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MYCORRHIZAL INOCULATION OF ONION (*ALLIUM CEPA* L.) IN THE EARLY DEVELOPEMENT STAGES

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ABSTRACT
The onion (*Allium cepa*) is one of the most widely cultivated vegetables. As a food item, it is usually served cooked, as a vegetable or part of a prepared savoury dish, but can also be eaten raw or used to make pickles or chutneys.
Mycorrhiza fungi are special fungi, which live in connection with plant roots. These are symbiotic fungi which have three main types: arbuscular mycorrhiza (AM), ectomycorrhiza and ectendomycorrhiza. AM are probably the most widespread plant symbionts and are formed by 80–90% of land plant species. Crops inoculated with AM have higher yield and quality, and those plants react to stress factors better (e.g. drought, high temperature). Several *Allium* spp. responded with growth promotion on AM inoculation. According to studies AM fungi also has a positive effect on nutrient content.
The aim of our experiment was to find an appropriate method for inoculation of onion seedlings, and define the minimum time for the appearance of symbiosis under greenhouse conditions.
The experiment took place at Szent István University Faculty of Horticultural Science, at the experimental glasshouse of the Department of Vegetable and Mushroom growing, from 11th September 2017. We used Daytona F1 onion seeds, the media was Latagro KB2 type peat and two types of mycorrhizal products: MycoGrow and Aegis Irriga. The experiment was made with 12 treatments with 30-30 seeds per each treatment. During the experiment, chemically treated and non-treated seeds were used. The seedlings were sampled 5, 7 and 14 weeks after sowing, 5-5 seedlings from each treatment per time. Altogether 96 painted and prepared samples were checked, each with 3-3 roots with a Zeiss Axio Imager A2 microscope.
The results showed the appearance of mycorrhizal fungi in a few treatments. In our examination not only typical AM fungi were found, but also in a treatment we found hyphaes with big amount of septas.
The results show opportunity of artificially infecting transplants after a few weeks with mycorrhizal products. However, continuing the experiment is necessary with some modification of the factors, to make the procedure more effective.

Keywords: arbuscular mycorrhiza, onion, seedling, inoculation, colonization

INTRODUCTION

The onion (*Allium cepa* L.) is one of the most widely cultivated vegetables. Its close relatives are the garlic, shallot, leek, chive, which all belong to the *Alliaceae* family. Onions are cultivated and used around the world. As a food item, these are usually served cooked, as a vegetable or part of a prepared savoury dish, but can also be eaten raw or used to make pickles or chutneys (WEB1).
According to FAOSTAT (2018), worldwide, in the last 10 years there were ups and downs both in the harvested area and yields, but due to it, in the last 6 years it seems to stabilize. In Hungary, onion is also one of the most widely cultivated and used vegetable. In 2016, the harvested area was around 2000 ha-s, the yield was 32 t ha^{-1}, but with intensive technology some growers could reach 60-70 t ha^{-1} yield (LEDÓ ET AL., 2017)
Mycorrhiza fungi are special fungi, which live in connection with plants roots. These are symbiotic fungi which have three main types: arbuscular mycorrhiza, ectomycorrhiza and ectendomycorrhiza. Arbuscular mycorrhiza (AM) has aseptated hyphae, colonizes the roots intracellular, most arbuscular mycorrhizal fungi belong to the *Glomero* taxa.

Ectomycorrhiza has septated hyphae, colonizes the roots extracellular, most ectomycorrhiza fungi belong to the *Basidio* or *Asco* taxa. Ectendomycorrhiza has septated hyphae, colonises the root intracellular and the most ectendomycorrhiza fungi belong to the *Basidio* or *Asco* taxa (SMITH AND READ, 2008).

AM are probably the most widespread plant symbionts and are formed by 80–90% of land plant species. This includes numerous important horticultural crops among the *Solanaceae* (e.g. tomato, eggplant or petunia), the *Alliaceae* (e.g. onion, garlic and leek), fruit trees (e.g. grapevine, citrus spp.), ornamentals and herbal plants (e.g. basil, thyme, rosemary) (ROUPHAEL ET AL., 2015).

Crops inoculated with AM have higher yield and quality, and those plants react to stress factors better (e.g. drought, high temperature). Several *Allium* spp. responded with growth promotion on AM inoculation. Onion (*Allium cepa*) plants inoculated with AM, grown in pots with mineral soil had a higher biomass than non-inoculated plants and reached marketable size (>25 mm bulb diameter) 2-3 weeks earlier. This AM inoculation caused furthermore firmer bulb formation of onions. Mycorrhizal inoculation led to 22% increased yields of onions compared to non-inoculated controls (BAUM ET AL., 2015).

