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Research Article

THE EFFECTS OF THE MYCORRHIZA ON PLANT GROWTH DURING ACCLIMATIZATION OF SOME *IN VITRO* GROWN SWEET CHERRY ROOTSTOCKS

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ABSTRACT. This study was conducted to investigate the effects of *Glomus mosseae* and *G. fasciculatum* on plant growth during acclimatization of micropropagated sweet cherry rootstocks. In order to determine the effects of mycorrhizal inoculation and growth media on plant growth, shoot length, and dry weight of roots and shoots were analyzed. The leaf content was significantly increased by mycorrhizal inoculation. The total root length and percentage of infected roots were investigated at the end of the acclimatization. As a result, survival rate of rootstock plantlets was not affected by mycorrhizal inoculation. The effect of inoculation was found significant on nutrient uptake and tissue P content. The results showed that mycorrhizal inoculation may be used at the *in vitro* rooting stage for better acclimatization. Mycorrhizae inoculated rootstocks grow better and increase Zn and P uptake.

Keywords: *Sweet cherry (Prunus avium L.) rootstocks, micropropagation, acclimatization, mycorrhizal inoculation, Zn, P uptake*

INTRODUCTION

After being worked on for thousands of years, agricultural lands have been exhausted due to the same old cultivation methods intensively practiced by the greedy human beings. Poor management practices such as over-fertilization and wrong pesticide applications have aggravated the productivity of the agricultural lands and have lead to lower production with higher inputs. Moreover, chemical applications by uneducated farmers have destroyed the natural balance of soil and upset the activity of the soil micro organisms. These problems have been more prominent by the end of the 20th century and alarmed farmers and researchers for solutions. New superior cultivars and organic farming have proved to be the feasible practices to remediate the problems. Researchers have shown that organic farming creates a well-balanced soil fauna and available nutrients both for the plant roots and for mycorrhiza fungi [1, 2, 3, 4, 5, 6]. Fungi involved in mycorrhizae associations exhibit the most common plant-microorganism symbiosis in nature. Botanically, mycorrhiza is the mutual relationship between the roots of higher plants and the soil fungi. Mycorrhizae fungi infect the root cortex and send out its hyphae (vesicular–arbuscular structures) into the cortex to become part of the inner root structure. Hyphae produce a network connecting the inside to outside of the plant and transferring nutrients from outside to inside and

carbohydrates from inside to outside [7]**.** Sustainability of the world population by the agricultural resources is of great concern as the population is expected to double by the end of the 21st century. This concern has led the developed countries to utilize new techniques and plant biotechnology to increase their agricultural yields. Plant propagation methods such as "plant tissue culture or micropropagation techniques" used in producing plants free of disease-causing agents are becoming more and more common. Micropropagation technique is a modern method to produce and also lets the fast-clonal propagation of plant species within a certain time [8, 9, 10]. By using the tissue culture or micropropagation method, disease-free plants expressing the desired characters can be produced in large amounts in a short period of time. Salamanca et al., [11] has demonstrated that AMF inoculation reduced the length of the micropropagated plant production cycle from 18 to 10 weeks. However, beside these advantages, the main problem in tissue culture is the acclimatization of the *in vitro* propagated plants to field environment. There are great losses when plants are transferred from the nearly 100 % humidity *in vitro* conditions to the field environment. The deaths are due to drying out which is caused by the fact that plants do not produce the water conserving cuticle layer under *in vitro* conditions. From the early stages of development, plant roots need the beneficial rhizosphere organisms, mainly the *Mycorrhizae* fungi [12, 13,14, 3, 15, 6, 5]. Rai, [16] indicated that arbuscular mycorrhizal fungi (AMF) improves bio priming of micropropagated plantlets and plays a significant role in ensuring the health of plantlets. Azcon-Aguilar et al. [17] reported that mycorrhiza formation appears to play a key role in favoring ex vitro development of micropropagated plants of avocado. A well-developed and strong root system aids the plant in water and nutrient uptake, and hence, improves the survival chances of plants which are produced by tissue culture methods. This research has analyzed the acclimatization techniques of cherry rootstocks propagated by tissue culture methods, and the effects of mycorrhizae in the acclimatization process. It has been reported [17, 18], inoculation of arbuscular mycorrhizal fungi (AMF) to the roots of micropropagated plantlets plays a beneficial role on their post-transplanting performance. Yadav et al. [19] indicated that an AM fungus during the initial period of the acclimatization phase has showed stimulatory effects for achieving better survival of micropropagated plantlets. Since period of micro propagated plantlets is short, for rhizosphere organism development it is sound to inoculate with mycorhizophere.

