

## Emergence seedling of soybean [*Glycine max* (L.) Merril.] as influenced by the arbuscular mycorrhiz fungi

Mina Ahmadi Memshi, Abbas Ghanbari Malidareh<sup>1</sup> and Mohammad Yazdani\*

Department of Agronomy, Ghaemshahr Branch, Islamic Azad University, Ghaemshahr, Iran; <sup>1</sup>Department of Agronomy, Tabriz Branch, Islamic Azad University, Tabriz, Iran

### Abstract

Mycorrhizae play a major role in the growth and development in different crops. In order to investigate the effects of arbuscular mycorrhiz fungi (AMF) and different manures on improvement of growth and development in soybean [*Glycine max* (L.) Merril.], an experimental was conducted at research farm of Islamic Azad University of Ghaemshahr using a factorial based a completely randomized design with three replications during 2011. The field experiment was laid out on factorial based on completely randomized design with three replications. Inoculation of arbuscular mycorrhizal fungi (*Glomus mosseae*) and without inoculation and three levels of phosphorus (consisting of 0, 50 and 100 Kg ha<sup>-1</sup>) were considered as treatment. In treatments, involving reduced fertilization (50 Kg.h<sup>-1</sup>), plants inoculated with AMF had significantly increased dry weight relative to the non-inoculated plants. Furthermore, with the low fertilizer dosage (50 Kg.h<sup>-1</sup>), the plant terminal emergence was increased in plants, which had been inoculated with the AMF. Under the conventional fertilizer dose (100 kg h<sup>-1</sup>), shoot length, dry weight of seedling was not influenced by AM formation, but it was significantly increased under reduced fertilizer dosage.

**Keywords:** Emergence seedling; arbuscular mycorrhiz fungi; soybean

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### Introduction

Conventional agriculture compensates for its unsustainable nature by the use of relatively high levels of external inputs such as fertilizers and pesticides. Improving sustainability of the agricultural sector will lead to production of nutritious and safer food, while minimizing environmental damage and consumption of non-renewable inputs. These perceptions prompted the rapid expansion of organic agriculture in many developed countries during the last two decades (Lockeretz, 2007). Phosphate, which is an essential mineral nutrient for plant growth, is one of the three main mineral nutrients applied excess application of phosphate fertilizers is an important cause of water eutrophication, and therefore improvement of phosphate uptake efficiency by plants is a priority. Inorganic phosphate has very limited diffusion capacities in soils and its rapid absorption from the soil

solution by plant roots generates Pi depletion zones at the root surface resulting in a decline of directly absorbed Pi by the plant surface (Roose and Fowler, 2004). In sustainable, low-input cropping systems the natural roles of microorganisms in maintaining soil fertility and biocontrol of plant pathogens may be more important than in conventional agriculture where their significance has been marginalized by high inputs of agrochemicals (Johansson et al., 2004).

The beneficial effects of arbuscular mycorrhiz fungi (AMF) on plant performance and soil health are essential for the sustainable management of agricultural ecosystems (Barrios, 2007). Enhanced uptake of P is generally, regarded as the most important benefit that AMF provide to their host plant, and plant P status is often the main controlling factor in the plant-fungal relationship (Ryan and Ash, 1999; Graham, 2000). AMF can play a significant role in crop P nutrition, increasing total uptake and in some cases P use

**Corresponding author:** Mohammad Yazdani, Department of Agronomy, Tabriz branch, Islamic Azad University, Tabriz, Iran

efficiency (Koide et al., 2000; Neumann and George, 2009). The mycorrhizal association is usually specific to soil type and climatic conditions. Under natural conditions, mycorrhizae facilitate plant growth by supplying plant roots with P when soluble P concentration cannot satisfy the needs of the plant (Meghvansi et al., 2008; Djebali et al., 2010). The evidence available suggests that this leads to increased AMF inoculums in soils, greater crop colonization and enhanced nutrient uptake. AMF might therefore be able to substitute for reduced fertilizer and biocide inputs in organic systems, though there is little evidence for increased yield resulting from high rates of AMF colonization in organic systems (Johansson et al., 2004; Gosling et al., 2006; Gianinazzi et al., 2010).

