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Efficiency of mycorrhizal fungi and phosphate solubilizing bacteria on phosphorus uptake and chlorophyll index in potato plantlets

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Abstract

To evaluate the effects of mycorrhizal symbiosis, phosphate solubilizing bacteria on phosphorus absorption and chlorophyll index, plantlets of two potato cultivars (*Agria* and *Sante*) derived from tissue culture were inoculated with two species of mycorrhizal fungi (*Glomus mosseae* and *G. fasciculatum*), two strains of Pseudomonas (*P173* and *P168*) and two strains of Bacillus (*Bacillus Subtilis* and *B.Megaterium*). A pot experiment was conducted using a factorial based on completely randomized design with four replications. Inoculation was carried out when plantlets were transferred to the greenhouse. In stolon initiation and tuber-inducing stages, chlorophyll index was assayed. In addition, phosphorus absorption was measured. Results showed that mycorrhizae inoculants had significant effect (on chlorophyll index and phosphorus absorption ($p \leq 0.05$). By mean comparisons, it was demonstrated that inoculation with *G. mosseae* had more positive effect on chlorophyll index and phosphorus absorption, compared with *G.fascisulatum*. Effect of cultivar showed that significant difference in P uptake and chlorophyll index in two stages.Sante cultivar demonstrated that superior except in chlorophyll content in tuber-inducing phase, compared with cv.Agria P sorption in Sante cv. occurred at a rate approximately twice cv.Agria. The highest amount of P was obtained in plantlets inoculated with *G. mosseae* and without PGPR's inoculation. Overall, inoculation of potato plantlets by the two species of mycorrhiza, Pseudomonas and Bacillus strains caused notable increase in nutrient absorption and chlorophyll contents. In turn, this effect improved the biomass of the plantlets and, consequently, helped them to acclimate better and be more efficient in minerals absorption.

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Introduction

Nowadays, application of bio-fertilizers has very importance because of increasing of crop production, maintaining sustainable soil fertility and developing sustainable farming systems. Optimal utilization of these resources, not only has positive effects on soil properties, but also has beneficial effects on the plant growth potential, retaining more soil organic-N and other nutrients in the plant–soil system, so reduces needs for consuming fertilizers. Economic and environmental benefits can result in increased income by high yields, reduced fertilizer costs and emission of the greenhouse gas (Gosling *et al.*2006; Kennedy *et al.* 2004; Dodd *et al.* 2000). Symbiosis between fungi and plant roots (mycorrhizal) by providing a source of organic carbon can leads to survival and reproduction of the fungus.This symbiotic relationship will improve plant growth, by enhancing nutrient uptake, water absorption, photosynthetic efficiency,synergistic relationship with phosphate solubilizing microorganisms, transferring elements through the hyphae to the root and plant resistance to pathogens(Frey and Scheub, 1992; Norris *et al.,* 1992; Rekha *et al.*, 2009).

Several species of *Pseudomonas* are known as the most efficient phosphate solubilizing bacteria. Bio-fertilizers improve nutritional status of the soil, secrete plant growth regulators and control soil-borne diseases (Henri *et al.* 2008; Vyaz and Gulati, 2000). So they can convert insoluble phosphate such as phosphate rock to soluble and available form for plant growth (Han and Lee, 2005).*Pseudomonas fluorescence* can promote plant growth by several mechanisms such as plant hormones production, increasing of P uptake, nitrogen fixation, production of metabolites and secretion of enzymes that regulate ethylene in plant.(Khan *et al.* 2008).