The goal of the research was to produce a well-developed and strong root system by using the symbiotic fungi and to overcome the difficulties in transferring the plants to field conditions and hence reducing losses. In addition, the stronger root systems will improve the absorption capacity of the plant supplying water and important nutrients such as phosphorus, and making the plant stronger against stress factors. Use of mycorrhizae as natural fertilizer is also an important goal as the chemical fertilizers are expensive and considered potentially harmful. In the light of these factors, the goal of this research is to improve the acclimation of cherry rootstocks to field conditions, and determining the optimum mycorrhizae and growth medium for the root system of the cherry rootstocks.

MATERIALS AND METHODS

The study included investigation effects of the mycorrhizae on plant growth during acclimatization of some micropropagated three cultivars of sweet cherry rootstocks. In

the experiment, all sweet cherry rootstocks (Gisela-5, Damil and Edabriz) were micropropagated and firstly acclimatization was made by the University of Çukurova, Faculty of Agriculture, and Department of Horticulture Laboratory for Plant Biotechnology**.** Also, mycorrhizal materials were supplied from University of Çukurova, Soil Science department which had used mycorrhizal species called *Glomus mossae* [19] and *Glomus fasciculatum* [21]**.**

Medium and Explants Establishment

In the experiment, Murashige and Skoog (MS) medium was used for micropropagation, growing and rooting [22]. The same procedure was used for shoot-tip culture. Shoot tip was isolated (0.1-0.5 mm) from shoots in a sterile bench and transferred into test tubes. Shoot tips taken to growth chamber with 16 h photoperiod (3000-4000 lux) at 26-28º C. Plants were transferred to new media per four weeks. After four weeks from *in vitro* rooting, the experiments were carried out greenhouse conditions.

Growth Substrata and Inoculums Properties

The plants were transferred into pots containing two types of growth substrates (GS);

- GS1; peat: perlite (1:1 v/v)
- GS2; Andesite: Soil: Compost (6:3:1 v/v).

The substrate was autoclaved at 121ºC for 2 h to use as growth substrate. Mycorrhizal inoculation was applied to plants when they were transferred from *in vitro* to *in vivo* same time. Control plants were transplanted into growth substrates without inoculum. Inoculum was obtained from cultures of *Glomus mosseae* and *G. fasciculatum* propagated on corn (*Zea mays*). Sand containing spores and infected root fragments were added to transplant substrates (1000 spores per plant).

Biomass Assessment and Nutrient Analysis

After three months, rooted plants were harvested and assayed for changes in biomass and root morphology. Roots were separated from the soil by washing with running and distilled water. Before drying the roots, small sub samples were taken for determination of root length and mycorrhizal infection using modified method from Koske and Gemma [23]. Washed root segments (1-2 cm) were cleared with 10% KOH for 30 min, rinsed with tap water, acidified in 5% HCl for 30 min and stained with trypan blue in lactic acid. Mycorrhizal colonization was determined using grid-line intersects method [24].

Shoot and root fresh and dry weights, survival rate, shoot height, and the number of leaves were recorded for each plant. Shoot and root fresh weights shoot and root dry weights were subsequently determined after drying at 65ºC for 48 h. Dry material from each pot was ground by Tema mill. Then 0.2 g of ground plant materials was ashed at 550ºC followed by dissolution in 3.3% HCl. After the digestion of the plant material, the concentration of P was determined by the Murphy and Riley, [25] method by using a spectrophotometer. The concentration of Fe, Zn, Cu and Mn was determined by atomic absorption spectrophotometer.

