SUPPRESSION OF ARBUSCULAR MYCORRHIZA'S DEVELOPMENT BY HIGH CONCENTRATIONS OF PHOSPHOROUS AT *TAGETES PATULA* L.

Brigitta SCHMIDT[1], Mónika DOMONKOS[2], Radu ŞUMĂLAN[1], Borbála BIRÓ[2]

[1]Banat's University of Agricultural Sciences and Veterinary Medicine of Timişoara, 300645 Timişoara, Calea Aradului 119, Romania
[2]Research Institute for Soil Science and Agricultural Chemistry of Hungarian Academy of Sciences, Budapest, Hungary.
Corresponding author: brigischmidt@yahoo.com

Abstract: *A pot experiment with hydroponic culture was conducted to study the effect of phosphorous content in rhizosphere on colonization rate of French marigold's (Tagetes patula L.) root system with arbuscular mycorrhizal fungi. Plants were watered with Hoagland's nutrient solution, in four variants: with normal phosphorous content, with double P content, with half and a quarter of the normal phosphorous content described in the recipe. Inoculation was realized with three different species of arbuscular mycorrhizal fungi: Glomus intraradices, Gl.etunicatum and Gl.claroideum. Root samples were collected, washed, cleared with KOH and stained with aniline blue to distinguish the specific mycorrhizal formations. After the growing period, we measured the plant development indexes (plant height, shoot and root fresh and dry weight, total leaf area, number and weight of flowers per plant) and mycorrhization's characteristics (rate of colonization and arbuscular richness) to find a correlation between concentration of phosphorous in medium, plant's and mycorrhiza's development. High phosphorous contents in nutrient solution determined a better growth and development of plants, resulting in an increased plant height, shoot and root fresh and dry weight, respectively, number and weight of flowers. A negative correlation was found between P and mycorhization, demonstrating the inhibitor effect on colonization and development of mycorrhizal fungi. Between plant development indexes and nutrient content there is a positive relation. Between plant height and total leaf area, shoot dry weight and root dry weight we found a very strong positive correlation. Also, shoot fresh weight and root fresh weight vary according to a positive correlation curve. Results show that an excessive fertilization with phosphates, even if it increases plants' productive performances, reduces in the same time the biodiversity of symbiotic fungi from soil and the development and extent of arbuscular mycorrhizal formations in root system of the host plants.*

Key words: *arbuscular mycorrhiza, French marigold, phosphrous nutrition*

INTRODUCTION

Arbuscular mycorrhizas are the most wide-spread mycorrhizal relationships in the world, being present at over 80% of terrestrial plant species (SMITH AND READ, 2008).

Mycorrhizal fungi enhance the nutrition of host plants, contributing to the absorption of minerals (P, N, K, Cu, Zn) and water (TAKÁCS AND VÖRÖS, 2003). The smaller diameter and the faster growth of hifae than of roots permit the exploitation of soil particles with smaller pores and the surpassing of the nutritional depletion zone which appears around the rhizosphere (SMITH AND READ, 2008). Earlier studies demonstrated that mycorrhization has positive physiological effects on host plant, increasing phosphorous content of plant tissues, biomass, chlorophyll content and photosynthetic rate (KHADE AND RODRIGUES, 2009, SCHMIDT AND ŞUMĂLAN, 2009, SUBRAMANIAN et al., 1997, SHENG et al., 2008).

Mycorrhiza contributes to protection against soil-borne pathogens by surrounding the roots and stopping the access of other microorganisms to the plant's radicular system (AZCON-AGUILAR AND BAREA, 1996).

The concentration of phosphorous in soil influences the mycorrhiza, by inhibiting the mycorrhizal dependency of plants when P exists in higher concentrations (TAKÁCS et al., 2006). High soil phosphate levels determines the reduction of hyphal growth and spore production of arbuscular mycorrhial fungi (AMIJEE et al., 1989, KOIDE, 1991). GUILLEMIN et al. (1995) demonstrated that in P-sufficient soils mycorrhizal infections were reduced by phosphate fertilization, meanwhile in a P-deficient soil fungal infection is not modified. TAKÁCS et al. (2006) suggested the use of rock phosphates instead of superphosphates for fertilization in sustainable agricultural practices, this kind of phophates disturbing less the infectivity and functioning of arbuscular mycorrhizal fungi.

