The influence of mycorrhizal fungi (*Glomus* sp.) on field pea plant survival and growth in drought caused stress conditions

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ABSTRACT

The influence of mycorrhizal fungi on field pea green mass yield, dry matter yield, grain yield, number of pods per plant, number of grains per pod, average number of plants per m^2, grain concentration of nitrogen, phosphorus and potassium were studied in the greenhouse during two investigative years. The best results with all parameters were obtained in both investigative years by seed inoculation with the mycorrhizal species *G. mossae*. The exceptions were mean green mass yield, dry matter yield and the number of grains per pod in the second investigation year (irrigation rate – 240 mm/m^2) where better results were achieved by seed inoculation with species *G. intraradices*. The highest green mass yield obtained by seed inoculation with mycorrhizal species *Glomus mossae* was 671.45 g/m^2, dry matter yield 59.40 g/m^2, grain yield 346.20 g/m^2 whereas grain nitrogen concentration was 4.08%. Far better results of all yield and quality indicators of this plant compared to non-mychorrized variants were accomplished by mycorrized variants in water lacking conditions.

Keywords: mycorrhizal fungi; *Glomus* sp.; field pea; drought stress; grain yield; nitrogen concentration

Mycorrhizas are associates of fungi, representatives of Zygomyceta, Basidiomyceta and Ascomyceta living on the higher plant roots. Albert Bernard Frank in 1885 (Brundrett et al. 1996) first discovered them. Mycorrhizal fungi fossil residues were found on the roots of the first vascular plants derived from the Devon period approx. 400 mil. years B.C. Botanists think that this symbiotic association played an essential role in developing and spreading vascular plants on the earth. However, regardless of their effect on vascular plants development of mycorrhizal fungi affected soil development. They produce humous components and an organic sticky substance (extra-cellular polysaharides) that hold soil into aggregates resulting in a well structured soil of good porosity and a large mycorrhizas intake in the total microbial bio-mass.

These fungi colonize roots in about 90% of the vascular plants. The symbiotic association between higher plants and mycorrhizal fungi is highly-interrelated whereby plants benefit from their symbiont whereas fungi gets photosynthesis-formed carbon compounds.

Owing to a dense hyphas network that interweaves large soil volume, an effective root absorption zone increases via hyphas (Barker et al. 1998). However, the mycorrhizal fungi also extracts chemical agents, thereby, making available elements such as phosphorus, iron, zinc, copper, boron, otherwise presented in low concentrations in a soluble form (Subramian et al. 1997, Gryndler et al. 2003). Mycorrhizas produce antibiotics protecting plants from parasitic fungi and nematodes whereas owing to hypha network around a root, a plant is protected against parasites. Mycorrhized plants are more resistant to a drought-caused stress, have better and more adequate root system and vascular tissue development. Due to the aforesaid, dry conditions are characterized by plants having higher survival percent and higher yield elements compared to non-mycorrhized ones (Martin and Jamieson 1996, Morte et al. 2000, Augé et al. 2001, Vosátka et al. 2003).

Thanks to the traits of this symbiosis and all benefits possessed by mycorrhized plants compared to non-mycorrhized, an agricultural production does not rely only on the indigenous strains of these fungi but inoculation of seedling seed and root is done by a compatible strain of mycorrhizal fungi. Inoculation is done by a dry treatment or wet one in a way that the inoculum suspended is water prior to inoculation (similar to legume seed inoculation with bio-preparations of the nodule bacteria).

This procedure is capable for ensuring effective symbiosis and reducing mineral fertilizer and pesticide requirements being very important from an economical and ecological aspect.

MATERIAL AND METHODS

The trial was conducted in the greenhouse within two investigative years (2002 and 2003) on Mollic Gleysols soil type (FAO 1998) by a completely randomised design in four replicates. Six variants of the seed inoculation by mychorrized fungi and various amounts of water added by irrigation were comprised by the investigation. French variety of the field pea Bacara was used. Basic plot size was 9 m^2. However, since marginal plot parts of 20 cm wide were not analysed the basic plot was 6.76 m^2. Sowing was carried out in the row space 20 cm apart, within the row 5 cm whereas sowing depth was 4–6 cm.

