Examinations of timing of AMF inoculations on micropropagated peach rootstock GF 677

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Examinations of timing of AMF inoculations on micropropagated peach rootstock GF 677 (Prunus persica X Prunus amygdalus)

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Abstract

Seven selected, home-isolated strains of arbuscular mycorrhizal fungi (AMF) were used for the artificial inculation of micropropagated peach rootstock GF 677 in two types of substrates (standardised nutrient-rich Pindstrup 1 media, and soil:zeolite = 3 : 1 mixture with a relatively low nutrient level). Survival and growth of the "ex vitro" plantlets and their later development were studied, using two different methods of inoculation:

i. Sterile plantlets were acclimatised and inoculated in one step in an AMF - containing (3 %) substrate (non acclimatized plants were inoculated).

ii. Plantlets were grown in Jiffy 7 containers during the acclimation of about 6 weeks preceding the inoculation trials (acclimatized plants were inoculated). It was noted that under nutrient stressed conditions the influence of AMF inoculation was more pronounced then under nutrient-rich condition. Mycorrhizal colonisation resulted in a lower growth rate in both substrates, when inoculation was carried out at the same time, then the transplantation (during the acclimation) from steril to greenhouse conditions. Some months later, however, a growth stimulating effect could be detected with some home-isolated endomycorrhizal strains. A better functioning arbuscularity of mycorrhization was found, when plantlets were inoculated after the acclimation phase.

Keywords: Endomycorrhizal fungi; inoculation methods; micropropagation; Jiffy 7 containers; rootstocks; substrates

1. Introduction

Micropropagation is a widely used method of mass production of several plant species, but it is especially important in clonal multiplication of fruit rootstocks and scion varieties as well. One of the most difficult problems during the procedure is the weaning stage, when plants are transplanted from sterile to semi-sterile glasshouse conditions. Transplantation stress can be a reason of serious losses (Kornova 1995).

Vesicular arbuscular micorrhizal fungi (AMF) are beneficial microbes to almost 90 percent of the higher plants, improving water and mineral nutrient uptake (Berta et al. 1990, Al-Karaki and Al-Raddad 1997), protection against biotic and abiotic stress (Biro et al. 1993) and certain pathogens (Gianinazzi et al. 1990, Cordier et al. 1996). Results of the beneficial effect of AMF inoculation on the salt and acid stress was also reported (Kung,U. J. B. 1995, Gupta and Krishnamurthy 1996). Inoculation, therefore with these selected microsymbionts may improve the survival rate and growth vigour of micropropagated plants (Calvet et al. 1989, Guillemin et al. 1991, Gianinazzi & Rancillac 1992, Uosukainen & Vestberg 1994). Fortuna et al. (1992) mentioned a two-week shorter acclimatisation time of micropropagated and AMF inoculated plum rootstock in comparison to the micropropagated and non-mycorrhizal ones.

Several self-isolated AMF have been tested for their infection frequency on white clover. They had different effects on survival as well as on growth of micropropagated peach plants in two substrates during the acclimatisation phase. The aim of this work was to examine the influence on growth of micropropagated peach GF 677 inoculated with endomycorrhizal strains in two different physiological stage of the plants in a nutrient rich and a poor substrate.

2. Materials and methods

GF 677 (Prunus persica X Prunus amygdalus) is a worldwide used rootstock for peach nursery trees, propagated in vitro in Hungary in large quantities. As Hungarian climate is much more extreme, then that of western Europe, inoculation in proper time with a proper AMF line can help to survive the hot and dry summer and the cold winter period.

Healthy shoot-tips of GF 677 peach rootstock were collected in the Research Station of the Research Institute for Fruitgrowing and Ornamentals, Budapest, Hungary. Actively growing 15-20 mm long shoottips were desinfected and initiated on MS (Murashige and Skoog 1962) medium supplemented with vitamins of Navatel (1980) and an auxin level of 1 ppm. GF 677 buds were transferred to a propagation medium, with salts lowered to one quarter of MS and the Cl^- ions omitted. 6-benzylaminopurine and adenin hemisulfate were added in a dosage of 0.75 mg/l respectively. Shoot cultures of GF 677 have to be elongated partly under cooled and dark conditions before rooting. The elongation takes place on the same medium as the initiation, which is followed by rooting on very low auxin concentration (Vértesy and Balla 1996).