Bacillus is one of the most important microorganisms. An experiment on clover showed that dual inoculation with indigenous AM fungus and indigenous PGPR *Brevibacillus* enhanced production of shoot biomass from 18% to 35% and nutrition content (N, P, Zn and Ni). Some interactions such as grazing of the external mycelium by soil organisms are detrimental, while with PGPRs can promote mycorrhizal functions (Hodge, 2000). Linderman and Paulitz (1990) reviewed the interactions between mycorrhizae and groups of bacteria on clover and reported that synergistic effects between fungi and bacteria increase the plant growth indices. Bacteria associated with mycorrhizal fungi adhere to fungal spores and hyphal structures and so spread the rhizosphere. Wu *et al.* (2005) have been evaluated the interaction between mycorrhizal fungi and some plant growth promoting bacteria and observed that application of biofetilizers increased the growth and nutrient uptake of maize, enhanced root colonization by AM fungi and improved soil properties. Prasad and Chandra,(2003) and Gunasekaran *et al.*(2004) was investigated chickpea dual inoculation of *Rhizobium* and *P. striata*or *B. polymyxa* (PSB) and reported that these materials can increase plant growth parameters, nodulation, nitrogenase activity, and N and P uptake. However, interactions between mycorrhizal fungi and PGPRs show that dual inoculation increase biomass, N and P accumulation in plant tissues (Veronica *et al.*2006). The aim of this study was to identify the efficiency of mycorrhizal fungi and phosphate solubilizing bacteria on phosphorus uptake and chlorophyll index in potato.

Materials and methods

Plantlets preparation

Stem cuttings as single nodes were taken from sterilized plant stems using Agria and Sante potato cultivars. They were transferred into a sterile MS culture media (Murashige and Skoog, 1962) and were exposed to 16 hour photoperiod [60 $\mu molm^{-2}s^{-1}$ Photosynthetic Photon Flux Density (PPFD) provided by cool white and red fluorescent lamps] and 22±2°C temperature regimes for 3 weeks. After 21 days, the single stem plantlet were subcultured and micropropagated on the same medium to produce sufficient numbers of plantlets for the experimental trials.

Project implementation

This study was conducted to investigate the effect of

application of arbuscular mycorrhizal fungi, phosphate-solubilizing bacteria (Bacillus and Pseudomonas) on phosphorus uptake and chlorophyll index in potato minituber in 2012. This study was done in Tissue Culture Laboratory Corporation of Crouch in Isfahan and SPCRI (Seed and Plant Certification and Registration Research Institute). Inoculation with mycorrhizae fungi and PGPR was carried out in greenhouse when plantlets were transferred to the greenhouse. A pot experiment was conducted using a factorial based completely randomized design with four replications.

This experiment was included four factors, two cultivars of potato (Agria and Sante) as the first factor, two species of vesicular arbuscular mycorrhizae (*Glomus mosseae* and *G. fasciculatum*) with control as second factor, two strains of Pseudomonas(*P116* and *P173*) and two strains of Bacillus(*Bacillus subtilis* and *B.meagterium*) with their control treatments as third and forth factors in four replications.Accordingly, the experimental unit was 216 plots.It was considered 3 pots in each experimental unit and 2 plantlets per pot. Pots with diameter and depth (20 cm) were half- filled with mixture of perlite and sterile peat moss as ratio (1:1 by volume). Ten days after the plantlets establishment were kept only one plantlet per pot. When planting plantlets, amount of 5 g of root colonization of species (120 propagules per 1g fungus) as inoculums was put adjacent of roots. Plantlets were placed in a suspension solution of two strains of Pseudomonas (*P173* and *P116*) with a respectively population of 1.6×10^9 and 1.8×10^9 live bacteria per each ml (CFU ml^{-1}), and also two strains of Bacillus(*B.subtilis* and *B.megaterium*) with population of 1.9×10^9 and 1.8×10^9 live bacteria per ml (CFU ml^{-1}) in 10 minutes. After plantlets inoculation were transferred to pots. Control treatment was considered without inoculation with fungi and bacteria inoculums.

Plantlets nutrition was fertilized with 0.3 grl^{-1} 20:20:20+TE Elite fertilizer (Yara Co.) every 15 days. Plant hilling was done by addition of more growth media 45 days after transplanting (DATP).

Light and temperature conditions of greenhouse are automatically set. The photoperiod was 14 h and natural light with Photosynthetically Active Radiation (PAR) of 350-650 $\mu molm^{-2}s^{-1}$ at the top of the plant canopy supplemented with alternating high pressure sodium lamps.

Measurement

Chlorophyll content by SPAD-502 was measured in the initiation of stolon and tuber. Evaluation was conducted on the terminal leaflet of the youngest fully expanded leaves of three randomly selected plants in each plot. Phosphorus uptake was measured by spectrophotometer in random leaf samples after drying in Oven (70 ℃ and 48 h) and extraction by dry ashing method (Emami, 1996).