The investigation elements were as follows: A. Seed inoculation (A_1 – non-inoculated seed; A_2 – seed inoculated with endomycorrhizal fungus *Glomus mossae* spora solution; A_3 – seed inoculated with endomycorrhizal fungus *Glomus intraradices* sporas solution); B. Amount of water added by irrigation (B_1 – total of 240 mm/m^2 during the growing season; B_2 – 30 mm/m^2 after seeding + 30 mm/m^2 in the 2–3 real leaves phase).

Soil samples were taken in early spring before the experiment was established. Soil analyses were carried out by standard methods (Table 1): organic matter content was determined by bichromate method, pH in H_2O and KCl, phosphorus and potassium content by ammonium-lactate method according to Egner-Riehm-Domingo (Page 1982).

Soil moisture was measured by tensiometer during field pea vegetation. Two weeks before seeding, soil moisture was approx. 60% of max. soil water capacity. At irrigation of 240 mm/m^2 (during the growing season), soil moisture was maintained within 70–100% of max. soil water capacity. While adding water of 30 mm/m^2 after seeding and 30 mm/m^2 at the 2–3 real leaves phase, soil moisture ranged within 70–75% after irrigation, 40–45% in the grain filling phase and dropped to approx. 30–35% of max. soil water capacity in the maturation phase.

Fertilization with 500 kg/ha NPK 15:15:15 was conducted prior to seeding. Due to weed occurrence, the experimental plot was treated with the herbicide (Basagran – 2 l/ha) after emergence. Green mass yield and dry matter per plant were determined in the blooming phase (approx. 80% of the blooming plants). Pea harvest was followed by determination of grain yield, mean count of pods per plant, mean count of grains per pod and as well as plant count per square. Nitrogen concentration in the grains was determined by Kjeldahl method, concentration of phosphorus was determined by spectrophotometry and of potassium by atomic absorption spectrophotometry.

The results were processed by modern statistical methods (ANOVA) using the computer program StatSoft Inc. (2001) STATISTICA (data analysis software system), version 6.

Table 1. Chemical properties of soil in experimental localities

Investigated properties layer (0–30)	Mollic Gleysols	
	2002	2003
pH (H_2O)	7.27	7.09
pH (KCl)	6.55	6.31
Humus (%)	3.22	3.31
P (mg/100 g soil)	17.40	19.20
K (mg/100 g soil)	24.10	22.90

RESULTS AND DISCUSSION

Obtained results show that the highest values of the investigated parameters were achieved in the variants of seed inoculated with mycorrhizal fungi. This difference is especially obvious at irrigation with 60 mm water/m^2 (Table 2).

The highest average yield of the green mass and dry matter was obtained in the mycorrhized seed variants with irrigation of 240 mm water/m^2. First investigative year at irrigation of 240 mm water/m^2 was characterized by the better results in seed inoculated with mycorrhizal fungus *G. mossae* whereas the second one with *G. intraradices*. Better results were accomplished by inoculation with mycorrhizous fungus *G. mossae* with added water of 60 mm/m^2 in both investigation years.

Mycorrhized plants (*G. mossae*, *G. intraradices*) obtained higher green mass yield by 118.8 –151.5% at irrigation of 60 mm water/m^2 compared to non-mycorrhized control i.e.127.6–145.2% higher dry matter yield.

