

Evaluation of the Effect of Two Symbiosis Fungi and Phosphorus Levels on Yield and Quality of *Rosa Hybrida* Cv. Dolce Vita in Soilless Culture

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ABSTRACT

According to the increasing the use of the organic fertilizers and because of the economical importance of cut-rose flower too, an experiment was designed in order to evaluate the effects of the different types of mycorrhiza and the different concentrations of P (0, 30, 50, 100) on Rose

This investigation was conducted in a greenhouse located at a *Technical*and Vocational Training center in Zibadasht area of Karaj in 1390. This experiment was arranged based on the completely randomized design with five replications. The effect of the mentioned treatments was evaluated on following factors: Phosphorus amount, membrane permeability, leaf chlorophyll, flowering stem length, viability of flower on plant, Petal sugar and Solution absorption also, the interaction effect of treatment and time on bud diameter and solute absorption.

Results of statistical analysis showed that the effect of treatment on the all of characters except the ratio of stem fresh weight on stem dry weight was significant in ($p < 1\%$) and also our result cleared that generally, the most effective treatment on evaluated factors was '*Glomus intraradices*' mycorrhiza and also, in this treatment, the concentrations of 30 and 100 had the similar operation. In conclusion the result of this experiment indicated the high effect of treatment by mycorrhiza on the studied factors.

KEYWORDS: *Rose hybrida*, Mycorrhiza, Phosphorus, Membrane permeability

1. INTRODUCTION

Roses (*Rosa hybrida*) are one of the most important ornamental plants in the world which are highly used in commercial flower industry. Throughout the history no other plant has had such a wide application and been the center of so much attention. Organic farming is becoming very necessary in today's world to control ecosystem health and to impart related human health benefits, world over there is growing demand for organic produce. In recent decades, mycorrhiza has been considered as a biological fertilizer, a cheap alternative, and environment compatible bio-solid or bio-organic for the replacement of expensive chemical fertilizer [1]. Mycorrhiza management is particularly important because it strongly influences the plant nutrition processes and the soil stabilization. The mycorrhiza is a symbiotic association between some fungi and the root of most plants [2]. Totally, the feeder root of most flowering plant growing in nature are generally infected by symbiotic fungi that do not cause root diseases; however, instead, are beneficial for their plant hosts. The infected feeder roots are transformed into unique morphological structures called mycorrhizae that is called "fungus roots" [3]. Its physiological and ecological importance in natural ecosystems and its beneficial effects on cultivated plant have been widely documented [4]. The nutrients phosphorus, nitrogen, zinc, copper and sulphur have been shown to be absorbed and translocated to the host by mycorrhizal fungi. In addition, the fungus appears to be able to absorb phosphorus at low concentrations than an uninfected plant root. Mycorrhizal fungi have active phosphate activity too. Moreover, mycorrhiza has an effect in plant growth by which changing in concentrations of growth regulating compounds such as; Auxin, Cytokinin, Gibberlins and Ethylene are made [5]. Furthermore, causing resistance in plant against pathogens has been mentioned as another important role of mycorrhiza too [6]. Phosphorus (P) is one of the key essential elements in modern agriculture. Fertilization of crops comprises the largest proportion of P used in agriculture. Phosphorus use has become increasingly prevalent during recent decades due to its depletion in soils used for crop and hay production. The importance of P to crop production systems is illustrated by the amount of fertilizer-P used during the last 35 years, which has doubled since 1960, stabilizing at slightly under two million tons/year over the last 10 year. Phosphorus has many important functions in plants, the primary one being the storage and transfer of energy through the plant. Adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are high-energy phosphate compounds that control most processes in plants including photosynthesis, respiration, protein and nucleic acid synthesis, and nutrient transport through the plant's cells. It is also known that phosphorus is essential for seed production, promotes increased root growth, promotes early plant maturity (less time for grain

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ripening), promotes stalk strength, promotes resistance to root rot diseases, and promotes resistance to winter kill. In addition to the importance of P in plant functions, the agronomic literature is full of examples of grain, fiber and forage yield increases due to proper maintenance of P fertility. Clearly, P is a necessary and beneficial input for modern crop production systems.