Data were analyzed by analysis of variance using of the Statistical Analysis System (SAS, version 9.0, 2002). Mean value were compared using Duncan's multiple Range Test at 5% probability level.

Results

According to the variance analyses (Table 1), there was significant difference ($P < 0.05$) between chlorophyll index at stolon initiation and tuber-inducing stages and also phosphorus uptake ($P < 0.01$). The effect of cultivar on uptake of phosphorus and chlorophyll index in its stolon initiation and tuber-inducing were significant at 1% level. Inoculation with *Pseudomonas* strains on chlorophyll index in tuber inducing stage was significant at 1% level but the other traits showed no significant difference. *Bacillus* bacteria had a significant effect on the absorption of phosphorus. The interaction between cultivars and mycorrhizal fungi in P uptake was significant at 1%. Interaction between variety and the strains of Pseudomonas in the statistical probability of 5% was significant on chlorophyll index at stolon initiation and also dual inoculation of mycorrhizal fungi and Pseudomonas strains. And also the interaction between mycorrhizal fungi and bacteria of the genus *Bacillus* on chlorophyll index and P uptake was significant at tuber initiation.

Table 1. Mean squares and statistical significance for the influence of mycorrhizal fungi and phosphate solubilizing rhizobacteria inoculation on chlorophyll index and phosphorus absorption of potato plantlets under greenhouse conditions.

SOV	DF	Mean Squres		
		SPAD (stolon initiation)	SPAD (tuber-inducing)	P Uptake
Cultivar(v)	1	164.97**	593.52**	0.257**
Mycorrihza (m)	2	18.31*	22.98*	0.180**
Pseudomonas (p)	2	7.81 ns	21.94*	0.002 ns
Bacillus (b)	2	3.77 ns	0.14 ns	0.022**
v×m	2	9.21 ns	4.35 ns	0.045**
v×p	2	25.76*	8.65 ns	0.004 ns
v×b	2	0.03 ns	1.35 ns	0.003 ns
m×p	4	3.81 ns	23.35*	0.001 ns
m×b	4	7.43 ns	18.97*	0.013**
p×b	4	10.81 ns	9.40 ns	0.002 ns
v×m×p	4	5.05 ns	13.42 ns	0.007 ns
v×m×b	4	9.17 ns	1.42 ns	0.004 ns
m×p×b	8	4.48 ns	3.17 ns	0.004 ns
v×m×p×b	12	2.02 ns	10.79 ns	0.005 ns
Experimental error	162	889.15	1145.82	1.26
CV	--	4.28	5.28	10.77

Chlorophyll Index at stolon initiation stage

According to the results of data analysis (Table 1), SPAD index leaves of potato cultivars were significantly different in the stolon initiation stage. Leaf SPAD index of cv.Sante was 3.17% higher than cv.Agria. Leaf SPAD index was similar in both phases by using of *G.mosseae* and this value was higher than *G.fasciculatum* applying and control treatment (Figure1). The comparison of means of interaction between variety and *Pseudomonas* strains in stolon initiation showed that the reaction of two varieties were different. So that, cv.Sante was superior compared with cv.Agria in all treatments. The highest values of this index was observed in cv.Sante compared to cv.Agria 5.17% and 3.34% ,respectively, by using of two strains of *Pseudomonas* (P173 and P116) and there was no meaningful difference with the control treatment. Agria cultivar showed the lowest SPAD index (52/84) with P173 inoculation and decrease value in this cultivar was 3/24% and 5/17% compared to control and cv.Sante, respectively, (Figure 2).

Chlorophyll Index at tuber-inducing stage

Results of this research showed that the maximum SPAD index (52.01) obtained with cv.Agria in this stage and was superior compared to cv.Sante (Figure3). *G.mosseae* inoculation showed maximum SPAD index in comparison with *G.fasciculatum* and control treatment. Among of phosphate solubilizing bacteria, the minimum of SPAD was observed in P173. According to significant interaction between mycorrhizal fungi and *Pseudomonas* strains on chlorophyll index at tuber- inducing was observed that the highest index was related to *G.fasciculatum* inoculation. SPAD index except for the compilation of *G.fasciculatum* with *P116* (Figure 4) were not significant and all were in the same statistical groups. There was significant interaction effect between mycorrhizal fungi and bacillus strains ($p<0.05$). The highest rate of chlorophyll index in the tuber-inducing was related to dual inoculation of *G.mosseae* and *B.subtilis* (51.43) that 3.75% increased in comparison with control (without inoculation with fungi and bacteria). Among of treatment combinations used in this study was determined dual inoculation of *G.fasciculatum* and *B.megaterium* and control treatment were the same with the minimum

indices. Other treatments were no statistically significant (Figure 5).