Achieved results are in harmony with the investigations results obtained by many authors (Martin and Jamieson 1996, Douds and Nagahashi 2000) who reported that plants inoculated with mycorrhizal fungi in the drought-caused stress conditions had higher survival percentage, thus, obtaining higher green mass and dry matter yield as well as higher phosphorus and nitrogen content in plant mass and grain. Namely, owing to morphological and physiological traits of these fungi, plants benefit from the

Table 2. Average values of investigated parameters during both investigation years

Investigated parameter	Year	Seed inoculation						LSD	
		non-inoculated seed		inoculated seed					
				G. mossae		*G. intraradices*			
		water added by irrigation (mm/m^2)						0.05	0.01
		240	60	240	60	240	60		
Green mass yield (g/m^2)	2002	521.10	143.20	671.45	390.50	584.70	312.45	6.16	9.62
	2003	517.45	158.90	526.38	369.20	550.00	348.65	4.72	6.64
Dry matter yield (g/m^2)	2002	42.65	12.50	59.40	32.00	51.70	28.25	1.17	1.88
	2003	40.05	13.60	54.22	31.50	55.29	31.16	0.49	0.86
Grain yield (g/m^2)	2002	301.80	120.60	346.20	270.40	330.27	253.10	3.92	5.17
	2003	274.80	95.45	320.80	268.10	309.65	250.05	4.16	6.08
Number of pods per plant	2002	6.08	2.12	7.42	4.65	6.12	3.80	0.67	1.14
	2003	6.06	2.34	6.85	4.38	6.31	4.12	0.22	0.36
Number of grains per pod	2002	5.36	3.07	6.16	4.31	5.68	4.01	0.29	0.41
	2003	5.27	3.11	5.73	4.16	5.92	4.29	0.12	0.17
Grain nitrogen concentration (%)	2002	3.41	3.17	4.08	3.66	4.01	3.58	0.04	0.07
	2003	3.38	3.14	3.84	3.59	3.71	3.61	0.06	0.11
Grain phosphorus concentration (%)	2002	0.470	0.438	0.501	0.477	0.490	0.472	0.004	0.007
	2003	0.462	0.433	0.496	0.469	0.491	0.476	0.003	0.005
Grain potassium concentration (%)	2002	1.490	1.219	1.778	1.586	1.712	1.505	0.042	0.056
	2003	1.499	1.207	1.715	1.560	1.714	1.520	0.006	0.009
Average number of plants per m^2	2002	92.10	28.60	96.30	78.25	93.60	71.00	1.14	1.72
	2003	87.85	32.20	92.75	74.50	92.70	72.55	0.37	0.52

symbiosis with them since water and nutrients from larger soil volume, otherwise being unavailable in dry conditions, are at their disposal.

The highest average values of grain yield, average number of pods per plant and average number of grains per pod were obtained in the variants of inoculated seed with mycorrhizal fungus *G. mossae,* regardless of the amount of water added by irrigation. The second investigative year was the exception with the highest average number of grains per pod obtained by using mycorrhizal fungus *G. intraradices*.

Grain yield was in significantly positive correlation with green mass yield (r = 0.975**) and dry matter yield (r = 0.961**).

Linderman (1994) stated that seed inoculated with arbuscular mycorrhizal fungi improved water and nutrients uptake in drought-caused stress conditions. He also reported that plants had better vigour, were more resistant to nematodes and disease agents resulting in higher yield elements.

The highest grain nitrogen ratio was achieved in the mycorrhized variants with irrigation of 240 mm water/m^2. In the first investigation year there was no statistically significant differences between variants inoculated with *G. mossae* and *G. intraradices* whereas in the second one better results were obtained by seed inoculation with mycorrhizal fungus *G. mossae*. Irrigation of 60 mm water/m^2 was characterized by grain nitrogen concentration in mycorrhized plants being higher by 13.9–14.9% compared to the same one in non-mycorrhized plants.

Although the seed was not inoculated with nodule bacteria, nodules were formed on the pea root in the blooming phase as a result of symbiosis with indigenous strains of *Rhizobium* bacteria. The nodules interior was red indicating active performance of atmospheric nitrogen uptake. Based on their investigation Xie et al. (1995) stated that symbiosis of the plant with nodule bacteria stimulated symbiosis development in the soybean plant between plants and mycorrhizal fungi.