The network of fungal mycelium connected to AM roots increases by several orders of magnitude the soil volume, which can be explored by a plant so that a mycorrhizal root is more efficient in phosphate uptake than a nonmycorrhizal root (Smith and Read, 2008). Under given field conditions, it has been estimated that a reduction of 80% of the recommended phosphate fertilizer could be supplemented by inoculation with AM fungi (Jakobsen, 1995). It is evident that such reductions in have important economical and environmental impacts (Hodge, 2000; Gosling et al., 2006). The exclusion of soluble mineral fertilizers and the very limited use of biocides in organic agriculture mean that it is reliant largely on biological processes for supply of nutrients, including the reliance on N<sub>2</sub> fixation as the main source of N to crops, and for protection of crops from pests and disease. Indeed, it is one of the central paradigms of organic agriculture that an active soil microbial community is vital for functioning of the agroecosystem (Lampkin, 1990). Within this paradigm, AMF are usually considered to play an important role and it is assumed that they can compensate for the reduced use of P fertilizers (Galvez et al., 2001).

Some evidence indicates that AMF are indeed capable of compensating for lower inputs of P fertilizer in organic systems (Johansson et al., 2004; Smith and Read, 2008). Gosling et al. (2006) found that AMF in an organically managed soil were as effective at increasing crop available P as superphosphate was on a conventional soil. However, this does not always translate into higher yields even when phosphorus use efficiency is higher (Ryan et al., 1994; Galvez et al., 2001). Mycorrhiza represents an important group because they have a wide distribution; may contribute significantly to microbial biomass and to soil nutrient cycling processes in plants. The mycorrhizal fungi are part of biofertilizers, recognized for their beneficial effects: improved plant nutrition, soil fertility improvement, root pest and disease control, improved water usage, amelioration of toxic effects in soils.

The increase in soybean cultivation in Iran is likely to improve the rural economy and socio-economic status of the Iranian farmers. Association of soybean with arbuscular mycorrhizal fungi increases the uptake of nutrients particularly phosphorus that improve growth and development (Xavier and Germida, 2003; Gavito et al., 2003). In addition, soybean suffers from various fungal diseases in its entire growing period from germination of seeds to the mature plant stage. Seedling emergence is an important trait that can limit commercialization of soybean seed. A rate of seedling emergence and leaf appearance is important in developing a soybean crop with earlier canopy closure and better seasonal light interception. Therefore, this study was design to find out the effect arbuscular mycorrhizal fungi (*Glomus mosseae*) and different phosphorus on emergence and seedling parameters of soybean in field conditions.

## Materials and Methods

In order to investigation effect of arbuscular mycorrhizal fungi and different phosphorus on growth and development in seedling of soybean [*Glycine max* (L.) Merril.], experimental was conducted at research farm of Islamic Azad University of AMF a factorial based a completely randomized design with three replications during 2011. Inoculation of AMF (*Glomus mosseae*) and without inoculation and three levels of phosphorus (consisting of 0, 50 and 100 Kg ha<sup>-1</sup>) were considered as treatment. Primary and terminal emergence of seedling was recorded on the 10 day after sowing. The plants was removed 10 day after sowing, and roots were washed using slow running water to remove soil particles and organic debris. The dry mass of shoot and root samples, root length and shoot length was determined after drying in an oven at 60 °C with forced air. Ten normal seedlings were selected at random from each treatment of the germination test on eight day and used for measuring seedling parameters. The root length was measured from the tip of primary root to the base of the hypocotyls and the mean root length was expressed in centimeters. The shoot length was measured from the base of the primary leaf to the base of the hypocotyls and the mean shoot length was expressed in centimeters. In addition, Seedling vigor index was recorded after 45 days. Seeds were considered germinated when the radical extended through the seed coat. Vigor index for each treatment was determined using this formula (Seedling Vigor = [root length + shoot length] × percentages of germination) developed by Abdul-Baki and Anderson (1973). Data were subjected to ANOVA using the SAS statistical software package using GLM and Duncan's multiple range tests was performed to compare the treatment means.