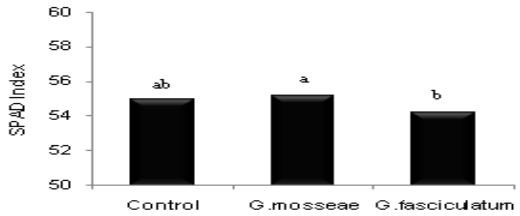

Fig. 1. Influence of Mycorrhizal fungi inoculationon chlorophyll index at stolon initiation stage.

Response of two cultivars differed in two stages with regard to chlorophyll index. Sante cultivar at the first stage and cv.Agria at the second stage had higher chlorophyll content. Overall, chlorophyll index at tuber-inducing stage was lower than stolon initiation stage (Figure 6).

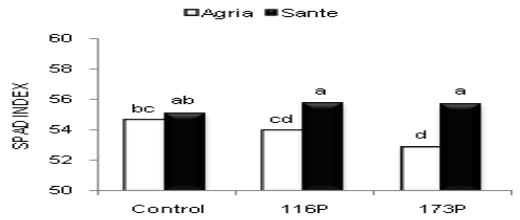

Fig. 2. Influence of Pseudomonas strains inoculation and cultivars interaction on chlorophyll index at stolon initiation stage.

Phosphorus Absorption

The greatest amount of phosphorus uptake was observed in the different treatments with mycorrhizal fungi without inoculation with *Bacillus* strains. So that in this experiment, P absorption by inoculation with *G.mosseae* and *G.fasciculatum* increased in comparison with control treatment,15.5% and 9.26%, respectively. Phosphorus uptake according to comparison of mean data of interaction between mycorrhizal fungi and *bacillus* strains were the highest (Figure 7). Interactions between mycorrhizal fungi and cultivars were statistically significant ($p<0.01$). P uptake of *G.mosseae* treatment in cv.Sante equal 25.8% and cv.Agria 14.2% increased in comparison with non-inoculated. Based on results, inoculation with *G.fasciculatum* led to P uptake increasing in cv. Sante and cv.Agria, 27.5% and 6.7%, respectively.

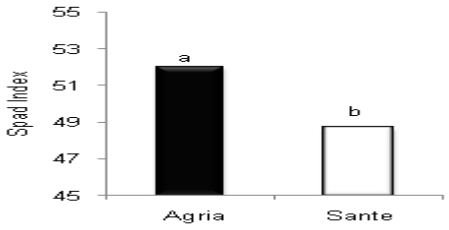

Fig. 3. Influence of cultivars on chlorophyll index at Tuber-inducing stage.

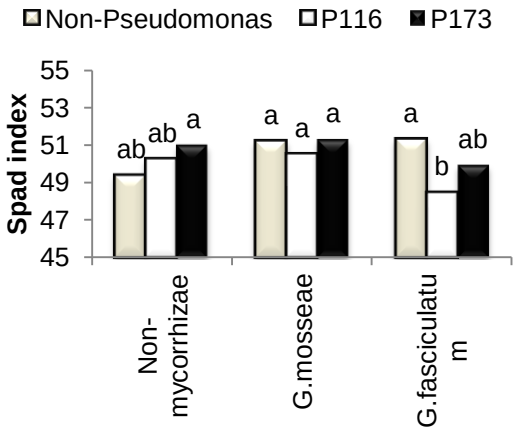

Fig. 4. Influence of Mycorrhizae and Pseudomonas strains inoculation interaction on chlorophyll index at tuber-inducing stage.