Experimental Design and Statistical Analysis

The experiments were analyzed with the MSTATC statistical programs [26] as Completely Randomized Designs with three replications and 10 plants for each replication and clones were used as covariate to control random variation. For all characteristics studied, the statistical significance of differences between means was determined using the Tukey-test.

RESULTS AND DISCUSSION

In order to observe the effects of the mycorrhizae and the growth substrate on the growth and development on the cherry rootstocks, the plants were grown for 12 weeks and then harvested. Although the findings indicated no significant effects of the mycorrhizal applications on the acclimatization of plants, the best results in both substrates (GS 1and GS 2) were obtained with *Glomus mosseae*. This application produced 89.82 % success with Gisela-5 cherry rootstocks.

Under the natural environment, majority of plants are colonized both by external and internal microorganisms; particularly such as beneficial bacteria and fungi, can improve plant performance under stress environments, and consequently enhance yield [27]. Other research carried out on field adaptation of indoor lab propagated plants support that mycorrhizal inoculation of the plants should be administered for higher survival rates. In addition, it is reported that the adaptation period is the most important step following the micro propagation techniques where the utilization of microorganisms might improve chances [28]. *In vitro* propagated plants are delicate and lacking vigor to survive the acclimatization shock with great losses observed frequently [29]. On the contrary, another research has reported that the infection was observed after the first 4 weeks, so that the inoculation did not have any effect on the survival rates of the micro propagated plants [30]. The same results were also observed in present research.

The effects of mycorrhizal applications and growth media on shoot length have been determined as well. Gisela-5 control plants showed greatest shoot growth (16.1 cm).

In this research, among the most important results, percentage of the infected root finding has been found. All investigation factors were significant and the highest infection rate was found *G. mosseae* application that belong Gisela-5 rootstock in GS-2 (% 89.82) (Fig. 1 and 2).

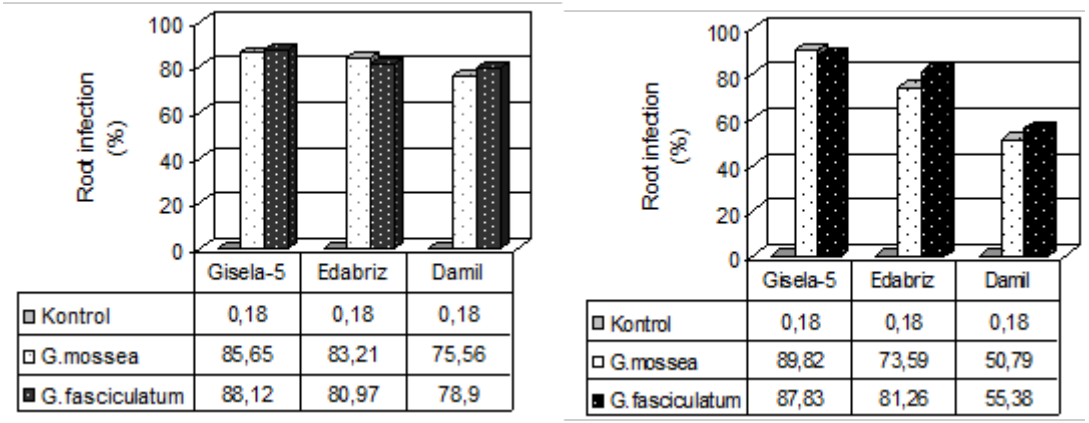

Fig. 1. *Cheery rootstocks percentage of the infected root finding grown on GS-1 and GS-2 (Respectively)*