The formation and function of mycorrhizal relationships are affected by edaphic conditions such as soil composition, moisture, temperature, pH, cation exchange capacity, and also by anthropogenic stressors including soil compaction, metals and pesticides. Much mycorrhizal research has investigated the impact of extremes in water, temperature, pH and inorganic nutrient availability on mycorrhizal formation and nutrient acquisition (ENTRY et al., 2002).

French marigold (*Tagetes patula* L.) is an annual ornamental plant with yellow composite flowers, small habitus and high stress tolerance. When marigold was inoculated with different species of AM fungi, LINDERMAN AND DAVIS (2004) observed varied responses in colonization and effectiveness (LINDERMAN AND DAVIS, 2004).

In this paper we present the results of the researches regarding the phosphorous concentration's effect on mycorrhization rate, arbuscular richness and some physiological indexes of French marigold, as: plant height, shoot and root fresh/dry weight, total leaf area, number and weight of flowers per plant.

MATERIAL AND METHODS

A pot experiment was conducted with hydroponic culture of marigold (*Tagetes patula* L., CNOS-VILMORIN, Polland) in steam sterilized perlite substrate. Plantlets were obtained by germinating surface sterilized (with $HgCl_2$ 0,2 %, for 3 minutes, followed by 5 rinses with sterile distillated water) seeds of marigold. Several seeds were placed in every pot, then plants were singled, keeping uniformly developed plants. Five repetitions (pots) were used for every variant.

The inoculation was realized with commercial inoculum INOQ Top, Germany, containing a mixture of three AM fungi species (*Glomus etunicatum* (BECKER & GERDEMANN, *Glomus intraradices* (SCHENCK & SMITH), *Glomus claroideum* (SCHENCK & SMITH)), carried by expanded clay balls.

The 0.5 liters pots, filled with growing medium, were placed in green-house, where temperatures were maintained at 27/18^{o}C day/night, with a photoperiod of 12h day lengths for the study period. Plants were watered with Hoagland's solution, using a quantity required by the relative size of the plant, and until the saturation of growing medium. Four variants of Hoagland's solution were used in the experiment: with 25%, 50%, 100% and 200% phosphorous concentration of the normal recipe.

Roots were collected and washed with tap water then deposited in 70% ethanol until examination. After storage, root samples were boiled in 15% KOH for 30 minutes, stained with aniline blue and fixed in 40% lactic acid (VIERHEILIG et al., 2005). Stained root samples were cut into 1 cm segments and assessed for colonization by the five-class system of Trouvelot (TROUVELOT et al., 1986) at 100x magnification (of more if necessary). The abundance of hyphae and arbuscules was determined in 30 root segments of each plant.

Plants' height, fresh and dry weight of shoots and roots, respectively, total leaf area (using portable leaf area meter LA 300, ADC Bioscientific Ltd.), number and weight of flowers per plant were measured.

Data collection was realized 11 weeks after seeding.

Data were statistically analyzed using ANOVA and correlations (CIULCA, 2002).

RESULTS AND DISCUSSION

Plant height is a value which indicates the characteristics of growing process, highly influenced by nutritional level provided by the nutrient solution. In our experiment, the only factor which varied was the level of phosphates in Hoagland's nutrient solution. The values for the height of plants show a linear ascendance with the growth of P level from nutrient solution. The highest plants were those with the greatest level of accessible phosphate, important for all the metabolic processes in vegetal organisms.

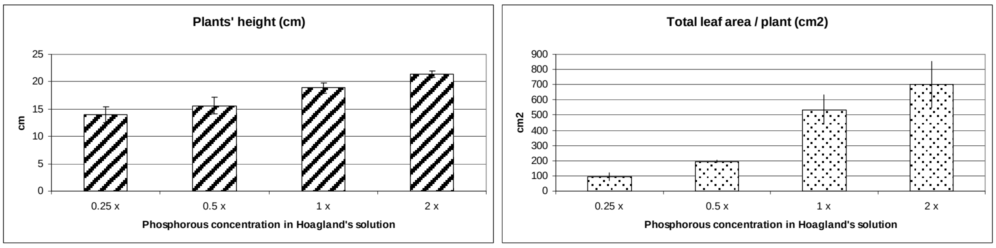

Fig. 1. Height (cm) and total leaf area (cm2) of plants.