Discussion

The study revealed that chlorophyll content had different reaction to symbiosis fungi and Plant Growth Promoting Rhizobacteria (PGPR) at different growth stages of potato. Leaf chlorophyll content in stolon initiation stage was superior to tuber inducing stage. It seems that, because of creating new sink and strong (tuber) in tuber-inducing stage and sudden increasing in required assimilates, the plant was facing to lack of nutrient supplies. At this condition, plant does not have enough nutrient reserves in its organs and forced to hydrolysis chlorophylls. Accordingly, in this stage, the occurrence of chlorophyll reduction compared with stolon initiation stage is predictable .Many researches has showed that chlorophyll content in potato leaves reaches the maximum levels in earlier stages of growth, then get lower (Majic*et al.* 2008). The use of mycorrhizal fungi during the growth stages increase the chlorophyll content and lead to increase in net assimilation rate. Wright *et al.* (1998) inoculated clover with

mycorrhizal fungi and determined that the rate of SPAD index of the youngest, fully expanded leaf was significantly higher than that of non-mycorrhizal plants. In this experiment the percentage rate of photosynthesis of mycorrhizal plants was increased compared with non-mycorrhizal plants.

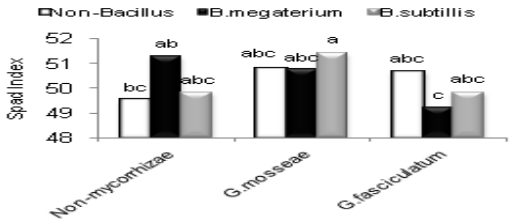

Fig. 5. Influence of Mycorrhizae and Bacillus strains inoculation interaction on chlorophyll index at tuber-inducing stage.

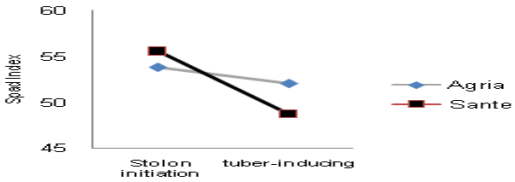

Fig. 6. Comparison of chlorophyll content at two stages.

Phosphorus is very effective in enhancing the number of potato tuber and biofertilizer inoculation caused more P absorption. Improving of P uptake was observed in our experiment with inoculation of mycorrhizal fungi and dual inoculation of mycorrhizal fungi and PSB P uptake was correlated with increase in chlorophyll content. Increment of leaf SPAD leads to increase in net assimilation rate, assimilates and ultimately will improve yield. Rudresh *et al.* (2005) announced that the availability of phosphorus had a significant effect on increase in chlorophyll content.They also cleared that mycorrhizal fungi can increase the concentration of phosphorus and rate of net photosynthesis in the host plant.

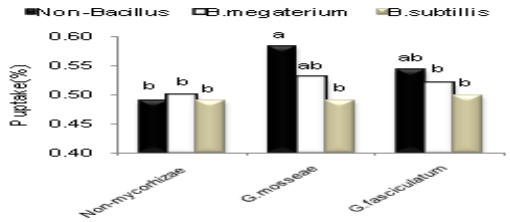

Fig. 7. Influence of Mycorrhizae fungi and Bacillus strains inoculation interaction on Phosphorus uptake

Sante cultivar demonstrated superiority almost double the amount of phosphorus uptake compared to Agria cultivar. Therefore, Sante is more efficient in phosphorus uptake than Agria. With respect to the importance of phosphorus supply for increasing production efficiency, it seems that phosphorus providing ability is one of the most achievements of bio-fertilizers.

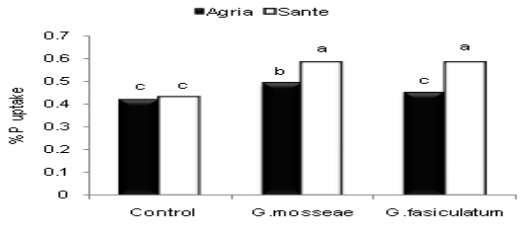

Fig. 8. Influence of Mycorrhizae fungi inoculation and Cultivars interaction on Phosphorus uptake.

Conclusion

The results of this study showed that inoculation of potato plantlets with symbiosis mycorrhizal fungi and phosphate solubilizing rhizobacteria improved chlorophyll content and efficiency of nutrients uptake, particularly in phosphate absorption.Therefore, the use of bio-fertilizers is an alternative to chemical fertilizers and should be introduced as a new approach in utilization of friendly environmental technology in sustainable agricultural.

Aknowledgment

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