at Agriculture faculty (Saba bacha), Alexandria University.

The mycorrhizal treatments were carried out by adding 50g of inoculum per pot of these treatments which was placed below the seeds. Non Mycorrhiza treatments received the same quantity of autoclaved inoculums. (Khan *et al.*, 2003) Inoculum (50g) was consisted of external mycelium, spores and colonized roots mixed with soil. Five of corn seeds were sown in each pot. After the seeds sprouted, two plants were selected from each pot based on their growth level. During the experiment, the evaporated water per pot was measured daily by weighting pots, and the young seedlings were provided with a specific amount of water so that the water content in the soil was maintained at 20%, 30% 40% or 50% of the available water, respectively. After 60 days, the plants were harvested. Root samples for

determination of root colonization with AM fungi were cleared with 10% KOH and stained with 0.05% trypan blue in lactophenol as described by (Phillips and Hayman 1970), and microscopically examined for AMF colonization by determining the percentage of root segments containing arbuscules and vesicles using a gridline intercept method (Giovannetti and Mosse 1980).

The plant height, the number of leaves, the fresh and dry weight of leaves, shoots, and roots per pot were measured. Leaf water saturation deficits were calculated by the saturation water content method. (Baker, 2010), transpiration rates and stomatal conductance were measured by a photosynthesis surveying instrument (Dutra *et al.*, 1996). The content of proline in leaves was measured with a Daojin UV-120 spectrometer (Piniar *et al.*, 2005). Water requirement (ml) was calculated through measuring the water consumption yielding 1 g of dry matter plants per pot.

## Statistical analysis

Data were subjected to analysis of variance using the ANOVA procedures according to (Snedecor and Cochran 1972) Statistical significance was determined at  $P < 0.05$ .

## RESULTS AND DISCUSSION

The AM fungi root colonization was noted in roots of corn, No colonization occurred in non-mycorrhizal corn seedlings. In the AM-inoculated plants, 96% of roots were colonized when the water content of the soil was at 50% of the available water. When the water content decreased, the percentage of AMF colonization likewise decreased. Specifically, the AM colonization of plants at 20% of the available water was less than that of plants at 30%, 40% and 50% of the available water (Table 1). The percentage of colonized seedlings remained above 76.8%. These results indicate that when corn seedlings are inoculated with *G. intraradiaces*, they are highly susceptible to colonization. These findings are consistent with the previous conclusion from field studies reporting a high

natural occurrence of AMF mycorrhizal fungi in *Malus hupehensis* plant(Liu 1989a).

This colonization by AM fungi may explain, in part, the ability of corn plant to resist the damage of stressed conditions. The plant height, the number of leaves, and the fresh weight and dry weight of mycorrhizal-inoculated plants were greater than those of the non-mycorrhizal-inoculated plants grown under the same soil water content conditions (Tables 2 and 3). Thus, the mycorrhiza significantly promoted corn seedling growth. The growth of mycorrhizal plants grown at 20% of the available water did not differ significantly from the growth of non-mycorrhizal plants grown at 30%of the available water. When compared with the non-mycorrhizal plants, the dry weight of mycorrhiza plants increased by 78%, 100% 121% and 150% at soil water contents of 20%, 30%, 40% and 50%of the available water, respectively.

**Table (1).** Mean percentage of mycorrhizal colonization in corn seedlings under various treatment conditions

| Inoculation                 | The available Water content of soil/% | Mycorrhizal colonization/% |
|-----------------------------|---------------------------------------|----------------------------|
| <i>Glomus intraradiaces</i> | 20%                                   | 76.8c                      |
|                             | 30%                                   | 82.6b                      |
|                             | 40%                                   | 95.2a                      |
|                             | 50%                                   | 96.1a                      |
| non-inoculated              | 20%                                   | 0                          |
|                             | 30%                                   | 0                          |
|                             | 40%                                   | 0                          |
|                             | 50%                                   | 0                          |

**Table (2).** Effect of fungi on the growth of corn seedlings under various soil water contents

| Inoculation                 | The available water content of soil/% | Plant height/cm | Numbers leaves |
|-----------------------------|---------------------------------------|-----------------|----------------|
| <i>Glomus intraradiaces</i> | 20%                                   | 8.69d           | 10.20c         |
|                             | 30%                                   | 14.32c          | 13.65b         |
|                             | 40%                                   | 16.40b          | 14.12b         |
|                             | 50%                                   | 19.20a          | 16.84a         |
| non-inoculated              | 20%                                   | 6.74e           | 6.43d          |
|                             | 30%                                   | 7.96d           | 7.87d          |
|                             | 40%                                   | 10.12f          | 9.89c          |
|                             | 50%                                   | 11.85f          | 10.34c         |