Inoculating horticultural crops with AM is becoming common practice, especially in intensive horticultural cropping systems. However, a high-quality inoculum is necessary for successful root colonization with AM (ROUPHAEL ET AL., 2015). According to HART AND FORSYTHE (2012), AM fungi also have a positive effect on nutrient content.

The aim of our experiment was to find an appropriate method for inoculation of onion seedlings, from treated and non-treated seeds and define the minimum time for the appearance of symbiosis under greenhouse conditions.

MATERIAL AND METHOD

The experiment took place at Szent István University Faculty of Horticultural Science, at the experimental glasshouse of the Department of Vegetable and Mushroom growing, 11th September 2017. We used Daytona F1 onion seeds, the medium was Latagro KB2 type peat. Two types of mykorrhizal products were used: MycoGrow and Aegis Irriga. The technical information of MycoGrow said that it could be given for the plant both irrigated and mixed in the media, while Aegis Irriga is just given with irrigation.

The experiment was made with 12 treatments with 30-30 seeds per each treatment. During the trial chemically treated and non-treated seeds were used. The *Table 1* shows the code of the treatments: if the seed is chemically treated or not, the method of the treatment (colonization) and the name of the product and the quantity.

The quantity of the products was calculated from the Technical information. In the case of MycoGrow we used 30 g m^{-2} ratio, while in the case of Aegis Irriga it was 10 g 100 m^{-2} ratio. Amounts of the mycorrhizal products for one tray were calculated from these ratios.

During the experiment, fertilizer and plant protection were not used. Seedlings were irrigated and supplementary lightings were used, when it was needed.

Temperature and humidity were measured during the experiment with a Voltcraft DL-181THP device. The maximum temperature was 28.85 °C, the minimum was 14.33 °C. The maximum humidity was 90.28%, the minimum was 59.94% during the experimental time. During the experimental time, the climate suited for the onion seedlings.

Table 1. Treatments used during the experiment

Treatment Code	Chemically treated +/-	Method of the treatment	Product name, quantity
A	+	mixed in the media	MycoGrow, 5.2 g
B	-	mixed in the media	MycoGrow 5.2 g
C	+	mixed in the media	MycoGrow 10.4 g
D	-	mixed in the media	MycoGrow 10.4 g
E	+	with irrigation	Aegis Irriga 0.017 g
F	-	with irrigation	Aegis Irriga 0.017 g
G	+	with irrigation	MycoGrow 5.2 g
H	-	with irrigation	MycoGrow 5.2 g
I	+	with irrigation	Two product mixed
J	-	with irrigation	Two product mixed
Control +	+	-	no treatment
Control -	-	-	no treatment

The seedlings were sampled 5, 7 and 14 weeks after sowing, 5-5 seedlings from each treatment per time. The first sampling was at 16th October 2017. The second sampling time was at 30th October 2017. The third sampling time was at 18th December 2017. Roots were washed and put in Falcon tubes, filled with 60% alcohol until further measurements.

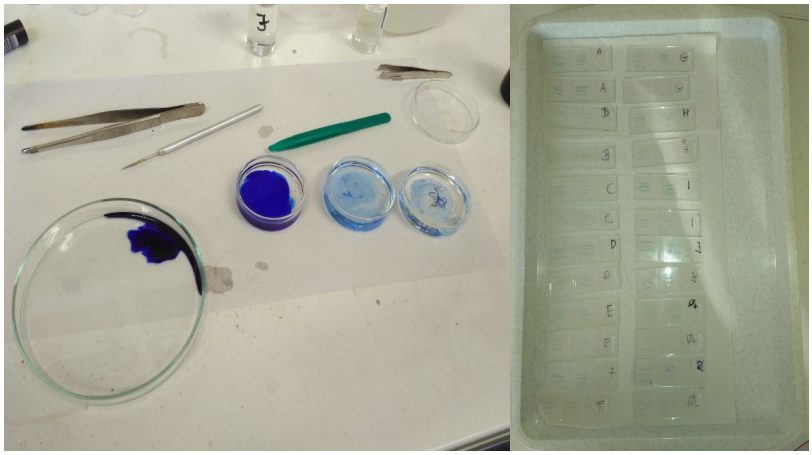

Figure 1. The method of painting; Dissections

To make mycorrhizal fungi visible, a special painting method was used. First, roots were cleaned from the remaining soil parts and from the alcohol with distilled water. Then roots were put in 10% (w/v) potassium-hidroxide and cooked in 60 °C for 60 minutes. Then potassium-hidroxide was washed away with distilled water and roots were put in lactic-acid for a night. The next day the roots were painted with aniline-blue. The roots were put in a bigger Petri-plate, paint was dropped on the roots and after a few seconds the