The rooted plantlets were transferred to glasshouse with high relative humidity. Two types of substrates, with a different nutrition level were tested for mycorrhizal inoculation on non acclimatized and on acclimatized plants:

i/ chernozem brown forest soil : zeolite - 3 : 1,

ii/ Pindstrup 1 (Pindstrup Moserbug A/S DK 8550 Ryomgard, Danmark), as a commonly used nutrient-rich standardised mixture. The non inoculated plantlets were transplanted for acclimatization at the beginning of the rooting into Jiffy 7 containers. They were acclimatized in this containers, and about 6 weeks later, together with the containers were transferred into the two different, inoculum containing substrates.

Plants were growing in this mixtures about three months and after that transplanted into commercial substrate for stonefruit species.

Intact spores of vesicular arbuscular mycorrhizal fungi have been collected by the wet sieving method of Gerdemann and Nicolson (1963). Desinfected spores were used for the artificial inoculation of the white clover plants in four types of steam sterilized soil. After colonisation assesment (frequency of AM fungi - F %, arbusculum content - a %) by trypan blue method (Kormanik et al. 1980) and the estimation of green mass production, the best seven strains were selected for further examinations on peach plantlets. The list of the strains used in this study is seen in Table 1.

Soil, containing infected roots was used for experimental mycorrhization, added as a 3 percent amount in a thin layer beneath the roots at the time of transplantation. The inoculated plantlets were transferred to outdoor containers under shade. Growth (plant height) and micorrhizal colonization were assesed after the acclimatisation phase and six months after the transplantation.

3. Results

3.1. Effect of AMF inoculation on the growth of non acclimatized peach rootstocks as a function of the two substrates

In Table 2 the effect of AMF inoculation is shown on the plant height at the end of the acclimation and after 6 months of growing period, when plants were grown in separated containers outdoor under shadow.

Growth of the plants was highly determined by the substrate used.

In the nutrient-rich standard Pindstrup 1 mixture, the growth of non-inoculated control plants was found to be much better, suggesting the hypothesis, that all of the essential substances for growth can be found in Pindstrup 1 for a certain growing period (Table

2). The presence and infection of AMF has retarded the growth of the plantlets at the very beginning of the acclimation phase. Following the growing period, five of the seven AMF lines effected better growth as the controll, and in four cases the difference became significant by the end of the season. (Table 2).

The soil : zeolite mixture as a less nutrient-rich substrate resulted a lower growth of the peach plants, especially of the control ones. In that case six of the seven strains had growth stimulating effect also during the acclimation too, compared to the non inoculated control plants in the same mixture. Among the AMF strains only three proved to be significantly beneficial for the later growth.

3.2. Effect of AMF strains on the growth of peach plantlets, as a function of the two inoculation methods

Two methods of inoculation were studied on the peach GF 677 plants in this report. Table 3 shows the growth of peach plantlets inoculated with the selected self-isolated AM fungi at different developing periods of the plantlets.

The time of inoculation had a significant effect on the later growth of peach plants. The height of the one-year old plants was inoculated with strain 109 on pindstrup 1 substrate was the double, and almost the double with strain 108 on soil:zeolite mixture than that of the uninoculated controlls, when the inoculation followed the acclimation phase. When the inoculation was carried out on non acclimatized plantlets, it retarded their later development. It can be supposed, that the small plantlets coming out of the sterile conditions meet too much stress effect at the same time, and they are not able to tolerate it for a long time. In that case there are no differences between the two substrates.

Significant growth stimulating effect of AM strains has been observed on both substrate when the acclimatized plants were inoculated. This beneficial effect lasted all over the later periods. Among the 7 strains 2 were appeared to be beneficial on both substrates, in case of the Jiffy used inoculation method.

Comparing the control plants of the two substrates, it can be ascertained, that after the first growing year there are no detectable differences caused by different nutrition content of the substrate. Thus the better growth of rootsocks is the influence of the certain AMF line, used for inoculation.

