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Determining the Mycorrhiza Type in *Cymbopogon olivieri*

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Abstract: To determine the type of mycorrhiza in the root of Cymbopogon olivieri and effect of drought stress in rate of this relation ,was used of the root of 45 and 60 days plants which were irrigated in 4 levels (control, 75% FC, 50% FC, 25% FC), The results showed that in the plants which were under the stress (50% FC, 25% FC), spores destroyed in the soil and mycorrhizal correlation will not occur with roots. But, mycorrhizal correlation was created in the 60 days stage and before flowering stage in the plants of control level. the colored images of root under the microscope can confirms the Vesicular Arbuscular mycorrhizal-correlation.

Key words: Mycorrhiza · Vesicular Arbuscular · *Cymbopogon olivieri* · Drought stress

INTRODUCTION

The genus of *Cymbopogon* was of Andropogonae race, Panicoidaea sub-family and Poaceae family. Up to now, 56 species and 120 varieties of this genus were presented. Two species of this genus in Iran e.g *C.parkeri* and *C.olivieri* were identified and reported that the genus of *C.olivieri* were selected for the study. Aromatic species of Cymbopogon of the millet family in the wide tropical regions of the world have been distributed especially in the South to South-East Asia. Some famous species, such as *Cymbopogon citratus*, *Cymbopogon nardus* and *Cymbopogon martini* by having the essence of lemon fragrance was used in the wide range of pharmaceuticals, cosmetics, health and food industries [1]. Plant growth is affected by different internal and external factors. One of the most important external factors that affect the plant is water stress (Drought). The imbalance between water stored in the soil and water requirements of plants so that the water in the soil to be below the plant needs, this is called water stress [2]. Drought stress occurs when the very little water amount is available to the plants. Such conditions may be due to the intense evaporation, the low osmotic potential of water in the saline soils and lack of adequate water uptake by plants in the shallow soil. Water stress is due to the imbalance between water uptake and lack of it that leads to various physiological responses. One of the factors of water uptake in roots is the presence of symbiotic fungi with the plant roots which are known as mycorrhizal fungi. These fungi are often the most important microorganisms in the soils which are not destroyed. So that about 70 percent of the microbial community biomass of soils is the mycelium of these fungi. Mycorrhizal fungi coexistence with vascular plants belong to three categories of, Basidiomycetes, Ascomycetes and Zygomycetes [3]. The result of this symbiosis, fungal activity in order to absorb and transport water and nutrients to the host plant and receive carbon compounds from the host plant photosynthesis [4]. Vesicular - arbuscular Fungi is the largest group of mycorrhizal fungi that penetrate the skin cells of roots and form Vesicular – arbuscular structures, arbuscular structures increase the metabolic compounds exchange rate in skin between the host plant and fungi [5]. It has been reported in many references, one of the most important reasons for the adaptation of plants in arid regions is the symbiosis ability with mycorrhizal fungi [5]. Mycorrhizal symbiosis in arid areas leads to increase the metabolic activity, effectiveness and ease of water uptake and transferring it into the plant [6]. Also specified that in terms of water shortage, stomatal resistance and water maintenance have been increased in mycorrhizal plants than the non-mycorrhizal plants [6]. Also, the symbiosis with mycorrhizal fungi can improve the plant nutritional status, increase of water absorption and resistance to drought through the water potential improvement [7].

MATERIALS AND METHODS

25 healthy seeds were cultured in plastic seedling pots with a diameter of 10 centimeters containing the

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loamy-sand soils, pH = 8 and electrical conductivity (EC) equal with 44 dS/ m and each one of pots series were irrigated with water amount of plant farm capacity. All stages of plant growth were under greenhouse conditions with 16 h light period and temperature of 82 ±2°C and 8 hours of darkness period with temperatures of 32 ±2°C. Light intensity was about 11,000 lux at plant surface and the humidity was 46%. when plants were in *Four-leaves* stage (thirty days after planting), the dry treatments were applied at three levels of 25% FC, 50% FC, 75% FC and the control (irrigation at FC level) in a completely randomized design with three replications.

Counting the number of fungal spores in the soil was done by *wet sieve* method and total spores were counted. The counting was not in a way that the different species of fungi can be separated and only the total amount of spores in the soil were counted. Procedures applied for isolation and enumeration of the fungi healthy living spores : 100 g of the soil should be suspended in the distilled water and fully stirred and then the soil, respectively first of all should be passed through a 50 mm sieve and then a 100 mm sieve and finally a 32 micrometer sieve and particles remaining on the 32 micrometer sieve were collected to continue the work and the collected contents was poured proportionally in centrifuge tubes to their sizes and tubes content centrifuged at 1800 rpm for five minutes and the upperside solution of tube containing the supernatant suspended materials and the dead spores were discarded and then the underside sediment with 440 grams per liter of sucrose solution were suspended and suspended sucrose solution again centrifuged at 1800 rpm and upper-side sucrose solution which contains the spores, passed through a 32 micrometer sieve to remain the spores on the sieve and then sieve surface washed with distilled water for being washed the sieve surface sucrose and osmotic pressure on the spores to be disappeared. Spores were collected on the 32 micrometer sieve by water flow and transferred into a petri dish and then observed and counted under a dissecting microscope [8].

The stages of staining the roots to observe amount of inoculation with the vesicular-arbuscular mycorrhiza: The roots are first washed with water and placed in the test tubes. 10% potassium hydroxide aqueous solution was spilled on them and tubes placed in hot water bath for 20 min and temperature of 90°C. Then, the roots were washed with water well and clean roots was placed in the 10% oxygenated water for 5 minutes. The roots were washed well with water and dipped in a 0.1 normal choloridric acid solution and then were acidized. After that the samples were placed 10 min in 0.1 % acidic color of fuchsin in the warm bath and after washing the specimens were placed in the decoloration solution (lactic acid: glycerol: water repectively in ratio of 14:1:1) in a period of one night. The stained samples were studied by optical microscope [9].