2. MATERIALS AND METHODS

2.1. Plant culture and treatments

The experiment was conducted under greenhouse conditions at a Technical and Vocational Training center in Zibadasht area in Karaj (Iran) in 2011-2012. The relative humidity was kept between 50 and 60%. The experiment was arranged based on the completely randomized design with five replications. Approximately, 120 scions were planted in pots in which were filled by coco peat, perlite and mycorrhiza (the mixture form of *Glomusmosseae* and *Glomusintraradices*) in volume ratio of 3; 1; 0.1 respectively. Phosphorus, in 4 levels (0, 30, 50 and 100%) was applied in pots too. Then plants were put in the green house by chance. The plants were irrigated every day regularly and about 11 days after planting nutrition solutions as follows were applied.

Stock A:

Ammonium nitrate	Fe (EDDHA6%)	Potassium nitrate	Calcium nitrate	Nutrient elements
25 gr	27gr	257gr	750gr	Dissolved in 1000 ml of water

Stock B (0% phosphorus)

Phosphoric acid	Ammonium nitrate	Copper sulfate	Boric acid	Zinc sulfate	Manganese sulfate	Potassium nitrate	Magnesium sulfate	Nutrient elements
0 cc	25 gr	0.2 gr	2.9 gr	0.85 gr	1.6 gr	200 gr	90gr	Dissolved in 1000 ml of water

Stock B (30% Phosphorus):

Phosphoric acid	Ammonium nitrate	Copper sulfate	Boric acid	Zinc sulfate	Manganese sulfate	Potassium nitrate	Magnesium sulfate	Nutrient elements
30 cc	25 gr	0.2 gr	2.9 gr	0.85 gr	1.6 gr	200 gr	90gr	Dissolved in 1000 ml of water

Stock B (50% phosphorus):

Phosphoric acid	Ammonium nitrate	Copper sulfate	Boric acid	Zinc sulfate	Manganese sulfate	Potassium nitrate	Magnesium sulfate	Nutrient elements
50 cc	25 gr	0.2 gr	2.9 gr	0.85 gr	1.6 gr	200 gr	90gr	Dissolved in 1000 ml of water

Stock B (100% phosphorus):

Phosphoric acid	Ammonium nitrate	Copper sulfate	Boric acid	Zinc sulfate	Manganese sulfate	Potassium nitrate	Magnesium sulfate	Nutrient elements
100 cc	25 gr	0.2 gr	2.9 gr	0.85 gr	1.6 gr	200 gr	90gr	Dissolved in 1000 ml of water

2.2. Flowering stems length

The flowering stem length was measured by a steel meter stick.

2.3. Petal sugar

In order to determine the petal sugar, approximately 0.2 mg of concentrated extract was mixed with 3 ml antrone and kept in a warm bathroom (100° c) for 20 minutes. The amount of light absorbed in 620 nm was measured [7].

2.4. Leaf chlorophyll

To evaluate the chlorophyll content of leaves, about 0.5 gr of leaves from each treatment were weighted and grinded in liquid nitrogen and by using 80% acetone the chlorophyll of leaves were extracted and by using a Spectrophotometer (Cary 50 UV/Vis Spectrophotometer, china) the absorption of solution was determined.

2.5. Membrane permeability

Approximately 1 gr of petals belonged to each treatment was weighted and immersed in distilled water and kept in 30° C. After 1 hour the leakage of ionic amount was recorded by an EC meter and then the mentioned solution was taken in an oven (121° C, 1.2 atm pressure) for 20 minutes and for the second time the EC of solution was measured.

2.6. Phosphorus amount

About 1 gr leaf of each treatment was weighted and kept in an oven (75°C for 72 hour). The samples were grinded and put in an electric oven for 24 hour in 55°C. 6 M HCL was added to the samples. The samples were added to the plastic containers and ammonium heptamolybdate, 40ml warm water, 30ml hot water and also 0.125 gr ammonium

vanadate added. 250 cc nitric acid 65% was added to the mentioned sample and a yellow solution prepared. The amount of light absorbed was determined by spectrophotometer in 410 nm. The amount of P was calculated by the following formula:

$$(A-B) \times v/2000w \times 100/D.M$$

A= the amount of P in prepaesample(mg/l), B= the amount of P in control, V= the final volume of extract in digestion time (ml), W= the dry weight of plant (gr)

2.7. *Solution absorbtion*

3, 7 and 11 days after sepals blooming, the volume of absorbed water, by measuring the difference of solution volume reduction was determined.

