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Effect of *Clariodeoglomusclariodeorum* on morphology and abundant of carrot root hairs in vitro

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***Abstract*— *The roothairs are important components of the root for absorbing nutrients for plants and also secreting the plant-produced secretes. Morphology and their number are influenced by various environmental and internal factors and are regulated by them. Mycorrhizal fungi are established through the root and their presence in the root can affect root's physical and chemical properties.The aim of this study was to evaluate the effect of the presence of the fungus in the roots on the characteristics of the capillary roots.In order to remove the effect of other microorganisms on the results of the experiment, this experiment was performed in vitro on the roots of the carrot secondary phloem tissue culture. In this experiment, the fungus could affect the characteristics of the root hairs: their number (22% decrease in the root hairs number in the root hair area of the root) and their length (A decrease of 21.3% in the length of capillary roots in mycorrhizal plants). These changes in the characteristics of capillary roots were also caused by the presence of fungal structures in the roots as well as by the decrease in the production of strigolactones. In this study, changes in the production of strigolactones calculated by using their effect on seed germination of Phelipancheaegyptiaca.*

Keywords*— *Phelipancheaegyptiaca, Strigolactones, Capillary root's length.

Highlight:

According to our results, the presence of mycorrhizal fungi can act as an alternative to root hairs. And their wide hyphae of these fungi can have the same function or better than the capillary roots for the plant. An important point in this regard is the sensitivity of the roots of plants from tissue culture after leaving the desirable condition of in vitro and entering the new environmental condition such as a field. Meanwhile, using Mycorrhizal fungi can provide a stronger guarantee for the ability of the roots of these tissue culture derived plants in the presence of mycorrhizal fungi inside them, in an out of glass environment.

Abbreviation:

In this experiment, which was carried out in tissue culture condition, the effect of mycorrhizal fungi on the number and length of capillary roots was investigated. The results showed that the presence of fungi in the root of the plant affected the amount of strigolactones production and reduced it. Following a decrease in the production of strigolactones, the number and length of root hairs also decreased. It was shown in this experiment that the presence of mycorrhizal fungi can act as an alternative to root hairs. And the wide hyphae of these fungi can have the same function or better than the capillary roots for the plant. An important point in this regard is the sensitivity of the roots of tissue culture plants after leaving the in vitro condition and entering the field. Meanwhile, using Mycorrhizal fungi can provide a stronger guarantee for the ability of the roots of these plants in the presence of mycorrhizal fungi inside them, in an out of in vitro condition.

I. INTRODUCTION

Mycorrhizaم arbuscular fungi (AM) belong to the Glomeromycota branch andGlomales class(Schusler, Schwarzott, & Walker, 2001). AM fungi are the compulsory coexistence of plants, and both sides benefit from this bilateral cooperation between fungi and plant (Smith & Read, 2008). In addition to enhancing access to mineral elements,

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almonds have other advantages such as increased tolerance to drought and heavy metals, protection against pathogens and plant breeding for plant host (Gange & West, 1994; Joner, Briones, & Leyval, 2000; Khan, Kuek, Chaudhry, Khoo, & Hayes, 2000; Augé, 2001). Also, they affect plant diversity and population structure and contribute to the growth and deployment of seedlings(Grime, Mackey, Hillier, & Read, 1987; Klironomos, 2003; Van Der Heijden, 2004; Van Der Heijden, et al., 2006). In the relationship between plant and AM fungi, there is a certain Chemical communication. These chemical connections include the release of a number of signals released into the environment from the plant and the production of signals emitted by the fungus(Oldroyd, 2013). The response of different plant species to colonization by Mycorrhizal fungi is different. This response usually depends on the degree of dependence of the plant species on the mycorrhizal communication. According to this, the morphological characteristics of the root will change(Hayman, 1983). Therefore, the fungus associated with the root can be related to the plant. In the meantime, the fungus needs to change the specific characteristics of the root so that it can produce its effective and beneficial effect on the plant. In this research, we have tried to determine the effect of these coexist fungi on the root by examining some morphological and chemical characteristics of the root.

II. MATERIALS AND METHODS

Isolation of mycorrhizal fungus:

Soil and root samples were collected from the rhizosphere area of carrot root in carrot fields in Isfahan in the fall of 2015. Spores of mycorrhizal fungi were isolated from the soil and were identified using articles and information founded on valid sites (www.zor.zut.edu.pl and www.invam.caf.wvu). From isolated spores, *Clariodeoglomusclariodeum*species was selected and propagated on corn roots in pearl perlite pots. The spores were then placed on a solid WA medium for germination. Germinated spores were removed with one millimeter of the surrounding culture medium and placed next to the root of the carrot culture from the previously cultured tissue culture in MS medium under conditions of in vitro to penetrate the root.