RESULTS

The results of counting the number of spores in the soil due to the applied drought stress level are shown in Figure 1. The presented results are the mean of three replications in each soil.

The results show that with increasing the stress intensity, the number of fungal spores decreases in the soil, because the presence of fungi in the soil was the evidence of mycorrhizal relationships in soil with different plants roots. In the study of lemon-grass roots according to the mycorrhizal inoculation images from the control plants (Figures 2, 3 and 4), the inoculation of this plant roots with Vesicular-arbuscular mycorrhiza can be confirmed.

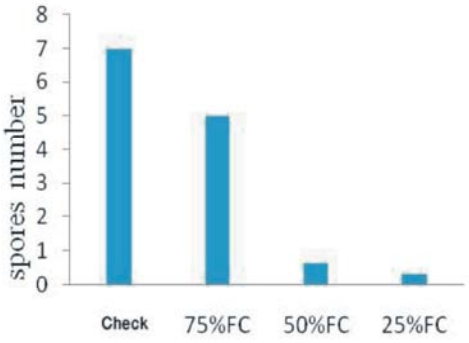

Fig. 1: Changes of spores number in the soil due to the rate of drought stress

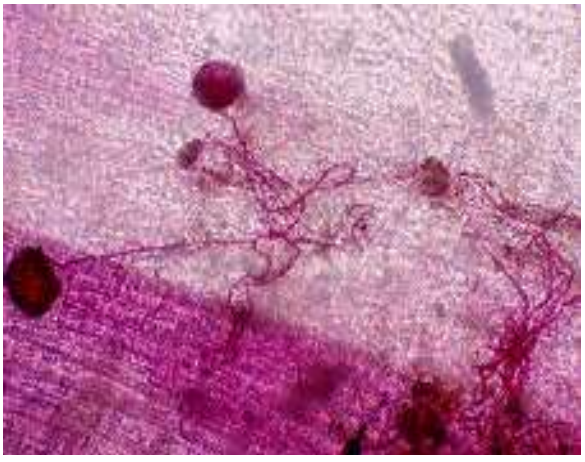

Fig. 2: Image of the vesicles in root of the control plant.

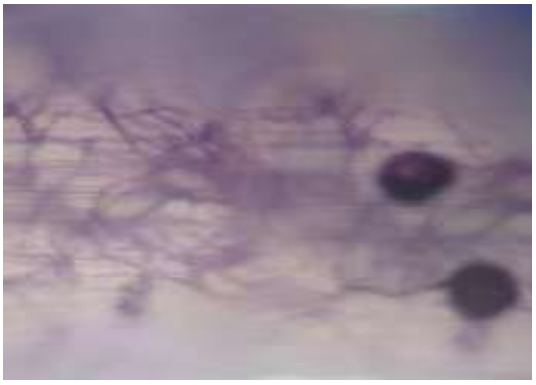

Fig. 3: Image of vesicles in plant roots under the stress of FC 75%.

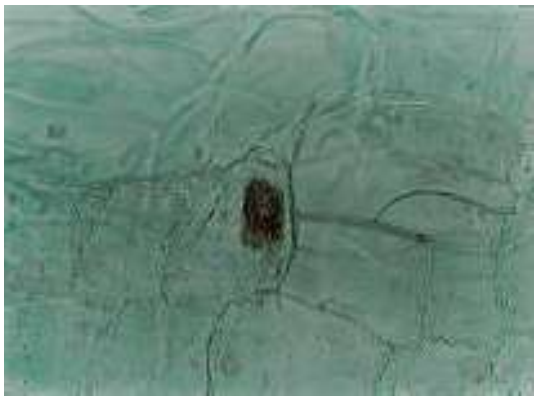

Fig. 4: Arboscules image in the root cells.

CONCLUSION

In recent studies, indicated that all of the millet (gramineae) have mycorrhizal Vesicular - arbuscular correlation [10], also the genus of Cymbopogon is stated that mycorrhizal correlation of *C. tortilis* and *C.winterianus* species was of Vesicular - arbuscular type [11, 12]. Mycorrhizal study of vesicular - arbuscular type was done about this species. In the root of *45-day* control plant and treatment group of 75%, filaments without cross walls were observed, but arbuscular or vesicles structures were not observed. In the roots of control plants under the stress of 75% and *60 days vesicles structure* was clearly detected and took photos, but the arboscules were not accurately determined in this growth stage. As can be observed from the results of spores number, in the root of plants under the stress of 25 % and 50 % until the time of 60^{th} *days* of plant life period, cannot be found any fungal filament (mycelium) or mycorrhizal correlation. The studies showed that plant rot inoculation to the mycorrhizl fungi is related to the planr species, mycorrhizal fungus correlated with it, plant age, stage of plant growth, root environment conditions including dryness, pH, phosphorus concentration around the roots, etc [13]. In explaining the results, it can be said that mycorrhizal Vesicular – arbuscular correlation with roots of this plant can be found but its time clearly was not determined in this study. It is likely that the amount of water in the soil is involved in the survival of spores of soil and the creating a mycorrhizal correlation. Because, this correlation was not observed in plants under the severe stress or at least the correlation has been postponed in them. Plant age in this correlation is effective, but by the results can be said that the lemon grass in the vegetative phase and before the reproductive phase and flower formation, has established the mycorrhizal correlation.

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