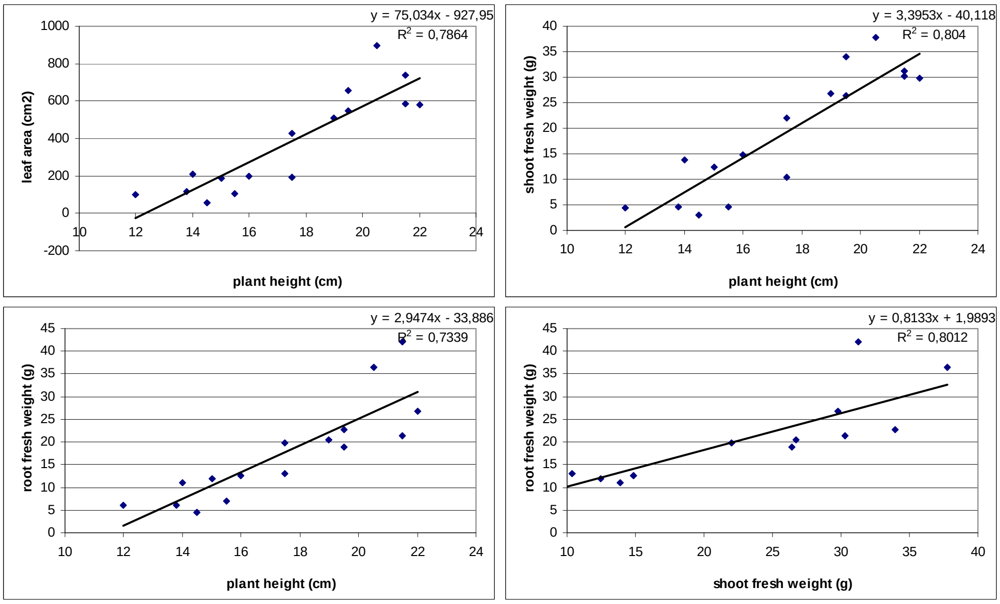

Fig. 2. Correlation between the main growing indexis of experiental plants.

Beside the plant height, the total leaf area per plant shows similar variation pattern. As it can be observed in Figure 1, both plant's height and leaf area have maximum values when phosphorous is found in double concentration than recommended for a general nutrient solution. Even if for plant height the differences were not significantly different, the leaf area shows great contrasts between treatments.

Plant height was in a strong correlation with all of the other physiological indexes, as leaf area (R^2=0.79), shoot fresh weight (R^2=0.80) or root fresh weight (R^2=0,73).

Fresh and dry weight of shoots varied in the same manner as those of roots (Fig. 3.). Analyzing the relation between the fresh weight of shoots and roots, it can be observed that there is a positive correlation between the two series of values (Fig. 2.). As fresh weight is influenced by plant height and mostly total leaf area per plant, the values are ascending with the growth of phosphorous content of nutrient solution. Being more phosphorous available for the plants, the biosynthesis processes are enhanced, determining a faster growth and development, which leads to a greater biomass and leaf area.

