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Inoculation with Mycorrhizal Fungi – a Feasible Biotechnology for Horticulture

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Summary

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Series of experiments focused on the inoculation of horticulture crop with arbuscular and ericoid mycorrhizal fungi proved that the mycorrhization could be a feasible biotechnology to improve plant growth and health status of several horticulture crop species: Cyclamen, Verbena and Rhododendrons when appropriate strains of mycorrhizal fungi are inoculated into the peat based media.

Introduction

Horticultural crop and flowers have been used as the host plants in several experimental tests for application of arbuscular mycorrhizal fungi (AMF) or ericoid mycorrhizal fungi (EMF) (CHANG 1994, STRIBLEY & READ 1974). Mycorrhizal fungi can stimulate plant growth especially in the soils with lower fertility and the positive effect of mycorrhiza on plants mainly due to improved phosphorus uptake has been documented (SMITH & READ 1997). AMF and EMF enable their host plant to tolerate environmental extremes such as nitrogen and phosphorus deficiency, drought, low pH, soil pollution, negative effects of some

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root pathogens etc. (SYLVIA & WILLIAMS 1992, MITCHELL & READ 1981). Since in most soils the indigenous populations of mycorrhizal fungi are present, the preinoculation of seedlings in inert substrates without native mycorrhizal symbionts give the introduced fungal strain the spatial advantage over the indigenous fungi which colonize the roots during cultivation period or after transplanting of plants into the field soil (POWELL 1984). In soilless substrates lacking the indigenous mycorrhiza or under the conditions where field soils are fumigated and most of the indigenous mycorrhizal fungi are eliminated, mycorrhizal inoculation is often successful and it can increase crop uniformity and reduce transplant mortality (VOSÁTKA 1995). The plants grown from cuttings or small seedlings can be inoculated with pure strains of effective mycorrhizal fungi with rapid colonization rates, which allows a successful colonization of newly formed roots after transplanting. Four experiments were performed to find the effects of inoculation with AMF on the growth of *Cyclamen persicum*, *Euphorbia pulcherrima*, *Verbena* sp. and inoculation with EMF on the growth of *Rhododendron* sp. in horticulture practice.

Materials and Methods

Experiment 1

Seeds of cyclamen (*Cyclamen persicum* var. 'Rosa mit Auge') were sown into the plastic boxes 30 x 20 x 10 cm filled with peat based media. Substrate in mycorrhizal treatment was inoculated with AMF inoculum containing spores, mycelium and colonized root fragments of three pure AMF cultures: *Glomus fistulosum*, isolate BEG23 from the Bank of European Glomales, G. mosseae BEG91 and *G. intraradices* BEG93. All cultures have been maintained separately on maize grown in Vermiculite-sand mixture (5:1) for 4 months in the greenhouse. The inoculum of three AMF cultures was mixed before use and 0.1 l of inoculum was applied per 10 l of substrate. Seedlings were transplanted after 10 weeks to the plastic pots (12 cm in diameter, volume 1.2 l) filled with a peat-montmorillonite clay (5:1) substrate. The experiment involved two treatments: uninoculated plants and plants inoculated with AMF. Each treatment consisted of 5 replicate blocks with 12 plants in each block. Replicates were randomly designed in the temperated greenhouse and plants were grown for 6 months. Plants were watered daily with tap water and fertilised weekly with 0.05 % solution of Kristalon Blue fertiliser. After cultivation all plants were subjected to a non-destructive measurement, where the following parameters have been estimated: plant mortality, plant height, width, number of leaves and number of flowers. Three plants from each replicate block were harvested and number and total area of leaves, number of flowers and buds, dry weight of leaves and flowers were measured. The root samples were stained according to PHILLIPS & HAYMAN 1970 and mycorrhizal colonization was determined by grid-line intersect method (GIOVANNETTI & MOSSE 1980).

Experiment 2

Cuttings of poinsettia *Euphorbia pulcherrima* var. 'Peter Star' were planted to the same substrate in the same treatments as in experiment 1 and cultivated under the same design and conditions. Number and total length of stems, number and total area of leaves and bracteas, dry weight of stems and leaves and bracteas were evaluated after 6 months of growth. The mycorrhizal colonization of the roots was evaluated as in experiment 1.

Experiment 3

Six weeks old cuttings of *Verbena* sp. Temari var. Pink were planted to the same substrate in the same treatments as in experiment 1 and cultivated in multiple trays with one compartment of 70 cm^3. There were 5 replication blocks with 20 plants in each block. After one month rooted cuttings were transplanted to 200 cm^3 pots and cultivated one more month in greenhouse under the same design and conditions as in experiment 1. Total length of stems and number of stems, shoot dry weight, leaf area, number of flowers and mycorrhizal colonization were evaluated as in experiment 1.