Data analysis:

This experiment was conducted in a completely randomized design with data analysis using IBM SPPSS Statistics software.

Measuring the amount of strigolactone in the root

To determine the production of strigolactones in the carrot roots, we used strigolactones in stimulating seed germination of the *Phelipancheaegyptiaca* parasite plant. This experiment is based on an experience conducted by Araco et al in 2013. Carrot root extract was applied to the seeds. In this experiment, the germination inducer, GR24 (10-10 and 10-11 M), was used as a positive control and water was used as a negative control. As a point, the germination rate of carrot root extract is always lower than that of GR24.

III. RESULTS

Number of root hairs in the mycorrhizal and non-mycorrhizal plants in vitro:

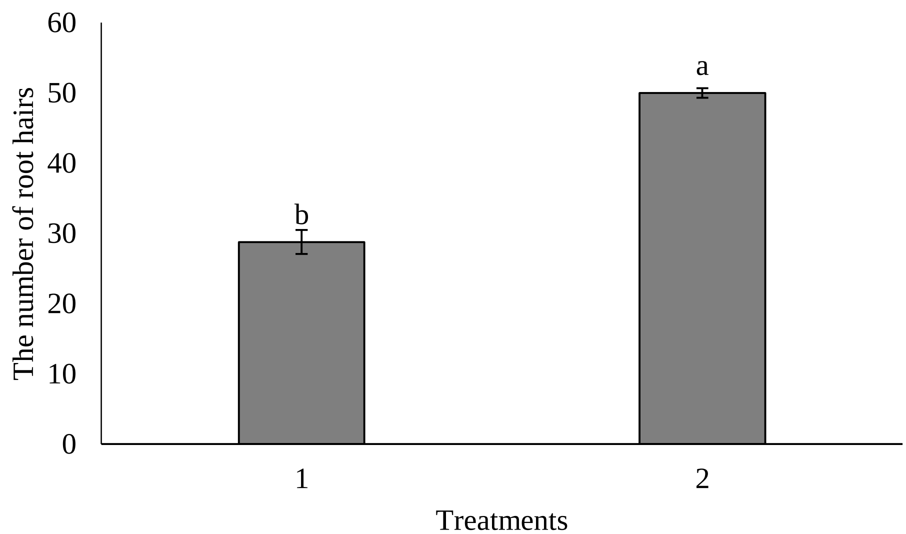

Fig.1: The number of root hairs in 100 micrometers of the root hairs area of root (P <0.05). 1) Mycorrhizal plant, 2) Non-.mycorrhizal plant.

As shown in figure 1, the presence of fungus in the root causes a 22% reduction in the number of roots hairs. According to the data analysis, this decrease was significant at the level of 0.05% .

The length of capillary roots in mycorrhizal and non-mycorrhizal plants:

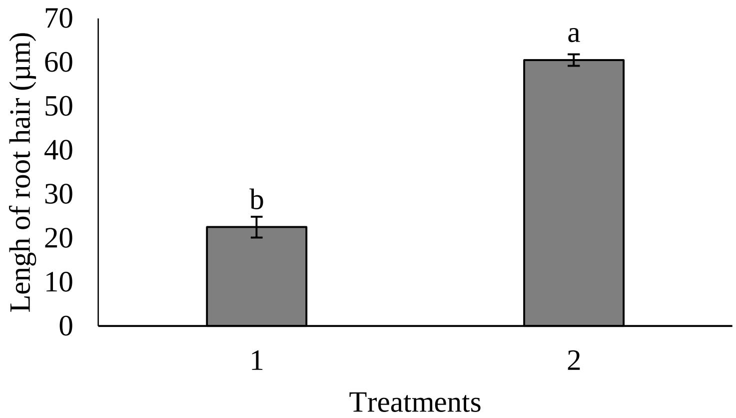

Fig.2: Comparison of the length of root hairs in mycorrhizal and non-mycorrhizal plants. 1) Mycorrhizal plant, 2) Non-mycorrhizal plant. (95% confidence interval of the difference).

Data analysis results showed a decrease of 21.33% over the root hairs length in the mycorrhizal plant (Figure 2). According to the data analysis, this decrease was significant at the probability level of 0.05%.

