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# $^{32}$P Isotope to Determine the Efficiency of Mycorrhizal Wheat Symbiosis Subjected to Saline Water

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*Phosphorus (P) behavior and its efficiency in mycorrhizal plants are of great importance. The objective was to evaluate the behavior of soil labeled P absorbed by different mycorrhizal wheat genotypes subjected to saline water. Three wheat genotypes including cultivar Kavir, the local cultivar Roshan, and the mutated line Tabasi T-65-7-1 were inoculated with different species of arbuscular mycorrhiza (AM) including* Glomus etunicatum, G. mosseae*, and* G. intraradices*. Plants were irrigated using saline water (electrical conductivity of 13.87 dS m$^{-1}$). The experiment was a factorial with 12 treatments and three replications under greenhouse conditions. Wheat genotypes and AM species significantly affected plant dry weight, specific activity, and total plant activity (*P = *0.01). A maximum of 1.49-fold increase in specific activity or P uptake per gram of plant dry matter and 3.53-fold increase in plant activity or plant total P uptake resulted by* G. etunicatum *as compared with control.*

**Keywords** Arbuscular mycorrhizal species, salinity stress, soil labeling, specific activity, wheat cultivars, $^{32}$P isotope



## Introduction

Soil salinity is one of the most common limiting factors affecting plant growth and yield, as approximately 7% of world soils are saline (Miransari and Smith 2007, 2009). The saline soils in Iran are about 44 Mha, of which 30% are located in the plains and 50% are under irrigated cultivation (El-Fouly, Zeinab, and Zeinab 2002). Salinity adversely affects plant growth by influencing soil-available water, ion imbalance, and the availability of nutrients such as phosphorus (P; Al-Karaki 2000; Miransari and Smith 2007, 2009).

Although soil microorganisms are an important part of the soil environment, their growth and activity under salinity has been less documented compared with plants (Weissenhorn 2002). Arbuscular mycorrhiza (AM) fungi are among the most important soil microbes, as their hyphae includes approximately 70% of the active soil microbial

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biomass (Mukerji and Chamola 2003). The presence of mycorrhizal fungi in saline soils and their symbiosis with their host plants indicate their tolerance to stress. This is improves nutrient uptake, which can also partially alleviate the subsequent yield reduction (Rejali 2007; Daei et al. 2009). According to Jindal and Atawal (1993), although under laboratory and field conditions, the rate of AM symbiosis decreases with increasing salinity; however, salt tolerance differs among different species and even among the isolates of AM species (Weissenhorn 2002; Daei et al. 2009).

High amounts of sodium chloride in the soil limit the potential of spore germination and also the AM hyphal growth and development. Under saline conditions AM symbiosis decreases due to the inhibiting effects of salinity on root growth, and also the ion balance and osmotic stress, thereby affecting hyphal growth (Daei et al. 2009). There are adequate data regarding the inhibiting effects of salinity on plant–AM symbiosis (Jindal and Atawal 1993; Al-Karaki 1998; 2000; Feng and Zang 2002); however, most of them were collected under controlled greenhouse conditions (Daei et al. 2009). Typically, plants differ in their sensitivity to salinity stress. Parameters such as salinity level, plant species, and growth stage determine the performance of tolerant plants under salinity compared with nontolerant plants (Neumann 1997).

The mobility of P is limited in the soil and becomes more limited due to salinity (Same, Robson, and Abbott 1983). Under saline conditions mycorrhizal plants are able to absorb greater rates of water and nutrients, and accordingly their growth is superior to nonmycorrhizal plants. Rejali (2007) found that in addition to enhanced plant growth, AM fungi are able to increase seed germination under salinity conditions, which has been attributed to enhanced nutrient uptake, especially P.

In a research work on *vicia faba* inoculated with *Glomus clarum*, with increasing salinity level to 6 ds $m^{-1}$, total nitrogen (N) decreased from 11.3 to 5.9 and from 10.9 to 3.9 mg $g^{-1}$ in inoculated and control plants, respectively. Also in all treatments the ratios of potassium (K) / sodium (Na), magnesium (Mg) / Na, and calcium (Ca) / Na decreased with increasing salinity; however, the reduction was less pronounced in inoculated plants (Rabie and Almadini 2005).