Experiment 4

Post-vitro rooted seedlings of *Rhododendron* sp., cv. Belle Heller were transplanted to the autoclaved peat media Lignocel. The effects of different EMF isolates, drought and liming on the growth of the host plants were investigated. The substrate was filled into the 4 liters plastic cultivation boxes and fertilised with Osmocote (No 5-6), 12 g per 4 l of substrate. 15 plants were grown in each box. EMF were grown on 6% malt extract (pH 4.5) liquid medium for 3 weeks. EMF were inoculated as 1 ml suspension of mycelia per plant (containing about 1.3 mg of dry weight of mycelia) pipetted bellow the plant roots. Four EMF isolates were used: *Oidiodendron* sp. (Oid1) isolated from the roots of Vaccinium myrtillus, Jeseníky Mts., CZ, O. sp. (Oid2) isolated from 40 years old *Rhododendron* sp., Pruhonice, CZ; Dark sterile mycelium - DSM (1) from *Loisaleuria procumbens*, Ukraine and DSM (2) from *Vaccinium myrtillus*, Jeseníky Mts., CZ. Liming stress was simulated by amendment of powdered limestone - 40 g of per 1l of the substrate. Drought stress was simulated by watering of treated plants only after decrease of substrate water status down to a level when plant wilting started. Plant vitality was estimated by counting of dead and alive plants (categorical data) and also by evaluation of plant health status according to a scale 0- 4 (0 - dead, 4 - healthy) - continual distribution. Membranes for estimation of EMF mycelium length in the substrate (Pragopor, ∅45mm, pore size 0.4μm) were inserted to the substrate for the last 6 weeks. Total length of mycelium attached to the membrane was estimated under the microscope (magnification 320x) after staining with 0.1% cotton blue in lactoglycerol by gridline intersect method. One intersect represented 36 μm, 10 fields of the area of 0.25mm^2 were measured on each membrane.

Data analysis

All data were tested for normality and analysed by the multiway ANOVA and by Duncan's Multiple Range test ($p<0.05$). The non-normal distributed data were transformed logarithmically. Non-parametric analysis (Kruskal-Wallis test and Connover test) of the non-normal distributed data was used. Mortality data were evaluated by G-test, incl. Williams's correction.

Results and Discussion

The inoculation with a mixture of three AMF isolates decreased substantially mortality of *Cyclamen* and increased the production of buds and total leaf area (Table 1). No substantial effects were found on any parameter of *Euphorbia* except increase in bracteas number (data not shown). Significant increase of flowering was found for inoculated *Verbena* plants, their leaf area, number of stems, total length of stems and dry biomass of plants (Table 2). No detrimental effects of AMF inoculation has been found in any treatments what favours practical applications and confirms results of most practical tests in horticulture (NEMEC 1987). Low specificity of AMF to host plant seems to be of a great advantage, however, chemical properties of cultivation substrate can influence development and efficiency of introduced symbionts. Particular care

should be paid to quality and purity of inocula at its preparation since AMF cannot be grown axenically and therefore there is a danger of contaminants e.g. root pathogens transfer from open pot cultures commonly used for inoculum production to inoculated substrate. As the experiment with *Euphorbia* has shown, there could be the cases where no significant effects of inoculation is found and therefore as suggested by CHANG 1994 the combination of crop-AMF should be tested for particular cultivation system before each large scale application.

Table 1. The effect of inoculation with arbuscular mycorrhizal fungi on mortality and growth of *Cyclamen persicum* plants (Experiment 1).

Treatment	Mortality	No of buds	No of flowers	No of leaves	Leaf area (cm^2)	Leaves DW (g)	Flowers DW (g)
Uninoculated	33.3 a	20.3 b	14.0 a	59.1 a	2477 b	20.0 a	5.7 a
Inoculated	16.3 b	27.5 a	14.8 a	64.7 a	2750 a	22.0 a	6.3 a

Means followed by the same letter are not significantly different within one column according to Duncan's Multiple Range test, $p<0.05$.

Table 2. The effect of inoculation with arbuscular mycorrhizal fungi on growth of *Verbena* sp. plants (Experiment 3).