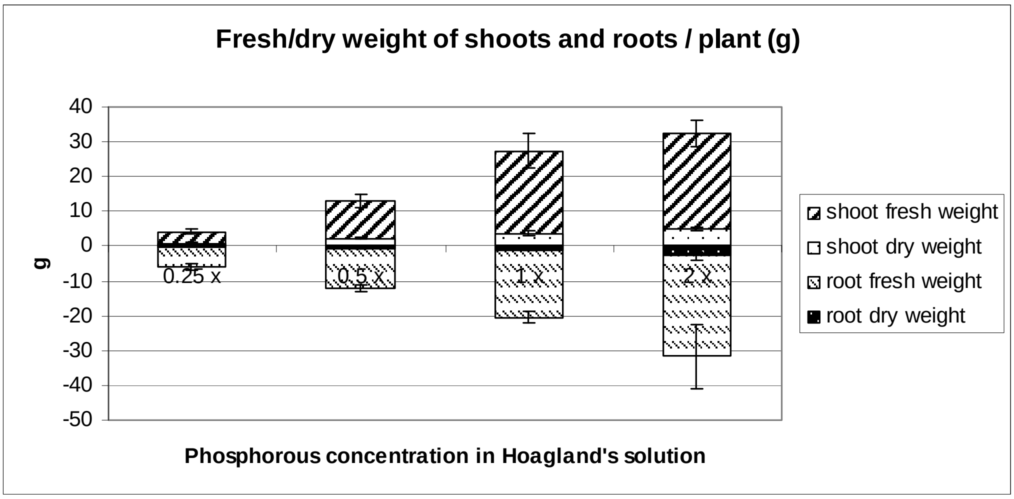

Fig. 3. Fresh and dry weight (g) of shoots and roots, respectively, of variants.

The proportion between shoot and root fresh/dry weight was varying excessively, no matter the concentration of phosphates in Hoagland's solution. The values of shoot/root fresh weight ratio varied between 0.7 and 1.33 and those of dry weight, between 2.03 and 3.52. Analyzing these data it can be observed, that fresh weight is nearly identical at sub- and supra-terrestrial parts, instead the shoots contain 2-3 times more dry matter than roots (Table 1.).

Table 1.

Ratio of shot and root fresh and dry weight, respectively.

Concentration of P in nutrient solution	Shoot/root fresh weight ratio (average±sd)	Shoot/root dry weight ration (average±sd)
25%	0.70±0.05	2.34±0.41
50%	1.07±0.21	3.52±0.75
100%	1.33±0.16	3.23±0.31
200%	1.08±0.28	2.03±0.96

Phosphorous is a macro-element indispensable for flowering and fruiting of plants (ŞUMĂLAN, 2009). Regarding the number and weight of flowers, both indexes were positively influenced by the higher concentrations of phosphorous in nutrient solution. The double concentration of phosphorous didn't determine a significant growth in the number of flowers. Instead, if we analyze the differences between the number of flowers at 200% and 25% of normal phosphorous content, the values are almost four times higher in the first case.

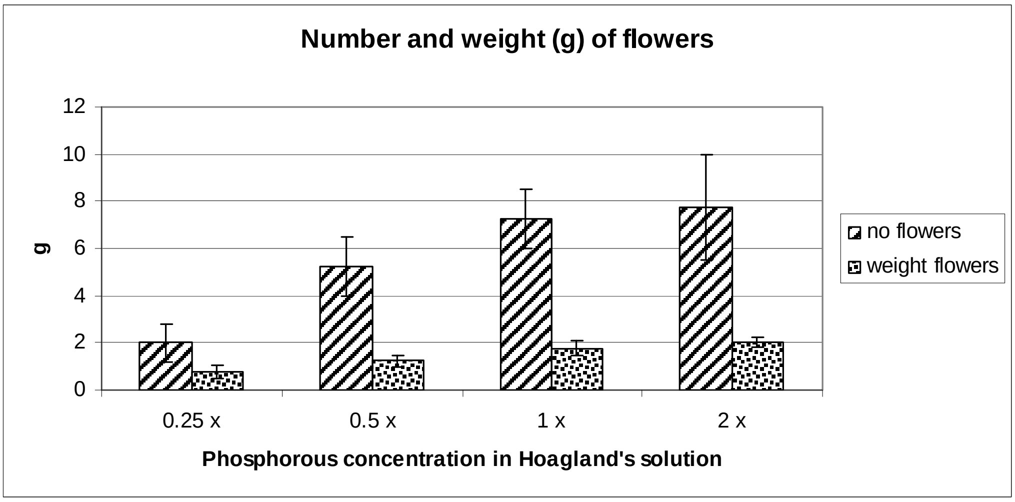

Fig. 4. Number and weight (g) of flowers from plants of different experimental variants

Even the weight of flowers, which indicates the dimension and implicitly the ornamental value, was considerably reduced by the low quantities of phosphorous administered to marigold plantlets.