3.3. Colonization of AM fungi on peach plantlets as a function of the substrates and inoculation methods

As there was not a real correlation detected between the AMF freqency data (F%) and the two different inoculation times and substrates only the arbuscularity is shown in the Table 4. The arbusculum content of the infected parts in the roots (a%) refer to the functionality of the plant /microsymbiont interaction, the success of the inoculation. It was controlled in two different times:

i/ Three months after the artificial inoculation, when the plants were transplanted into normal substrate, used in the nursery for stonefruits, without any sterilisation, into outdor containers.

ii/ One year old plants were examined.

The control plants, which were practically completly sterile at the time of transplantation into the stonefruit substrate became inoculated spontaneously, since this substrate contains AMF strains and the GF 677 rootstock variety has suspectibility to this type of infection. Among the colonization capacity of the seven selected strains there were big differences depending on the used mixture and on the time of the inoculation.

In case of Pindstrup 1, there was perfect harmony with the growth data (Table 2.,3.) The colonization was not very quick, but by the end of the first growing year the arbusculum content of the roots was very similar to the soil : zeolit substrate. It is especially true, when acclimatized plants were inoculated. The inoculated non acclimatised plants in the good structural substrate Pindstrup1could find all the needed nutrients, moisture and air for their survival and growth, in contrary to the soil : zeolit mixture. So that this plants were not looking for any connection with AMF strains the substrate contained. The same tendency was observed, when the inoculation was carried out on acclimatized plants, but on higher inoculation level. According to the functionality of the AM fungi it was strightly more efficient when the plantlets were inoculated after the acclimation grown in Jiffy containers.

Comparing the colonizations datas of the three months old plantlets no arbusculum were found in case of three strains, wich developed later (Table 4). In contrast a positive effect on the growth of these plants were measured (Table 2). Probable during the metabolism of the plants and AMF they become in connection earlier, then the really symbioses chould be detected, but more examinations are neccesary about it.

Among the seven examined strains only one (strain 109) turned out to have positive effect in all of investigated treatments.

4. Discussion and Conclusions

The present results confirm that mycorrhizal inoculation by different AM fungi is effective in promoting plant growth and development of micropropagated GF 677 peach rootstocks. Results also suggest the importance of AMF colonisation of peach micro-plants in case of the stress-conditions of low nutrient at the transplantation stage. The efficiency depends, however strongly on the strain origin (Balla et al. 1998), on the substrate quality and the physiological stage of the plants at the time of inoculation, and on the method used and presented here.

The screening of different AM fungi, for their effect on the survival and growth indicated however specificity of the isolates, suggesting the necessity to select AM fungal isolates and also to test them possibly among various environmental conditions, as stated also by Powell (1982).

Although mycorrhizal inoculation had not always a positive effect on the survival and growth of plantlets at the very beginning of acclimation (Balla et al. 1988.), but it improved their further development, mainly in case of the nutrient poor soil : zeolite mixture. Regarding to the nutrient content of the substrates the same result were presented by Vidal et al. (1992), suggesting the importance of potting mix and AM fungus if maximum benefits were to be gained after transplanting the plantlets into the greenhouse.

Levels of infection observed with trypan blue staining were not found to be in a positive correlation with plant growth, when inoculating non acclimatized plants, similarly to the results of Fortuna et al (1992). When the inoculation was evaluated after the weaning stage, a positive correlation was found between the growth (height) of peach plantlets and the functioning of the inoculated AMF strains.

Our results suggest, that the early and also the later periods of endomycorrhizal inoculation using the proper AMF strain can enhance growth of micropropagated peach microplants at the weaning stage and also at the later periods. (See the effect of strain 109)

A higher plant growth stimulating effect was measured in the nutrient poor substrate, where colonisation with AM fungi showed a necessery help for better nutrient supply. After 6 months an improved growth could develope with some AMF local isolates of peach origin. The starter effect of a better nutrient supply (standard Pindstrup 1 media) has lasted all along the growing period, suggesting the importance of plant nutrient supply in the plant health and fitness.