## Results and Discussion

Effect of arbuscular mycorrhizal fungi (AMF) and different rate of phosphorus on emergence and early growth of soybean is shown in Table 1. Statistical analysis showed significant differences in treatments at  $P \leq 0.05$  levels. Application AMF were significant on primary and terminal emergences, root length, chlorophyll content (SPAD), dry weight and seedling vigor. Shoot and root length of soybean seedlings were significantly lower when they were grown with AMF. Treatment of different amount of P was effected on seedling growth (Table 1). Conventionally fertilized plants had a higher weight than plants receiving a reduced dosage of fertilizer, the factor fertilization being highly significant. In contrast, the values of primary emergences and chlorophyll content (SPAD) were not significantly different from control in different amount of P treatment (Table 1).

In treatments, involving reduced fertilization (50 Kg.h<sup>-1</sup>), plants inoculated with AMF had significantly increased dry weight relative to the non-inoculated plants (Table 2). Furthermore, with the low fertilizer dosage (50 Kg.h<sup>-1</sup>), the plant terminal emergence was increased in plants, which had been inoculated with the AMF. Our results further demonstrate that, under reduced fertilizer dosage (50 kg h<sup>-1</sup>); AMF inoculation resulted in could not an improvement in shoot length and seedling vigor (Table 2). AMF are chiefly responsible for phosphorus (P) uptake – the plants may be able to use insoluble sources of P when inoculated with mycorrhizal fungi but not in the absence of inoculation - and early inoculation at the seedling stage has been proven beneficial (Medina et al. 2011).

Under the conventional fertilizer dose (100 kg h<sup>-1</sup>), shoot length, dry weight of seedling was not influenced by AM formation, but it was significantly increased under reduced fertilizer dosage. Excessive use of even these organic sources of P can result in suppression of

the AMF community (Jordan et al. 2000). Kahiluoto et al (2001) demonstrated reduced AM colonization of roots and AMF spore density in soil with increasing P fertilization for several crops on two soils with low and intermediate concentrations of available P. Careful nutrient budgeting should enable this to be avoided, optimizing fertility rather than maximizing it (Johansson et al. 2004; Gosling et al. 2006). Because of the strong influence that host P status has on the AM association, use of P fertilizers has a significant impact on the relationship between the plant and the fungus. In some areas of intensive agricultural production P fertilizer use has been well in excess of crop requirements, resulting in a buildup of total and in some cases easily available P in the soil (Withers et al. 2001; Kogelmann et al. 2004; Gosling et al. 2006; Powlson et al. 2011). However, the growth promotion mediated by AMF was decreased at this fertilizer rate. Gianinazzi et al (2010) hypothesized that when plants are grown under optimal conditions growth promotion by AMF is unlikely, whereas under suboptimal conditions enhanced growth can be achieved. It is widely accepted that AM establishment induces transcriptional changes in the host plant (Hohnjec et al. 2005; Liu et al. 2007; Fiorilli et al. 2009). Some of the changes in the host are related to modifications in the relative abundance of plant hormones, most of which are thought to play a role in the symbiosis (Hause et al. 2007). Among plant hormones, ethylene, salicylic acid, abscisic acid, and jasmonic acid are known to be key elements in fine-tuning the plant defense response during interaction with other organisms (Pieterse et al. 2009).

Treatment of different phosphorus showed that, in zero and 50 Kg.h<sup>-1</sup> of phosphorus decreases the terminal emergence of seedling and Root length, shoot length and dry weight. Bulluck et al (2002) found greater fungal species diversity under organic cultivation than under conventional cultivation with inorganic fertilizer. Enhanced plant vigor has also observed following