Asghari et al. (2005) evaluated the effects of mycorrhizal inoculation on the growth and nutrient uptake of *Atriplex nummularia* and found that in the sixth week of the experiment at the salinities of 2.2 and 12 ds $m^1$ plant dry matter increased by 78 and 175%, respectively, compared with noninoculated plants. This indicates that there had been significant interaction effects between AM fungi and salinity levels and that the alleviating effects of AM fungi on plant growth was intensified with increasing stress (Miransari et al. 2007, 2008).

To reach sustainable agriculture, the use of biological products such as AM fungi, which are appropriate partial replacements for P fertilizers, is of great significance. Hence it is important to determine nutrient behavior such as P in the soil and in the plant under different conditions for the appropriate estimation of fertilizer rates. This is significant both from agricultural and environmental perspectives (Bucher 2006; Miransari 2011).

The use of isotopic techniques is suggested for the precise measurement of nutrients, absorbed by the plant and for the evaluation of their movement in the plant–soil interface (Ardakani et al. 2004). This method has not been tested much for the evaluation of mycorrhizal symbiosis, particularly under salinity stress. Such method can precisely indicate the contribution of mycorrhizal fungi to plant P uptake under different stresses such as salinity. This can have important and significant implications for agriculture and environment as the control of stress become more feasible. In this background, the technique of

$^{32}$P isotope was used to precisely measure the movement and uptake of $P^{32}$ by mycorrhizal wheat genotypes, under saline conditions. Hence, the objective was to evaluate the behavior of soil labeled P absorbed by different mycorrhizal wheat genotypes subjected to saline water.

## Materials and Methods

### *Experimental Setup*

Because of the use of $P^{32}$ isotope, the experiment was conducted in a greenhouse in a semi-arid climate. Soil properties including soil texture (silty clay loam, hydrometry method; Gee and Bauder 1986), pH (7.4; Rhoades 1982), organic carbon (0.74%, wet oxidation method; Nelson and Sommers 1982), total N (0.05%, Kjeldahl method; Nelson and Sommers 1973), P (12 mg $kg^{-1}$, sodium bicarbonate extraction method; Olsen 1954), and potassium (K; 12 mg $kg^{-1}$, flame photometer method, emission spectrophotometery; Knudsen, Peterson, and Pratt 1982) concentrations were determined. Water pH (7.44), electrical conductivity (EC; 13.87 dS $m^{-1}$), sodium absorption ratio (15.5%, SAR), and the concentrations of bicarbonate ($HCO_3$; 183 mg $L^{-1}$), $Na^+$ (1893 mg $L^{-1}$), and chloride ($Cl^{-1}$; 2852 mg $L^{-1}$) were also measured (Nelson and Sommers 1982).

The experiment was a factorial on the basis of a completely randomized design with two treatments (three wheat cultivars and four fungal treatments), resulting in the total of 12 experimental treatments in replications. Wheat cultivars including cultivar Kavir (K), the local genotype Roshan (R), and the mutated Tabasi line T-65-7-1 (M) and AM species including control (M0) *G. etunicatum* (M1), *G. mosseae* (M2), and *G. intraradices* (M3) were used in the experiment.

The fungal inoculum at 100 g was weighed for each pot on 11 December 2005. A 5-cm layer of the topsoil in each pot was removed and the inoculum was applied, which was then covered with a 3-cm soil layer. Ten wheat seeds were planted in each pot and covered with two cm of soil. Before planting, urea and triple superphosphate fertilizers were applied to each pot at 0.5 g according to soil testing. The pots were irrigated with the saline water. Twenty-three days after seeding plants were thinned to five plants in each pot. The dissolved N in water, using urea at 0.3 g to each pot, was applied before stemming during plant growth.