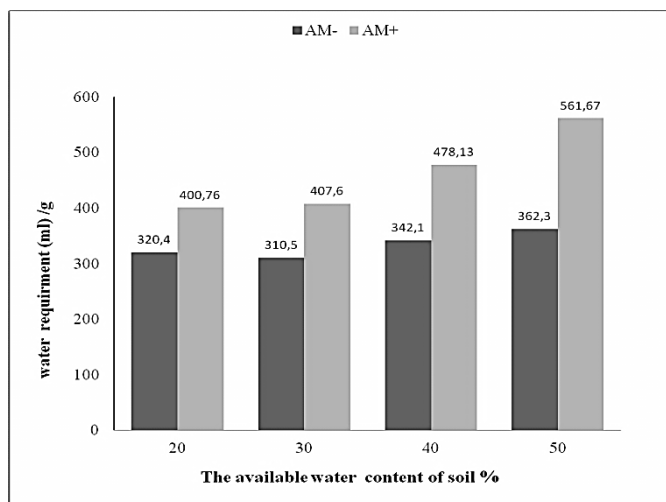
Mycorrhizal plants can significantly increase corn seedlings growth. When the soil water content was at 20% of available water, the mycorrhizal plant dry weight was 78% of the weight of non-mycorrhizal plants. Moreover, when the soil water content was 50% of available water, the mycorrhizal plant dry weight was 150% of the weight of non-mycorrhizal plants. This effect of mycorrhiza on the growth of corn seedlings is more effective than its impact on the growth of other plants such as peach and *Avena sativa*(Khan *et al.*, 2003). The fresh weight and the dry weight of mycorrhizal plants grown under the condition of 20% of available water were not significantly different from the weights of non-mycorrhizal plants grown in that of 30% and 40% water content.

Mycorrhizal corn seedlings required less water than the non-mycorrhizal plants to produce 1 g of dry matter. Specifically, when compared with the non-mycorrhizal plants, mycorrhizal plants required less water at 20%, 24%,28% and 35%of available water when grown in soil with a water content of 20%, 30% , 40% and 50%of available water , respectively (Fig. 1). These findings indicate that the mycorrhizae enhanced the water utilization efficiency. Leaf water saturation deficits in the mycorrhizal plants were lower than in the non-mycorrhizal plants grown under identical water content conditions.

Proline is an osmoregulatory key element in plants experiencing conditions of water stress. Under some

conditions, various plants produce a large amount of proline to enhance osmosis and prevent dehydration. Thus, the quantity of proline produced can reflect the degree of water stress (Piniór *et al.*, 2005). Our results in table (4) shows that concentration of proline in leaves increased as the soil water content decreased. Furthermore, proline concentrations in the mycorrhizal plant leaves were significantly lower than in the non-mycorrhizal plant leaves. These results suggested that the quantity of proline in corn leaves increased as the water content of the soil decreased. When grown under the same water conditions, mycorrhizal plant leaves contained less proline than the non-mycorrhizal plant leaves. Thus, under the same degree of water deprivation, the mycorrhizal plants are less physiologically stressed than the non-mycorrhizal plants. Notably, when the soil water content was 40% or 50% of available water, the stomatal conductance in the mycorrhizal plants was significantly higher than that in the non-mycorrhizal plants. When the soil water content was at 20% of available water, the stomatal conductance in mycorrhizal plants was still higher than that in the non-mycorrhizal plants, but the difference did not reach significance. Furthermore, when compared with the non-mycorrhizal plants grown under the same water content conditions, the mycorrhizal plants

exhibited higher transpiration rates (Table 4). Stress from dehydration can cause stomata close, and a decrease in stomatal conductance and transpiration rates in leaves, which may prevent the roots from absorbing and transporting water (Kaya *et al.*, 2003). This may eventually result in a decrease in the dry weight of plants. Our study indicated that mycorrhizae may improve stomatal conductance, transpiration rates in leaves and biomass, and significantly enhance drought tolerance in corn seedlings. Findings in the current study suggest three mechanisms by which mycorrhizae enhance drought tolerance in plants. First, hyphae can absorb soil water directly. Under drought-stressed conditions, hyphae can utilize the soil water that is not accessible by the roots. Thus, the water supply to the plant is improved and effectively enhancing drought tolerance. Second, hyphae can absorb nutrients including phosphorus, zinc and many other elements. In this way, plant nutrition can be improved, and plant growth can be increased likewise. Ultimately, the mycorrhizal plants have more roots capable of absorbing water. Finally, mycorrhizae can regulate the balance of internal hormones in plants to indirectly influence water metabolism in the affected plant (Murakami-Mizukami *et al.*, 1991, Dutra *et al.*, 1996, Lu *et al.*, 2007).