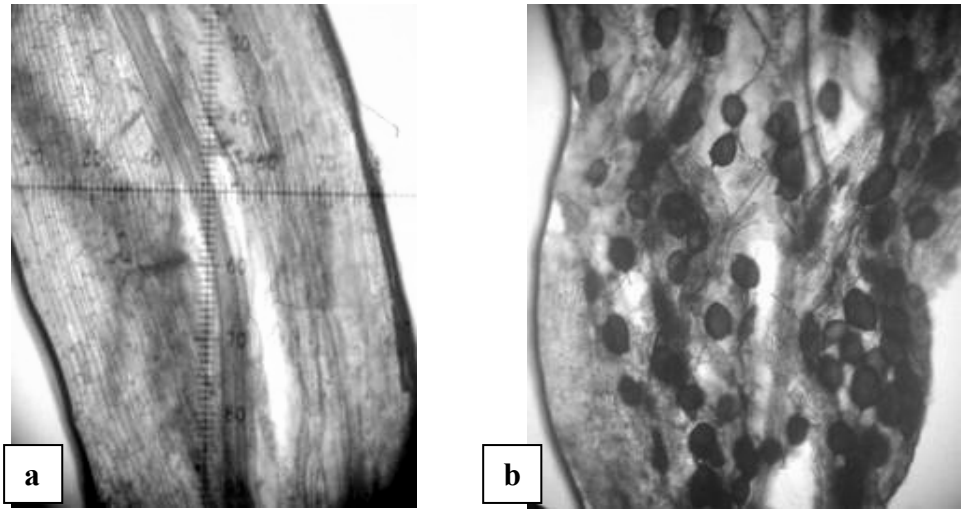

Fig. 2. a: *(-) non-mycorrhizal and* ***b:*** *(+) mycorrhizal root inoculation*

The effect of mycorrhizal application on total root length has been determined in order to show positive effects over the control plants. According to the shoot dry weights of cheery rootstocks, the highest shoot dry weights were determined by interactions of the variety+application+growth media. This rate was determined as 1.056 g by Gisela-5 control plants. Also, the type of GS-2 has been determined as the best media for shoot dry weights (Fig. 3).

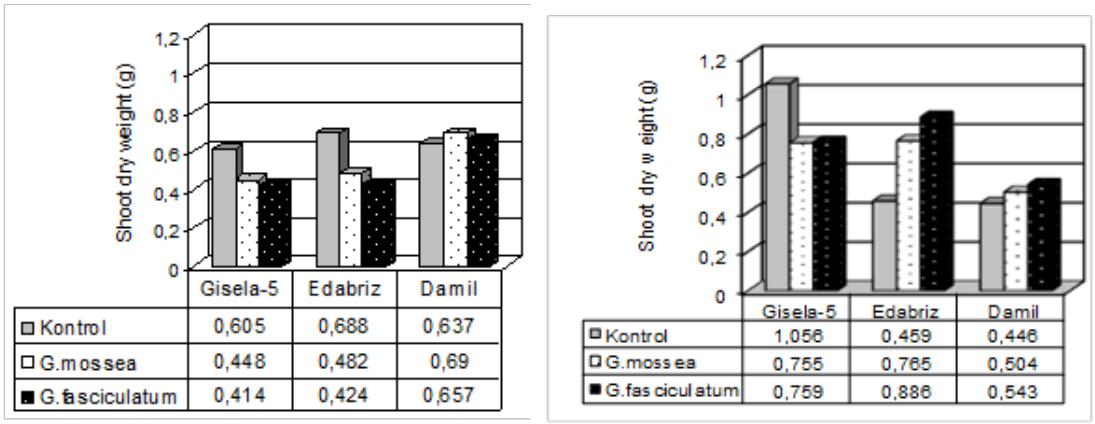

Fig. 3. *Shoot dry weights of Cheery rootstocks grown on GS-1 and GS-2 (Respectively)*

The highest root dry weights were determined by interactions of the variety + application+ growth media. This rate was determined as 0.88 g by Gisela-5 rootstocks. In generally for both growths medium non inoculated control plants produced high root dry weight. Also, the type of GS-2 has been determined as the best media for root dry weights (Fig. 4).