### *Application of $P^{32}$ Isotope*

A total of 3 Mci ($\times$ 3.7 $\times$ $10^{10}$ = MBq) $P^{32}$ isotope was made to label the pot soil using orthophosphoric acid, 96 d after planting on 18 March 2007. This concentration of isotope was diluted using distilled water and applied at 1 mL $pot^{-1}$. The activity of each pot at the time of application was 58.72 Nci ($\times$ 37 = NBq). After distributing the isotope on the soil surface, water was applied to each pot according to the pot moisture so that the isotope completely reached the plant roots and no water drained.

The labeled plants were harvested at grain filling stage on 10 May 2007, after 53 days of crop growth. All plants were harvested from the soil surface, placed in bags, and dried for 48 h at 70 $^{o}$C using an oven. The plants were then weighed and milled. From each treatment, 1 g of plant dry matter was placed in a still container (plunchet). The labeled samples were then placed in a multi-low-level β counter (model FHT 770, Ember Line Co.) for the activity measurement. The samples were placed in the instrument for 1000 s and the amount of radiation was calculated for each one (based on the number of count/s, CPS).

Because the calibration of the counter method, with respect to the efficiency of standard springs for $P^{32}$ isotope, was conducted using the gas proportional system (GPS), the instrument efficiency was equal to 0.36. Using the following formula the activity of each plant sample (1 g dry matter) was calculated based on becquerel (Bq) unit:

$$\text{Activity of 1g dry matter} = \frac{\text{number of radiations/s (CPS)}}{0.36}$$

$$\text{Plant activity} = \text{activity of 1 g plant dry matter} \times \text{shoot dry matter}$$

### *Statistical Analysis*

Using SAS (SAS Institute Inc. 1988) data were subjected to analysis of variance (ANOVA) and the effects of treatments and their interactions on plants measured parameters were tested. Also comparison of means were made using Duncan's multivariate test (Steel and Torrie 1980).



## Results

The effects of wheat genotypes, mycorrhizal species, and their interaction on plant dry matter, specific activity, and plant activity were significant (Table 1). Tabasi resulted in the greatest plant dry matter (2.55 g $plant^{-1}$), specific activity (117.63 Bq $g^{-1}$), and plant activity (323.93 Bq $plant^{-1}$) followed by Kavir and Roshan (Table 2).

Compared with other AM species, *G. etunicatum* affected all measured parameters at the most. The related differences were significantly different from other species for plant dry weight and plant activity, and from *G. intraradices* for specific activity (Table 2). Compared with Roshan cultivar, Tabasi mutated line resulted in 146 and 225% increases in specific activity (P uptake per gram of plant dry matter) and plant activity (plant total P uptake), respectively (Table 2). The maximum rates of P contribution to P uptake per gram of plant dry matter were 49 and 33% by *G. etunicatum* and G. mosseae, respectively, compared with control. The corresponding values for plant total P uptake were 253 and 135%, respectively (Table 2).

**Table 1**
Analysis of variance for the effects of different experimental factors on measured parameters

| Source of variation | Degree of freedom | Mean of sum squares | | |
|---|---|---|---|---|
| | | Shoot dry weight | Specific activity | Plant activity |
| Wheat | 2 | 0.42* | 14180.89*** | 143990.39** |
| AM | 3 | 4.95** | 2271.86** | 101669.48** |
| Wheat × AM | 6 | 0.32* | 6773.94** | 60233.40** |
| Error | 21 | 0.10 | 202.75 | 256.50 |
| CV (%) | — | 13.80 | 17.63 | 7.74 |

*Significant at $P = 0.05$.
**Significant at $P = 0.01$.

**Table 2**
Mean comparisons for different plant parameters, affected by different treatments

| Treatment | Specific activity (Bq/g) | Plant activity (Bq/plant) |
|---|---|---|
| K | 72.73b | 184.22b |
| M | 117.63a | 323.93a |
| R | 47.76c | 99.58c |
| M0 | 67.79b | 100.60c |
| M1 | 101.26a | 355.38a |
| M2 | 89.93a | 236.79b |
| M3 | 65.59b | 147.75c |

*Notes.* K, Kavir; M, Tabasi; R, Roshan; M0, control; M1, *G. etunicatum*; M2, *G. mosseae*; M3, *G. intraradices*.