remaining paint was washed away (*Figure 1*). Then the roots were put in lactic-acid for a night, to properly wash away the unnecessary paint. This painting method can vary a little due to the roots thickness.
After painting, dissections were made from the roots. Aproximately, 1-1,5 cm long pieces from the roots were cut and put in a few drops of lactic-acid on a slide. Three roots were put under each coverslip (*Figure 1*). Altogether 96 painted and prepared samples were checked, each with 3-3 roots with a Zeiss Axio Imager A2 microscope.

RESULTS

The result of the mycorrhizal colonization could be seen in the microscopic pictures. In treatment „C", which was chemically treated, and mixed double dose of MycoGrow in the peat, a long hyphae can be seen. In the hyphae there are no septas (*Figure 2*). These hyphae are long enough, to say, at this treatment the colonization was successful.

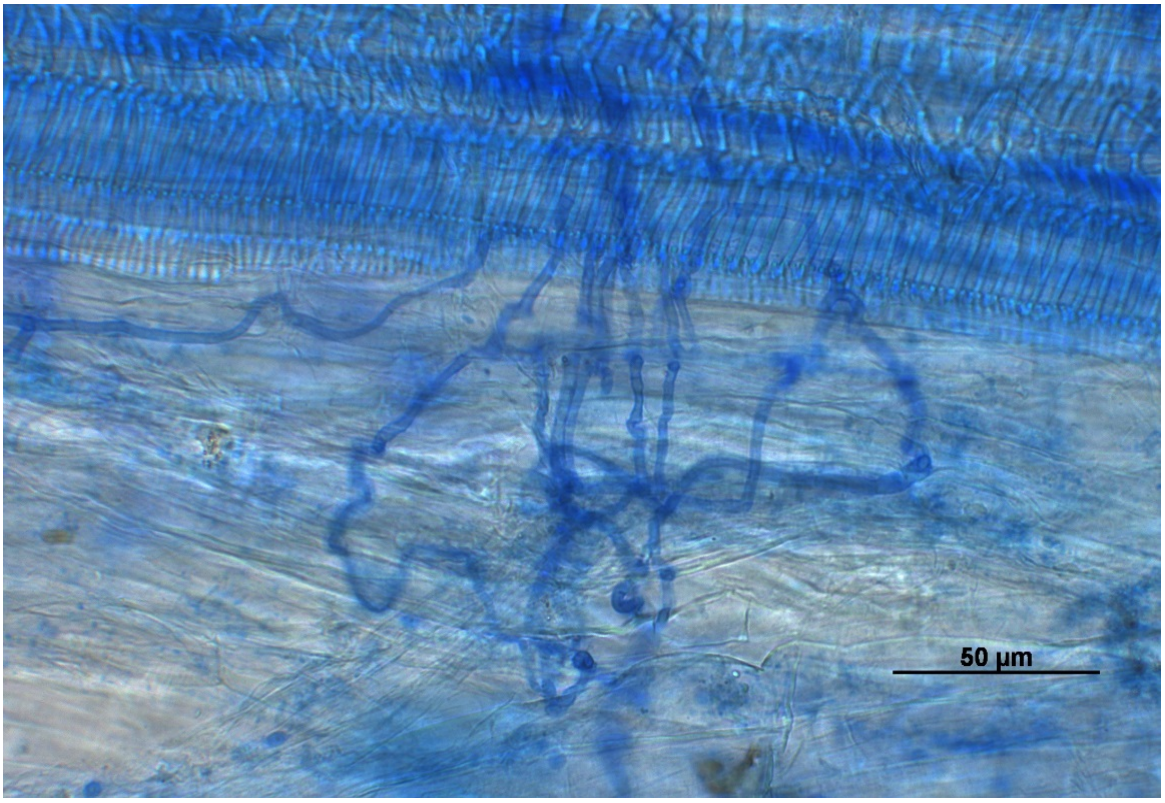

Figure 2. Treatment „C", 2. sampling

There are also mycorrhizal hyphae on *Figure 3* which was taken from Treatment „C" from the second sampling time. In this picture, septas in the hyphae could be seen (arrows), so this was not that type of mycorrhiza fungi we looked for, but its spore could be in the mycorrhizal product beside the other type of mycorrhiza fungi.
In other cases, we did not find any inoculated root from 96 examined roots. Also, there were no remarkable difference between treated and non-treated seeds. However, it is intersting to find an inoculated root in a treatment in which chemically treated seeds were used.