**Table1: Effect of AMF and different rate of phosphorus on emergence and early growth of soybean**

Source variation	Primary Emergence	Terminal Emergence	Root length	Shoot length	Dry Weight	SPAD	Seedling vigor
AMF							
No-inoculation	27.88 b	40.00 b	8.24 a	13.62 a	0.48 b	36.95 b	21.75 b
inoculation	37.2 a	58.00 a	8.94 a	12.08 a	0.54 a	43.69 a	29.21 a
Phosphorus							
0 Kg ha <sup>-1</sup>	31.16 a	46.01 b	6.48 b	11.72 b	0.41 c	39.44 a	18.16 c
50 Kg ha <sup>-1</sup>	30.50 a	48.12 b	7.05 b	12.57 b	0.50 b	40.73 a	24.15 b
100 Kg ha <sup>-1</sup>	36.00 a	54.06 a	12.28 b	15.34 a	0.62 a	40.75 a	34.13 a
Significant							
AMF (A)	363.24**	1586.7**	0.97 ns	0.27 ns	31.11**	233.5**	250.8**
Phosphorus (B)	54.05 ns	102.72*	61.34**	21.34**	0.61**	3.36 ns	390.66
A * B	67.16 ns	215.05**	0.54ns	3.94*	0.019**	2.24 ns	292.4 **
Error	23.78	20.55	1.79	0.91	0.002	3.68	14.32
CV (%)	14.98	9.17	15.58	7.25	9.70	4.87	14.85

Levels of significant: \*  $P < 5\%$ , \*\*  $P < 1\%$ , NS = not significant

**Table 2: Effect of AMF and different rate of phosphorus on emergence and early growth of soybean**

Source variation	Terminal Emergence (%)	Shoot Length (cm)	Dry Weight (g)	Seedling Vigor ( )
No-inoculation + 0 Kg.h <sup>1</sup>	38.00 c	10.72 d	0.35 c	17.66 b
No-inoculation + 50 Kg.h <sup>1</sup>	40.33 c	11.83 cd	0.43 bc	24.06 b
No-inoculation + 100 Kg.h <sup>1</sup>	41.67 c	15.85 a	0.66 a	22.34 b
inoculation + 0 Kg.h <sup>1</sup>	50.33 bc	12.73 b-d	0.47 b	18.66 b
inoculation + 50 Kg.h <sup>1</sup>	56.00 b	13.32 bc	0.57 a	24.25 b
inoculation + 100 Kg.h <sup>1</sup>	70.00 a	14.83 ab	0.59 a	45.93 a

In each treatment, means followed by the same letter are not significantly different at  $P \leq 0.05$ .

application of AMF to soybean. Results showed that application of AMF, were not affected of root length and shoot length compared the control. Nevertheless, this treatment increased terminal emergence of seedling, dry weight chlorophyll content (SPAD) and seedling vigor. Seed vigor, an important agronomic trait defined as the potential to produce vigorous seedlings. This characteristic of Seed is a measure of the quality of seed, and involves the viability of the seed, the germination percentage, germination rate and the strength of the seedlings produced (Leck et al. 2008). In theory, seed vigor may influence crop yield through both indirect and direct effects. The indirect effects include those on percentage emergence and time from sowing to emergence. These influence yields by altering plant population density, spatial arrangement, and crop duration.

Furthermore, according to results application of AMF and reduced P application by 50 Kg ha<sup>-1</sup>, significantly increased root length and dry matter of seedling compared to other plots without the AMF (Figure 2). Promotion of growth by AMF is a result of increased root area allowing the roots to explore larger volumes of soil to access nutrients, and increased solubility of insoluble compounds as well as increased availability of micronutrients (Hanson, 2000; Leck et al. 2008).

## Conclusions

The present study concludes that AMF have potential to enhance the seedling emergence in soybean, which can be useful to enhance the growth and development of soybean seedling. In other hand, AMF promoted the growth of soybean plant with increasing seedling vigor. This study demonstrated that by proper selection for AMF that are compatible with the host plant, the growth and development of the legume crop like soybean can be significantly enhanced. Low input systems such as (50 Kg ha<sup>-1</sup>) are generally more favorable to AMF have the potential to substitute for the fertilizers and biocides that not permitted in organic systems. In conclusion, the reduced-input system was more dependent on AMF than the conventionally managed, higher-input system. The effect of the AMF was influenced by the fertilizer dose.

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