Analysis of the interaction effects indicated that the combined effects of all cultivars and *G. etunicatum* resulted in the greatest plant dry weight. The combined effects of Tabasi and *G. mosseae* (183.53 Bq $g^{-1}$) and Kavir and *G. etunicatum* (147.85 Bq $g^{-1}$) on specific activity were significantly greater compared with other treatments. Regarding plant activity, the symbiosis of *G. etunicatum* and genotypes Kavir (561.62 Bq $plant^{-1}$) and Tabasi (458.24 Bq $plant^{-1}$) and the symbiosis of *G. mosseae* and Tabasi (493.33 Bq $plant^{-1}$) significantly enhanced plant activity compared with other treatments (Table 3).

The effects of all combinations on plant dry weight and plant activity were significantly greater than the control; however, for specific and plant activity just for Tabasi the differences were significant. Regarding the specific activity for Roshan the combined effect of cultivar and control resulted in numerically greater values than the combined effect of cultivar and AM species (Table 2).

**Table 3**
Means comparison for the interaction effects of experimental factors on measured parameters

| Treatment | Plant dry matter (g) | Specific activity (Bq/g) | Plant activity (Bq/plant) |
|---|---|---|---|
| $KM_0$ | 0.93g | 88.73de | 113.08e |
| $KM_1$ | 3.80a | 147.85b | 561.62a |
| $KM_2$ | 2.50cde | 30.77f | — |
| $KM_3$ | 2.05de | 36.55f | 76.60f |
| $MM_0$ | 1.93ef | 54.07ef | 104.25ef |
| $MM_1$ | 3.23ab | 132.03bc | 458.24c |
| $MM_2$ | 2.67bcd | 183.53a | 493.33b |
| $MM_3$ | 2.37cde | 100.90cd | 239.87d |
| $RM_0$ | 1.33fg | 60.57ef | 84.47ef |
| $RM_1$ | 2.90bc | 39.43f | 115.02e |
| $RM_2$ | 2.40cde | 38.25f | 92.25ef |
| $RM_3$ | 2.33cde | 49.63f | 104.16ef |

*Notes.* K, Kavir; M, Tabasi; R, Roshan; M0, control; M1, *G. etunicatum*; M2, *G. mosseae*; M3, *G. intraradices*.

## Discussion

### *Shoot Dry Weight*

The greater shoot dry weight of Tabasi genotype can be attributed to the greater uptake of P (Bucher 2006). The following reasons account for significantly enhanced P uptake by mycorrhizal plants, compared with nonmycorrhizal plants (van der Heijden et al. 2006; Miransari et al. 2009a, 2009b): (1) The greater density of mycorrhizal hyphae, up to 15 times more, compared with root density, resulting in a more effective soil exploration, (2) hyphae can grow in soil pores, not available even to the finest root hairs (Smith and Read 2008; Miransari et al. 2007, 2008), (3) phosphatase production by mycorrhizal hyphae and hence P availability from mineral and organic products (Joner and Johansen 2000), (4) much greater kinetic factors ($K_m$ and $V_{max}$) for mycorrhizal hyphae, compared with roots (Jakobsen, Smith, and Smith 2002), (5) fungal interaction with root architecture (Koide 1991), and (6) mycorrhizal alteration of plant diversity (van der Heijden et al. 1998).

Because Tabasi was able to develop an efficient symbiosis with AM species, it absorbed greater rates of water and nutrients including P particularly under saline conditions (Daei et al. 2009). The enhancing effects of AM species on shoot dry weight is through increasing water and nutrient uptake, resulting in enhanced rate of photosynthesis (Tarafdar and Marschner 1994; Miransari et al. 2009a, 2009b) and hence greater production of photosynthates and improved plant growth (Miller, Megoingle, and Addy 1994). Usually under stress plants allocate more carbon to their roots (Miransari and Smith 2007, 2008; Miransari et al. 2007, 2008).

Compared with the other species of AM fungi, *G. etunicatum* was able to make the most efficient symbiosis with the wheat plants, resulting in greater shoot dry weight (Miransari et al. 2007, 2008, 2009a, 2009b; Daei et al. 2009). Under salinity stress *Sesbania* sp. inoculated with AM fungi resulted in significantly greater shoot dry weight compared with nonmycorrhizal plants (Bhoopander and Mukerji 2004; Asghari et al. 2005).