Hair root area in carrot root under an optical microscope:

Fig.3: The number of root hairs in mycorrhizal and non-mycorrhizal plants under optical microscopy (Model BM-22). a) Non-mycorrhizal plant, you can see here the abundant number of root hairs and their high length of them in absent of mycorrhizal fungus, b, c, and d) Mycorrhizal plant. Here you can see a small number of root hairs in the mycorrhizal plant. They are also much smaller than non-mycorrhizal plants in length.

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The effect of Mycorrhiza fungus on the rate of production of strigolactones:

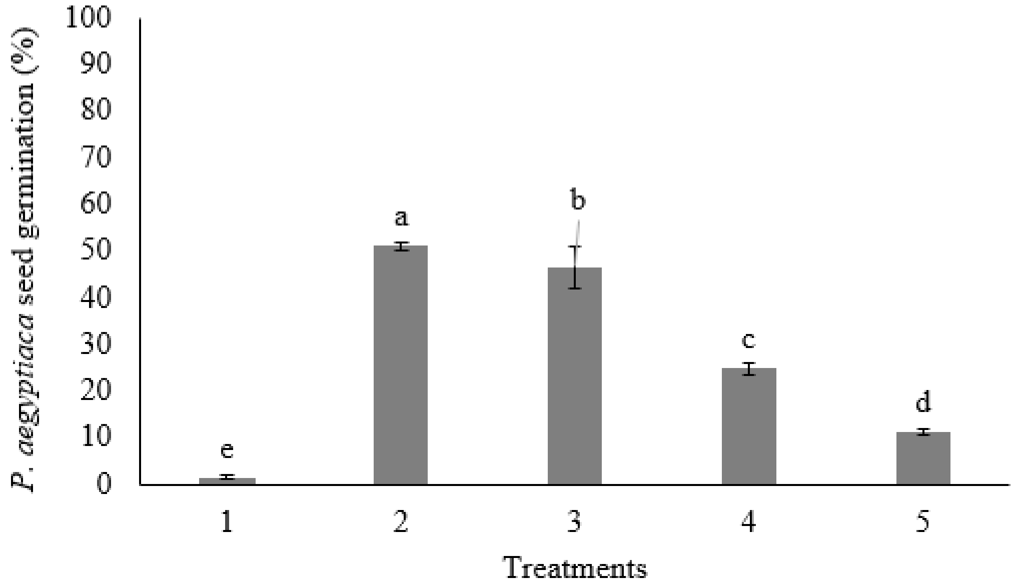

Fig.4: Percentage of P. aegypicae seed germination in mycorrhizal and non-mycorrhizal plants. 1) Water (Control -), 2) GR24 (Control+) 10^{-10}M, 3) GR24 (Control+) 10^{-11}M, 4) Non-mycorrhizal plant, 5) Mycorrhizal plant.

As shown in Figure 4, the presence of the extract of carrot root has a significant effect on germination of *P. aegypicae* seeds, however, this increase in germination in the mycorrhizal plant is significantly lower than that of the non-mycorrhizalplant.

IV. DISCUSSION

According to earlier studies, as with the results of this experiment, there is always a correlation between the amount of capillary roots of the plants and their need for mycorrhizal coexistence. The presence of mycorrhizal fungi in the plant directly reduced the number of capillary roots and their length. Their absence resulted in normal and natural growth of capillary roots. Akiyama et al. (2010) found that there is a linear relationship between the root structure and the mycorrhizal roots. The length of the capillaries root and their abundance in the root is a good guide to detect the degree of dependence of the plant on the mycorrhizal coexistence, as shown in Figure 2 and 3, in the mycorrhizal plants, the length of the capillary root, as well as their abundance in this area had a significant decrease. Plants with large roots and a smaller number of capillary roots, such as citrus, strongly depend on mycorrhizal fungi. On the other hand, plants such as Gramineae, having narrower roots and more root hairs, have reported less interdependence with these types of fungi, according to reports. Plants that have more affinity for the coexistence have a roughly rooting system, the root-to-shoot ratio is almost constant, and a much smaller number of root hairs formed(Hetrick, 1991). In a number of studies, it has been pointed out that a large root system with long capillary roots can be sufficient for the plant as mycorrhizal plants do, and reduces the plant's dependence on these fungi (Schweiger, Robson, & Barrow, 1995; Baylis, 1970). In this experiment, it can be seen that root characteristics can be changed in relation to the presence of mycorrhizal fungus. However, external hyphae of these fungi, even when they have the same length with capillary roots, can be much more efficient in absorbing nutrients and water than root hairs. This is due to the fact that these fungi have the ability to penetrate into smaller pores of the soil, and also hyphae do not compete in phosphorus absorption, while adjacent roots compete themselves in sources(Baldwin, Tinker, & Nye, 1972; Barber, 1995). Lee et al. (2014) obtained similar results to the results of this experiment and found that mycorrhizal roots could act as a substitute for capillary roots. According to this, it is possible to justify the reducing inthe number of root hairs in the presence of mycorrhizal fungi.Baylis (1970) also said that plants with shorter capillary roots are heavily dependent on arbuscular fungi, in our experiment, with root colonization, in addition to reducing the number of capillary roots in this area, their length also diminished. Crush (1974) also reported that the decrease in dependence on arbuscular fungi resulted in an increase in the length of capillary root in the three leguminous plants. Manjunatfa andHahte(1991) also found that capillary root length is one of the most important criteria for determining the dependence of the plant on arbuscular fungi, which is identical to the results of this experiment.