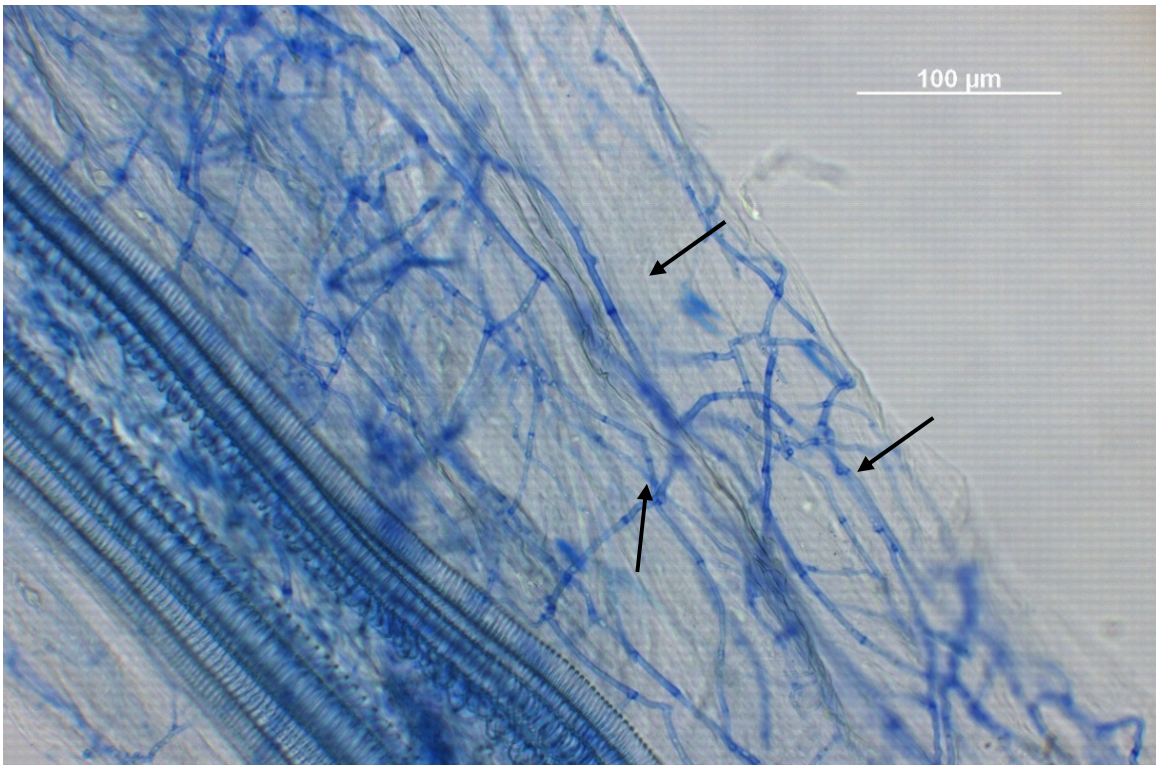

Figure 3. Treatment „C", 2. sampling

CONCLUSIONS

The results showed the appearance of mycorrhizal fungi in a few treatments after 7 weeks from sowing. In our examination not only typical AM fungi were found, but also in a treatment we found hyphae with big amount of septas. Symbiont activity could not be high in our experiment, however in other studies a higher value of colonization was recorded. For example, TAWARAYA ET AL. (2012) had a 94±3% (n=4) colonization on *Allium fistulosum* 58 days (approx. 8 weeks) after sowing. The experiment took place in Japan. There the medium was andosol, the fungal inoculum consisted not only spores, like in our experiment, but also extraradical hyphae and infected roots with *Glomus* R-10. Amount of the inoculum was 75 g kg^{-1}.

Results show opportunity of artificially infecting transplants after a few weeks with mycorrhizal products. However, continuing the experiment is necessary with some modification of some factors (like type of substrate, or used inoculation method), to make the procedure more effective.

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WEB1: https://en.wikipedia.org/wiki/Onion