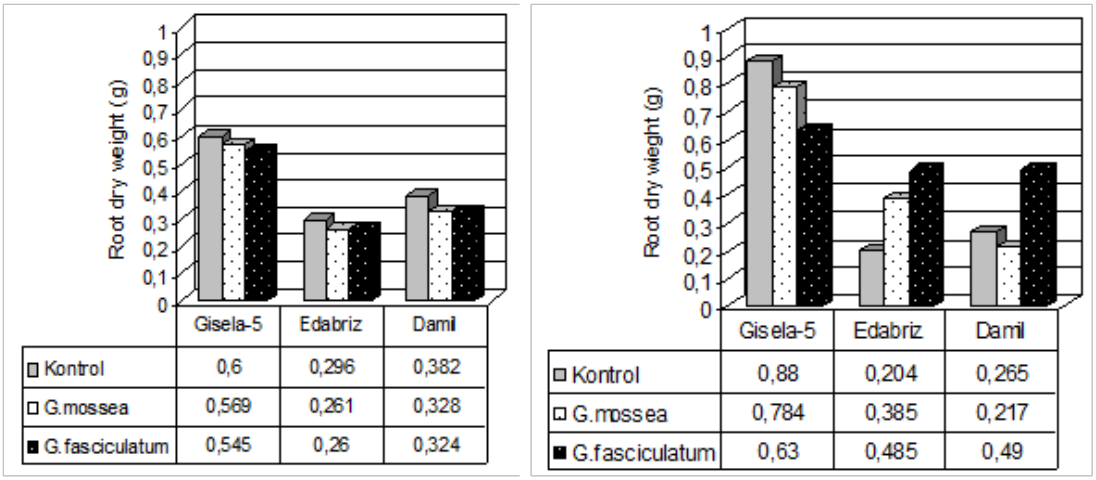

Fig. 4. *Root dry weights of Cheery rootstocks grown on GS-1 and GS-2 (Respectively)*

From these results it seems that the effects of isolates are different. Previously it has been reported that isolates of the fungi differ in their ability to benefit plants and this can be modified by both plant species and environmental condition [31]**.** Previously Hooker et al., [31] indicated that mycorrhizal inoculation usually resulting in root systems which are more branched and therefore likely to have a higher capacity for uptake of nutrient and water.

According to the leaf analysis, significant results have been obtained. The leaf content of P, Zn, Cu and Fe were tested and the highest iron (Fe) concentration was determined by interactions of the variety+application+growth media as 574.7 mg kg^{-1} with *Glomus fasciculatum* (Fig. 5).

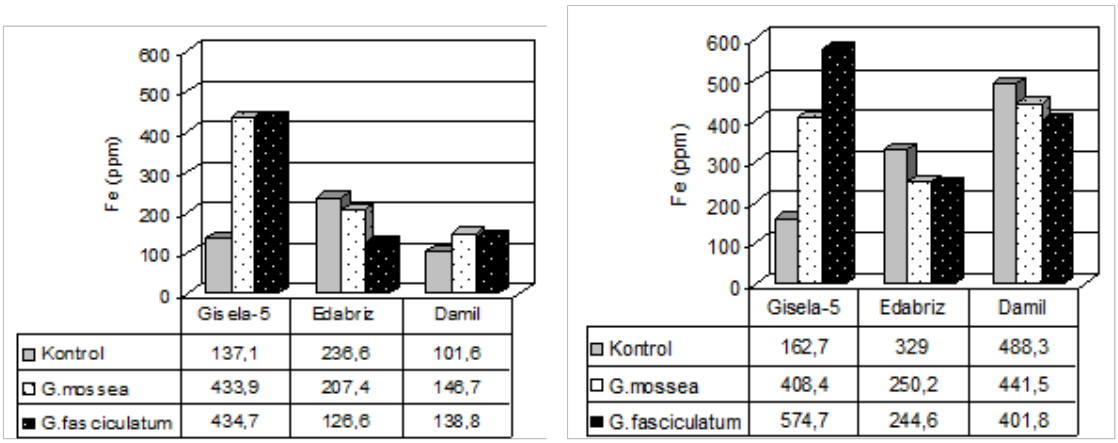

Fig. 5. *Cheery rootstocks Fe content (ppm) grown on GS-1 and GS-2 (Respectively)*