The influence of existence of mycorrhizal fungi on the amount of strigolactones production:

Changes in root morphological characteristics, such as the number and length of capillary root that were examined in this experiment, are due to several factors. These factors include the effect of the existence of fungal structures in the root and changes in the root growth factors. One of the factors affecting roots is the amount of strigolactones production(Kapulnik, et al., 2011). In this experiment, it was shown that mycorrhizal fungi can affect the amount of strigolactones production and reduce their amount.

Strigolactones are newly discovered metabolites that are considered to be phytohormones. These hormones play a role in plant biology. And according to studies, Strigolactones are known to play a role in the morphology and architecture of the plant. They affect the formation of lateral roots and capillary roots and lift up their length. So they adjust the growth of the plant in different environmental conditions (Gomez-Roldan, et al., 2008; Umehara, et al., 2008; Vogel, et al., 2010). Therefore,they are as the negative regulators of the lateral roots and the positive regulator of the lengthening of the capillary roots under stress conditions (for example, low levels of nutrients), respond to growth conditions (Kapulnik, et al., 2011).In non-stress conditions, the presence of these fungi within the root reduces the production of strigolactones. In the same work, the presence of mycorrhizal fungi in the pea reduced the germination percentage of *Orobanche* and *Phelipanche* species. That it showed a reduction in strigolactones(Fernandez-Aparicio, GArcia-Garrido, Ocampo, & Rubiales, 2010). In addition, previouslyhad been shown that in tomato mycorrhizal plants, seed yield increased and analyzes showed that this increase was related to a decrease in strigolactones(Fernandez-Aparicio, GArcia-Garrido, Ocampo, & Rubiales, 2010). Therefore, the decrease in the production of strigolactones by the fungus appears to be a phenomenon that is different in different plant species. It seems that the regulation and controlling of root colonization by the fungus was done in this way, and this is a plant strategy that prevents excessive colonization. In some cases, the plant is able to respond to the presence of *G. intraradices* in the form of secretion of strigolactones. Auxin and Ethylene as phytohormones play a key role in regulating lateral roots formation and also the development of capillary roots that affect the level of Pi deficiency(Rubio, et al., 2009). This is similar to the effect that this Pi deficiencyhas on the rate of colonization by the arbuscular fungi. It has recently been shown that strigolactones, along with Auxin, play a role in the development of lateral roots and branches in Arabidopsis. This depends on the level of Pi in the growth medium (Ruyter-Spira, et al., 2011).

Kapulnik et al, (2011) also obtained similar results with our experiment and concluded that strigolactones have a direct effect on capillary rootsmorphology and abundance.

In the meantime, it may be necessary to say that other microorganisms that have a relationship with the roots of plants, especially microorganisms that have a symbiotic relationship, can have a significant effect on the number and morphology of the root and especially root hairs. For example, in a study conducted by Heidstra et al in 1997, Rhizobium in legumecaused an increase in the number of root hairs.

V. CONCLUSION

In this experiment, which was carried out in tissue culture condition, the effect of mycorrhizal fungi on the number and length of capillary roots was investigated. The results showed that the presence of fungi in the root of the plant affected the amount of strigolactones production and reduced it. Following a decrease in the production of strigolactones, the number of root hairs also decreased by 22%. In addition to numbers, their length also decreased. It was shown in this experiment that the presence of mycorrhizal fungi can act as an alternative to root hairs. And the wide hyphae of these fungi can have the same function or better than the capillary roots for the plant. An important point in this regard is the sensitivity of the roots of tissue culture plants after leaving the in vitro condition and entering the field. Meanwhile, using Mycorrhizal fungi can provide a stronger guarantee for the ability of the roots of these plants in the presence of mycorrhizal fungi inside them, in an out of in vitro condition.

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