**Fig. (1)** The amount of water required to produce 1 g of dry matter in AM and non-AM corn seedlings grown in the soil water content of 20%, 30%, 40% and 50%; respectively

**Table (3).**Effect of AM fungi on the biomass of corn seedlings under various soil water contents (gpot<sup>-1</sup>)

| Inoculation                | The available water content of soil/% | Leaf   |       | Shoot |       | Root   |        | Plant DW |
|----------------------------|---------------------------------------|--------|-------|-------|-------|--------|--------|----------|
|                            |                                       | FW     | DW    | FW    | DW    | FW     | DW     |          |
| <i>Glomus intraradices</i> | 20%                                   | 4.19e  | 2.01d | 2.31d | 1.76d | 6.22d  | 2.71e  | 6.48e    |
|                            | 30%                                   | 8.26c  | 3.46c | 4.35c | 3.05c | 15.52c | 7.00c  | 13.51c   |
|                            | 40%                                   | 12.01b | 5.24b | 5.92b | 4.18b | 26.40b | 10.63b | 20.05b   |
|                            | 50%                                   | 13.65a | 6.54a | 7.11a | 5.42a | 31.22a | 14.20a | 26.16a   |
| non-inoculated             | 20%                                   | 2.31g  | 1.34e | 1.65e | 1.21d | 2.56de | 1.09f  | 3.64f    |
|                            | 30%                                   | 3.66f  | 1.76e | 2.95d | 1.87d | 4.63d  | 3.12e  | 6.75e    |
|                            | 40%                                   | 4.22e  | 2.60d | 3.01d | 1.96d | 6.15d  | 4.50d  | 9.06d    |
|                            | 50%                                   | 6.33d  | 3.31c | 5.22c | 3.06c | 7.33d  | 4.11d  | 10.48d   |

**Table (4).** Effect of AM fungi on water physiology of corn seedlings

| Inoculation                 | The available water content of soil/% | Leaf water Saturation deficit/% | Concentration of proline in leaves/% | Stomatal conductance /( $\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) | Transpiration rates/( $\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) |
|-----------------------------|---------------------------------------|---------------------------------|--------------------------------------|---|---|
| <i>Glomus. Intraradices</i> | 20%                                   | 13.06b                          | 0.056b                               | 25.23b  | 2.11b   |
|                             | 30%                                   | 10.13b                          | 0.032c                               | 46.86a  | 3.40a   |
|                             | 40%                                   | 7.39c                           | 0.027c                               | 50.72a  | 4.11a   |
|                             | 50%                                   | 5.67c                           | 0.016cd                              | 53.62a  | 4.62a   |
| non-inoculated              | 20%                                   | 32.54a                          | 0.320a                               | 14.70b  | 1.68c   |
|                             | 30%                                   | 15.56b                          | 0.082b                               | 21.68b  | 2.32b   |
|                             | 40%                                   | 9.63b                           | 0.067b                               | 23.63b  | 2.67b   |
|                             | 50%                                   | 6.34c                           | 0.046bc                              | 26.12b  | 2.89b   |

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## تأثيرات فطر *G. intraradices* على مقاومة الجفاف ونمو نبات الذرة (*Zea mays L.*)

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**المستخلص:** أجريت هذه التجربة في صوبة زجاجية بكلية الزراعة (سابا باشا) جامعة الإسكندرية لاختبار فرضية دور فطر الميكوريزا *Glomus intraradices* في مقاومة الجفاف ونمو نبات الذرة، ولتحقيق ذلك تم تنمية نباتات الذرة في تربة ملقحة بفطر الميكوريزا وإضافة أربعة أنظمة من المحتوى المائي 20% و30% و40% و50% من الماء المتاح، أوضحت النتائج أن عزلة فطريات الميكوريزا لها تأثيرات معنوية مختلفة على نمو نبات الذرة من حيث (ارتفاع النبات، مساحة الورقة، وزن النبات الطازج والجاف) بالإضافة إلى تحسين المحتوى المائي في الورقة، ومعدلات النتج وبالتالي قدرة النبات على مقاومة الجفاف، ولوحظ أن الاستهلاك المائي للنباتات الملقحة بفطر *G.intraradices* قليل لإنتاج واحد جرام من المادة الجافة بنسبة 20%-35% بالمقارنة مع النباتات غير الملقحة تحت نفس الظروف، ونستنتج من هذه الدراسة إن لفطر الميكوريزا آليات حماية النباتات من الجفاف.

**الكلمات المفتاحية:** *G. Intraradices*، نبات الذرة (*Zea mays L.*)، مقاومة الجفاف.