Treatment	No of flowers	Leaf area (cm^2)	No of stems	Stems length (cm)	Shoot DW (g)
Uninoculated	0.07 b	234 b	4.6 b	84.1 b	2.0 b
Inoculated	0.82 a	275 a	5.6 a	108.4 a	2.4 a

Means followed by the same letter are not significantly different within one column according to Duncan's Multiple Range test, $p<0.05$.

The screening of EMF for their effects on the growth of *Rhododendron* (not all data shown) showed a high specificity of the symbionts since only 3 out of 12 isolates stimulated plant growth compared to uninoculated control, while the other had a neutral or slightly negative effects. Opposite to that, no strict host specificity was found for these fungi (XIAO & BERCH 1995), but different growth responses of EMF isolates were found for various host plants (POWELL 1982). The growth stimulations can be found for plants inoculated with some EMF (Table 3) what agrees with findigs of PEARSON & READ 1973. However, no changes in mortality of *Rhododendron* seedlings were observed after EMF inoculation or environmental stresses tested (Table 4). Stimulatory effects found under normal conditions were not always obtained when plants were exposed to simulated stress (no stimulation of shoot dry weight under stress conditions, opposite to that found in nonstressed controls). Nevertheless, the stimulations of plant health status and number of leaves per plant were observed in inoculated treatments of stressed plants. The effects of both stress factors themselves were, however, far more pronounced than the effects of inoculation. Differences in mycorrhizal growth response in stressed and nonstressed conditions can be caused by the fact that EMF may exploit bigger volume of the substrate and that inoculated plants may benefit

from accessing more water, after all in drought treatments. Previous research showed stimulation of nutrient uptake by plants due to EMF (PEARSON & READ 1973). Despite an evidence of metabolic capabilities of the EMF (LEAKE & READ 1990), it is still unclear, what is the mechanism of the increased nutrient uptake by mycorrhizal plants, either a specific transport or only a mass flow caused by increased uptake of water. From an ecological point of view the symbiotic fungi may also act as a buffering agent reducing the negative effects of environmental stresses due to toxins degradation or sequestration of heavy metals (BRADLEY & al. 1982).

Table 3. The effect of inoculation with ericoid mycorrhizal fungi (Oid 1.2 - *Oidiodendron* spp. and DSM 1.2 - dark sterile mycelia),on mortality, health status and growth of *Rhododendron* sp. plants (Experiment 4).

Fungus	Mortality	Health status	Shoot DW (g)	No of leaves	Leaf area (cm^2)
Uninoculated	16.7	3.3 a	0.68 b	16.5 b	118 b
Oid 1	26.7 ns	2.3 b	1.24 a	20.9 a	201 a
Oid 2	26.7 ns	2.7 ab	0.74 b	16.4 b	128 b
DSM 1	26.7 ns	2.7 ab	1.07 a	14.9 b	147 b
DSM 2	13.3 ns	3.2 a	0.65 b	13.1 b	101 b
G - test	ns	-	-	-	-
ANOVA	-	ns	*	*	*

Means followed by the same letter are not significantly different within one column according to Duncan`s Multiple Range test, $p<0.05$.
Significance of inoculation: ns – nonsignificant; * $p<0.05$.

Table 4. The effect of inoculation with ericoid mycorrhizal fungi (Dark sterile mycelium 1), liming (higher pH) and drought stress on the growth Rhododendron plants (Experiment 4).

Treatment	Mortality	Health status	Shoot DW (g)	No of leaves	Leaf area (cm^2)	EMF mycel. (No inters.)
Inocul./pH 4.9	26.6	2.7 ab	0.74 a	16.4 a	128 a	71 a
Uninoc./pH 4.9	13.3	3.3 a	0.65 a	13.1 b	101 a	82 a
Inocul./pH 6.1	38.5 ns	1.5 d	0.12 c	10.3 bc	9 b	20 b
Uninoc./pH 6.1	30.8 ns	1.6 cd	0.10 c	8.5 c	7 b	38 b
Inocul./Drought	23.1 ns	2.4 bc	0.25 bc	12.3 b	29 b	40 b
Uninoc./Drought /	38.5 ns	1.7 cd	0.32 b	10.7 bc	35 b	30 b
G - test	ns	-	-	-	-	-
One way ANOVA	-	**	***	**	***	***

Means followed by the same letter are not significantly different within one column according to Duncan`s Multiple Range test, $p<0.05$.
Significance of inoculation: ns – nonsignificant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

The mycorrhizal inoculation can be a feasible biotechnology used in horticulture for better exploitation of nutrients from substrates and for improvement of growth of horticultural crops, however, carefull tests for an appropriate strains of AMF and particularly EMF should be undertaken before large scale applications.

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