The leaf content of copper (Cu) has increased by mycorrhizal applications and the highest leaf content of Cu was obtained by interactions of the application + variety + growth media. This rate was determined as 22.71 mg kg^{-1} by Damil with *G. mosseae*. The type of GS-2 has been determined as the best media for leaf content of Cu (Fig. 6).

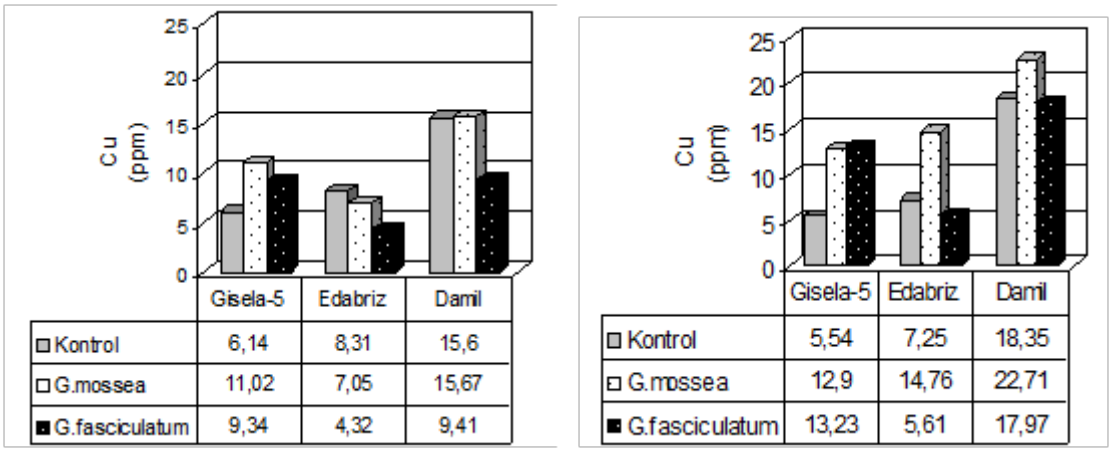

Fig. 6. *Cheery rootstocks Cu content (ppm) grown on GS-1 and GS-2 (Respectively)*

The leaf content of Zn has increased by mycorrhizal applications and the highest leaf content of Zn showed interactions of the variety+application+ growth media. G. *mosseae* more effective than *G. fasciculatum*. The primary effect of AM on their host plant is an increase in plant P and Zn uptake [32]. It seems that *G. mosseae* is the efficient inoculum for Gisela 5 and Edabriz rootstocks zinc uptake. This rate was 38.09 mg kg^{-1}. This result was obtained for the type of GS-1 (Fig. 7).