Endomycorrhizal inoculation with selected strains in proper time can improve the development of trees under stressed conditions, but further investigations are neccesary on the selection of strains, on the inoculation of the combination of the strains and on the survival capacity of the inoculated strains emong the native microorganisms.

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References

Kornova, K. 1995. Investigation of in vitro rooting of some peach varieties. Plant Science. 32: 109-111

Berta, G., Fusconi, A., Trotta, A., Scannerini, S. 1990. Morphogenetic modifications induced by the mycorrhizal fungus Glomus strain E 3 in the root system of Allium porrum L. New Phytol. 114: 207-215.

Al-Karaki G.N., Al-Raddad A. 1997. Effects of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of two wheat genotypes in drought resistance. Mycorrhiza. 7: 83-88.

Biro, B., Voros, I., Koves-Péchy, K., Szegi, J. 1993 Synergistic effect of microsymbionts (Rhizobium and AM fungi) on recultivated mine spoils. Geomicrobiol. J. 11,279-286.

Gianinazzi, S., Trouvelot, A., Gianinazzi-Person, V. 1990. Role and use of mycorrhizas in horticultural crop production. In: 23th IHC Plenary Lectures. Int. Soc. Hort.

Sci., Florence, Italy, 25-30.

Cordier, C., Trouvelot, A., Gianinazzi, S., Gianinazzi-Pearson, V. 1996. Arbuscular mycorrhiza technology applied to micropropagated Prunus avium and to protection against Phytophthora cinnamomi. Agronomie. 16: 679-688.

Kung'U. J. B. 1995. Effect of VA-mycorrhiza inoculation on growth performance, coppicing ability and drought resistance of two agroforestry tree species. Thesis (Ph.D. UPLB-Coll. of Forestry Library, College, Laguna (Philippines).

Gupta R., Krishnamurthy K. V. 1996. Response of mycorrhizal and nonmycorrhizal Arachis hypogaea to NaCl and acid stress. Mycorrhiza. 6: 145-149.

Calvet, C., Pera, J., Estaun, V., Camprubi, A. 1989. Vesicular-arbuscular mycorrhizae of kiwifruit in an agricultural soil inoculation of seedlings and hardwood cuttings with Glomus mossae. Agronomie, 9: 181-185

Guillemin, J.P., Gianinazzi, S., Gianinazzi-Pearson, V., 1991. L'endo-mycorrhization de vitroplants d'Ananas comosus:mise en évidence d un effet mycorrhizien. Fruits 46:355-358.

Gianinazzi, S. and Rancillac, M., (eds.)1992.:COST 821Congress on Micropropagation and Endomycorrhizae, Dijon, France 21-23 May 1992. Special issue, Agronomie 12: 741-924.

Uosukainen, M., and Vestberg, M., 1994. The effect of growth substrate and fertiliser on the growth and vesicular-arbuscular mycorrhizal infection of three hosts. Special issue, Agricultural Sci. in Finland. 3:225-314.

Fortuna, P., Citernesi, S., Morini, S., Giovanetti, M., Loreti, F. 1992. Infectivity and effectiveness of different species of arbuscular mycorrhizal fungi in micro-propagated plants of Mr 215 rootstock. Agronomie 10: 825-829.

Murashige, T. and Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-497.

Navatel, J.C., 1980. Production de l'amandier-pecher INRA GF 677 porte-greffe du pecher par multiplication in vitro. C.T.I.F.L./S.A.P. Centre de Balandran: 1-10.

Vértesy, J. and Balla, I., 1996.: Micro-propagation of some peach rootstocks and varieties. The First Egyptian-Hungarian Horticultural Conference, Kafr El-Shikh; Egypt, Book of Abstracts: 34.

Gerdemann, J.W., Nicolson, T.H. 1963. Spore of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46: 235-244.

Kormanik, P.P., Bryan, C.W. and Schultz, R.C. 1980. Procedures and equipment for staining large numbers of plant root samples for endomycorrhizal assay. Can. J. Microbiol. 26: 536-538.