Also under drought stress the concentration of nutrients such as P in the shoot part of mycorrhizal wheat was greater than in noninoculated plants, resulting in the enhanced shoot growth and dry matter (Al-Karaki et al. 2004; Smith, Smith, and Jakobsen 2004; Rejali 2007; Daei et al. 2009). According to Feng and Zang (2002), under salinity, mycorrhizal plants alleviated the unfavorable effects of $Cl^{-1}$ on plant growth by increasing root and shoot dry weights. This has been attributed to the increased concentration of chlorophyll, P, and soluble sugars in mycorrhizal plants compared with nonmycorrhizal plants (Same, Robson, and Abbott 1983). In addition mycorrhizal plants had greater water efficiency than nonmycorrhizal plants, affecting shoot dry weight (Al-Karaki 1998).

### *Specific Activity*

The specific activity can be a suitable indicator of symbiosis efficiency because the amount and method of distribution and also the time of application for tested $P^{32}$ were the same for all the experimental treatments. Accordingly, it can be stated that the observed differences in the amount of activity per plant dry matter or the $P^{32}$ taken up can greatly indicate the activities of AM species, absorbing greater rate of nutrients including P through extending their hyphal network (Miransari et al. 2009a, 2009b).

The genetic differences in different wheat genotypes including differences in root architecture, biomass, and P transporters (Burleigh, Cavagnaro, and Jakobsen 2002; Smith, Smith, and Jakobsen 2004; Poulsen et al. 2005; Li et al. 2006) can also account for some of

the variation related to the stated differences. The enhanced plant dry matter by AM inoculation and suppressed specific activity in cultivar Roshan may indicate that AM species have been able to increase plant growth by the uptake of other nutrients rather than P.

Using $^{32}P$ isotope on white clover, AM fungi significantly increased P uptake compared with control plants (Mehravaran, Mozafar, and Frossord 2000). It was also found that mycorrhizal plants might receive greater amounts of P due to their enhanced P-absorbing surface (Poulsen et al. 2005; Bucher 2006; Li et al. 2006; Javot, Pumplin, and Harrison 2007). Majd and Ardekani (2004) examined the effects of AM species on wheat nutrient uptake using $P^{32}$ isotope. They found that the greater $P^{32}$ uptake was related to the treatments with more efficient symbiosis, indicating how the interaction effects between wheat genotypes and AM species may influence the symbiosis.

### *Plant Activity*

As the effect of treatments on plant growth, dry matter, and P uptake is more efficient, the plant activity becomes greater. Plant activity can clearly indicate plant total uptake of $^{32}P$ and plant growth. Hence, treatments resulting in greater dry weight or nutrient uptake are of greater activity or more efficient. The greater activity of mycorrhizal plants, compared with nonmycorrhizal plants, can be attributed to their enhanced root distribution and volume by AM hyphae and the previously mentioned factors (Smith and Read 2008). In addition, plant activity represents mycorrhizal efficiency, meaning that plants with greater activity have a more efficient symbiosis with mycorrhizal fungi. This is especially related to the uptake of P by the mycorrhizal host.

## Conclusion

Among different cultivars, the Tabasi mutated line was the most efficient one to absorb P; *G. etunicatum* resulted in both greater plant dry weight and greater specific activity. Regarding plant activity, which indicates the efficiency of AM symbiosis, the Tabasi mutated line could develop an efficient symbiosis with the AM species, particularly with *G. etunicatum*. Also, Kavir made an effective symbiosis with *G. mosseae,* resulting in the greatest plant activity (561.60 Bq $g^{-1}$), followed by the symbiosis of the mutated Tabasi line with *G. etunicatum, G. mosseae*, and *G. intraradices.* In addition to the determination of mycorrhizal efficiency, the right combination of AM species and wheat cultivar is also determined by this research work, which can have important implications (Daei et al. 2009). For example, with regard to the level of P in the soil, the appropriate AM species and wheat cultivar can be selected.

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