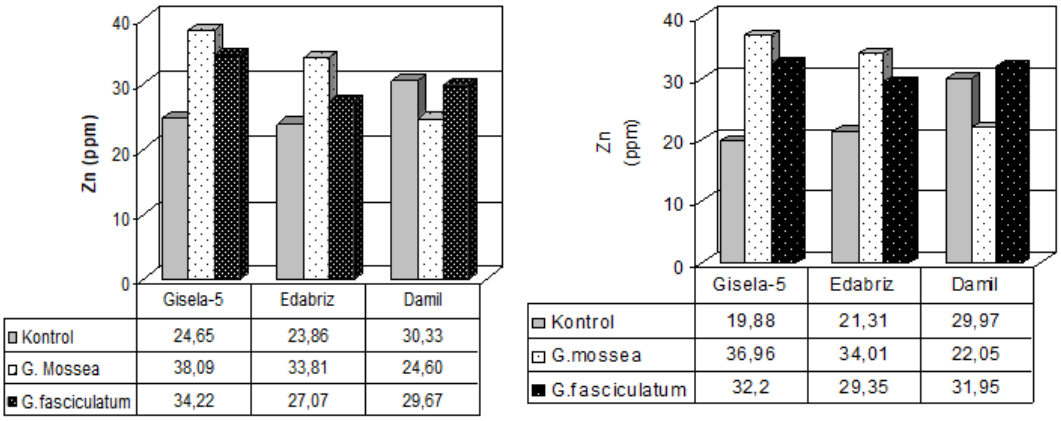

Fig. 7. *Cheery rootstocks Zn content grown on GS-1 and GS-2 (Respectively)*

Lastly, the effect of mycorrhizal applications on the leaf content of Phosphorus (P) has been showed to be double amount of concentration according to the control plants. From those varieties Edabriz showed the highest concentration. The other highest concentrations were determined on Gisela-5 and Damil. The type of GS-1 has been determined as the best media for leaf content of P. General appearance of P content is shown in *Fig. 8.* It seems that *G. fasciculatum* is the one of the efficient inoculums for P uptake. It has been reported the inoculation with AM in horticultural plants can improve growth by increasing the uptake of P, Zn and other minerals [33, 34]**.**

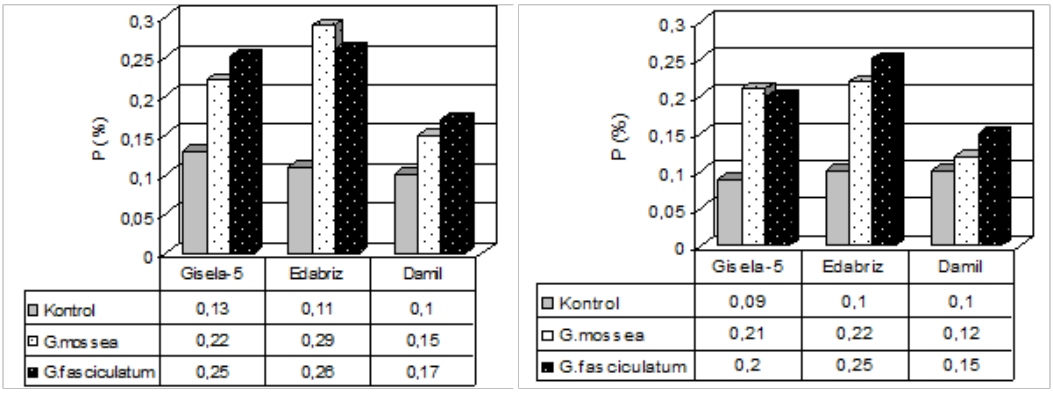

Fig. 8. *Cheery rootstocks P content (%) grown on GS-1 and GS-2 (Respectively)*

CONCLUSION

In recent years, the demands for micro propagated plants in the modern fruit cultivation have increased very much. Furthermore, before establishing of orchards, it is necessary to produce and use healthy plants, especially if the natural and non-chemical materials are not used for the healthy plant production, it can be very useful for the plant growth and human health. The results of this experiment indicated that micropropagated cherry rootstocks are significantly inoculated with mycorrhizal species under several growth media. It seems both inoculums are efficient mycorrhizal species for both cherry rootstocks and growth mediums. Results have shown that GS-2 has produced more dry weight and feed plant better than the GS-1. It was determined that the mycorrhizae affected sweet cherry rootstocks were very healthy and plant tissues are very rich in plant nutrients, and these results seemed to be in favour of the previous research. It is better to use early several mycorrhizae species with several levels of inoculum to find the best inoculum time and ratio of inoculum use for further research. The results are shown that AM species have significant effect on nutrient uptake of cherry rootstocks.

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