The highest average concentration of pea grain phosphorus was in the first year accomplished in the variant of seed inoculated with mycorrhizal fungus *G. mossae*. The second investigative year was known for absence of statistically significant differences between plants inoculated with mycorrhizal fungi *G. mossae* and *G. intraradices*.

Plants mychorrized with the fungus *G. mossae* had higher grain phosphorus concentration compared to non-mycorrhized plants (control) at irrigation of 240 mm water/m^2. This difference was higher (8.7%) at irrigation of 60 mm water/m^2. Plants mycorrhized with fungus *G. intraradices* were characterized by 5.8% (240 mm water/m^2) i.e. 9% (60 mm water/m^2) higher grain phosphorus concentration compared to non-mycorrhized control.

Douds and Nagahashi (2000) and Graham and Abbott (2000) reported that mycorrhizal fungi extracted strong chemical agents into the soil, thereby, made elements like phosphorus, iron, zinc, copper, boron… (otherwise present in low concentrations in a soluble form) available. Furthermore, on the basis of their investigation results, Miller et al. (1997) reported that mycorrhizal fungi favourably affected better and more adequate development of root system and vascular tissue increasing effective root absorption zone through hyphas. This trait is especially pronounced in the nutrients – poor soils and in the water shortage conditions.

In congruence with pea grain phosphorus concentration, average grain potassium concentrations were obtained in the variants inoculated with mycorrhizal fungus *G. mossae* in both investigation years. The concentration of plant grain potassium inoculated with *G. mossae* and *G. intraradices* was by 16.9%, i.e. 14.6% higher compared to the control variant at irrigation of 240 mm water/m^2. This difference was 29.7% (*G. mossae*), i.e. 24.7% (*G. intraradices*) at irrigation of 60 mm water/m^2.

The highest number of plants per m^2 was achieved in the first investigative year in the variant of the seed inoculated with mycorrhizal fungus *G. mossae*. In the second investigative year there were no statistically significant differences between the inoculated variants. Inoculated variants had by 3.5–5.1% higher average number of plants per m^2 compared to non-inoculated control at irrigation of 240 mm water/m^2. This difference was significantly higher (36.12–51.3%) at irrigation of 60 mm water/m^2.

Douds and Nagahashi (2000) reported that based on their investigation results mycorrhizal fungi improve uptake of less mobile soil nutrients in the water shortage conditions. They also improve water absorption due to hyphas mass interweaving a large soil volume. Finally, the aforesaid brings about higher percent of plants survival in the drought conditions as well as better quality of the obtained agricultural product.

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ABSTRAKT

Vliv mykorhizních hub (*Glomus* sp.) na přežití a růst rostlin hrachu setého ve stresových podmínkách způsobených suchem

Byl studován vliv mykorhizních hub na výnos zelené hmoty, celkové sušiny a semen, na počet lusků na rostlině, počet semen v lusku, průměrný počet rostlin na m^2 a na obsah N, P a K v semenech. Prezentovány jsou výsledky dvouletých pokusů realizovaných v definovaných skleníkových podmínkách. Nejlepší výsledky sledovaných parametrů byly v obou letech získány u osiva inokulovaného mykorhizním druhem *Glomus mossae*. Výjimkou byl průměrný výnos zelené biomasy, výnos sušiny biomasy a počet semen v lusku ve druhém pokusném roce (závlahová dávka 240 mm/m^2), kdy lepších výsledků dosáhla varianta s osivem inukulovaným druhem *G. intraradices*. Výnos zelené hmoty hrachu při inokulaci osiva mykorhizním druhem *Glomus mossae* dosáhl 671,45 g/m^2, výnos sušiny 59,40 g/m^2, výnos semen 346,20 g/m^2, obsah N v semenech činil 4,08 %. Výrazně lepších výsledků u všech výnosových a kvalitativních znaků těchto rostlin v porovnání s variantami neošetřenými mykorhizními houbami bylo dosaženo za podmínek nedostatku vody v půdě.

Klíčová slova: mykorhizní houby; *Glomus* sp.; hrách setý; stres suchem; výnos semen; obsah dusíku

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