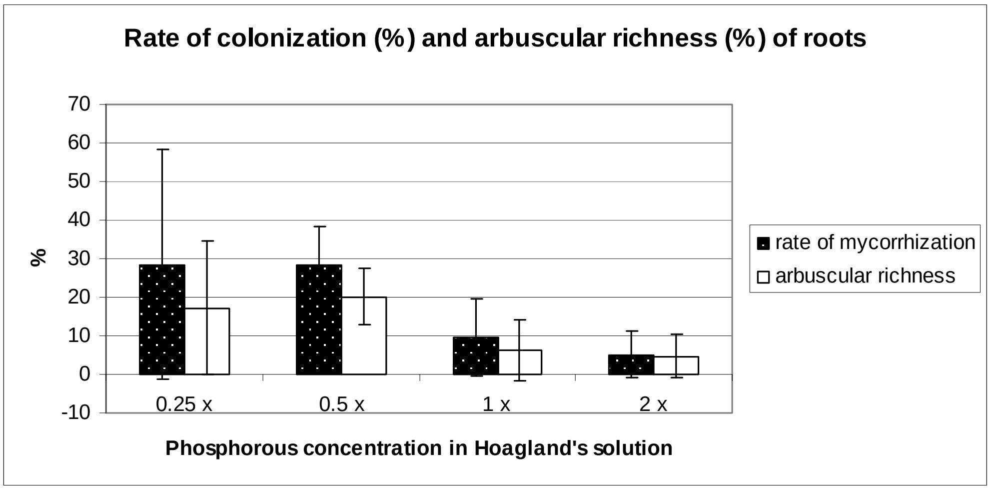

Fig. 5. The rate of colonization (%) and arbuscular richness (%) of roots determined with Trouvelot method (1986)

The percentage of colonization in roots and the arbuscular richness were negatively influenced by the concentration of phosphorous available for plants. Between treatments with 25% and 50% of the normal P content in Hoagland's nutrient solution we couldn't observe significant differences in rate of mycorrhization, but the percentages were drastically reduced when phosphates were in double or even normal quantity.

The arbuscular richness was the highest in roots of marigold plants watered with nutrient solution containing only half of normal P content, followed by the variants with 25% P treatment. Even the highest values for mycorrhization rate or arbuscular richness were relatively low, indicating that other elements than phosphorous can inhibit mycorrhiza. Also, earlier studies on *Tagetes erecta* L. indicates higher infection rates in root, between 46-50% (ABOUL-NASR, 1996), even if the age of plants was similar. Instead, CALVET et al. (1993) obtained similar values for colonization percentage when *Tagetes erecta* L. was inoculated with *Glomus mosseae* (between 20-35% or even less, depending the experimental treatments). HOWEVER, LINDERMAN and DAVIS (2004) found very great differences in rate of colonization (between 10-70%) between *Tagetes erecta* and *T. patula* cultivars, respectively.

These differences in mycorrhization rate can be attributed to the variation of fungi/host plant genotype and of nutritional level.

Both values, rate of colonization and arbuscular richness were in negative correlation with the concentration of phosphorous in nutrient solution (R^2=0.72 and 0.83 respectively). Instead, the fresh weight of shoots was in positive correlation with phosphorous content (R^2=0.79).

CONCLUSIONS

Mycorrhizal relationships are worldwide present, but their effectiveness is highly influenced by environmental factors. The concentration of nutrients in rhizosphere, especially high phosphorous content, can become limiting factors for the colonization with AM fungi. Even if the normal or double concentration of P in nutrient solution can determine an increase in plant height, total leaf area, fresh/dry weight of shoots and roots, number and of weight of flowers, the rate of colonization and arbuscular richness are suppressed in there conditions.

In agricultural ecosystems, high amounts of fertilizers are used, which has a negative effects on environment, determining leaches of chemicals or reduction of biodiversity. The composition of microbial species in rhizosphere suffers severely because of intensive agricultural practices.

The future tendencies are linked to biological approaches, as use of bio-fertilizers or biological protection against pathogens. The supplemental inoculation with arbuscular mycorrhizal fungi is becoming a world-wide recognized practice, which can assure a better management of nutrients and plant protection, especially in soil where the local microbial community is destroyed.

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