Balla, I., Vertesy, J., Biro, B., Voros, I. 1998. Survival and growth of micropropagated peach inoculated with endomycorrhizal strains. Poc. IS on Replant Problems Eds. Utkhede, R., Veghelyi, K. Acta Hort. 477. ISHS 115-121.

Powell, C.L., 1982. Selection of efficient VA mycorrhizal fungi. Plant Soil. 68:3-9.

Vidal, MT., Azcon-Aguilar,C., Barea, J.M., and Pliego-Alfaro, F. 1992. Mycorrhizal inoculation enhances growth and development of micropropagated plants of avocado. Hort. Sci. 27:785-787.

Table 1

Code and origin of the AMF strains investigated in this study

Lab code	Species	Host 1	Host 2	Origine
105	Glomus sp.	Peach	White clover	Érd+
106	Glomus intraradicis	No information	Red clover	Érd
107	Glomus fasciculatum	Peach	White clover	Érd
108	Glomus sp.	Peach	White clover	Érd
109	Glomus sp.	Peach	White clover	Érd
110	Glomus sp.	Peach	White clover	Érd
111	Glomus sp.	Cherry	White clover	Érd

+ Chernozem brown forest soil

Table 2.

Effect of AMF inoculation on the growth of the peach rootstocks measured after 6 weeks or 6 months of inoculation in two different substrates

	Plant height (cm), substrates and growth periods			
AMF	Pindstrup		Soil : zeolit = 3 : 1	
Strains	6 week-old plants	6months-old plants	6 week-old plants	6 months-oldplants
Control	14.2	26.0	2.36	15.22
105	6.35	**33.7**	2.15	14.25
106	8.79	15.8	**4.81**	**19.54**
107	5.48	16.0	**5.75**	**17.36**
108	2.65	**34.6**	2.83	6.53
109	3.48	**32.8**	**3.35**	**18.32**
110	4.10	27.2	**4.05**	14.35
111	4.28	**32.4**	**4.28**	12.70
Mean +	5.01	27.5	3.88	14.72

+ Out of control

Table 3.

Effect of AMF inoculation on the height of the one-year old peach plants as a function of the substrates and the inoculation methods (non acclimated or acclimated plants)

	Plant height (cm), substrates and inoculation times			
AMF	Pindstrup 1		Soil : zeolit = 3 : 1	
Strains	Non acclimated	Acclimated	Non acclimated	Acclimated
Control	39.3	**43.7**	35.2	**49.3**
105	46.6	34.6	29.9	n. d.
106	32.8	31.9	35.0	40.5
107	44.5	**50.2**	38.2	38.9
108	49.7	49.0	17.3	**74.0**
109	44.3	**81.5**	28.6	**59.2**
110	37.6	**56.4**	21.0	**39.6**
111	44.5	**61.6**	26.4	**44.6**
Mean	42.4	**51.1**	28.9	**49.4**

Table 4.

Colonisation of AM fungi (a %) on GF 677 peach plantlets inoculated before or after the acclimation in pindstrup or soil:zeolit substrate (tested on 3 month and 1-year old plantlets).

AMF Strains	AM colonization (a%), substrates and inoculation times							
	Pindstrup 1				Soil : zeolit = 3 : 1			
	Before acclimation		After acclimation		Before acclimation		After acclimation	
	3 months	1 year	3 months	1 year	3 months	1 year	3 months	1 year
Control	0	5.3	0	32.8	0	37.8	0	25.1
105	0	24.3	30.4	48.5	2.5	14.5	n.d.	n. d.
106	n.d.	6.8	48.3	61.6	n.d.	28.8	40.9	41.6
107	3.4	15.3	23.5	49.0	9.9	46.7	15.1	44.1
108	1.0	39.8	16.3	50.6	4.3	17.8	**40.2**	**72.5**
109	**7.2**	**21.0**	**51.1**	**70.8**	**8.3**	**54.4**	**42.4**	**82.3**
110	0.4	4.2	0	45.9	0	13.3	0	**79.3**
111	n.d.	4.9	0	58.7	n.d.	31.3	0	56.8
Mean	2.4	15.2	24.2	52.2	3.6	30.5	23.1	51.1

n.d. - not determined

a % - arbusculum content in the AMF infected parts of the roots