2.8. *Viability of flower on plant*

To determine the viability of flower on plant, flowers longevity on plant after sepal blooming, were recorded.

2.9. *Statistical analysis*

Statistical analysis was performed by SAS software (SAS Institute Inc., 1990). All data sets were tested for normal distribution and variance homogeneity (P = 0.05).

viability of flower on plant (Day)	flowering stem length (cm)	leaf chlorophyll (µg/ml)	membrane permeability (µs/cm)	Phosphorus amount (mg/kg)	Petal mg/gr)sugar (dry weight	df	S.O.V
^{ns} 3.01	301.93 *	^{ns} 77.91	^{ns} 5.36	^{**} 22077.55	355.034 ^{**}	2	Types of mycorrhiza
42.46 ^{**}	^{ns} 246.45	218.43 ^{**}	322.19 ^{**}	28217.33 ^{**}	22.83 ^{**}	3	Different concentrations of Phosphorus
6.208 ^{**}	194.01*	216 ^{**}	292.285 ^{**}	25444.95 ^{**}	17.261 ^{**}	6	Types of mycorrhiza × Different concentrations of Phosphorus
1.161	95.696	49.83	88.613	367.98	0.38	47	Error
11.021	14.777	21.861	11.430	19.68	6.265	-	cv

3. RESULTS AND DISCUSSION

Table 6. The effect of all treatments on evaluated factors

Solution absorption (ml)	df	S.O.V
501.694 *	2	Types of mycorrhiza
403.081*	3	Different concentrations of Phosphorus
^{ns} 112.543	3	Time
1072.768 ^{**}	6	× Type of mycorrhiza Concentrations of Phosphorus
^{ns} 800.064	6	Time ×Type of mycorrhiza
1785.304 ^{**}	27	× Type of mycorrhiza Concentrations of Time ×Phosphorus
124.722	96	Error
14.301	-	CV.

3.1. *Flowering stems length*

Mycorrhiza treatment significantly affected flowering stem length; the highest flowering stem length (71.116 cm) was determined by *Glomusmosseae* whereas the lowest flowering stem was obtained by *Glomusintradices*. The flowering stem length was not significantly affected by phosphorus treatment butthe best result was obtained by Phosphorus treatment in 50% concentration and the lowest size was in control. Both mycorrhiza and P significantly affected flowering stem length, the highest flowering stem length was obtained by *Glomusmosseae* + P 100%. It has frequently been reported in different papers that symbiotic plants has more significant increase in their growth than non-symbiotic plants [7]. It seems that symbiotic plants can absorb nutrient elements more efficiently than others [8].

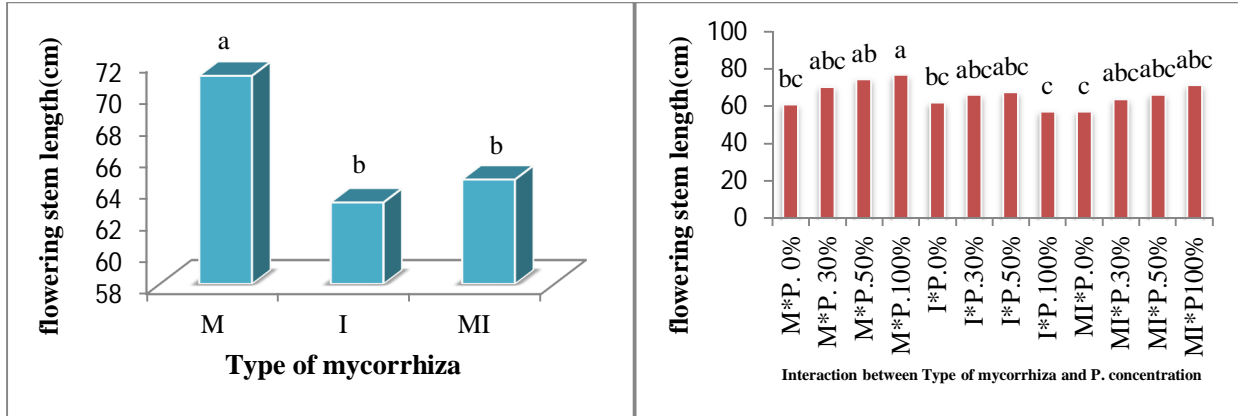


Figure 1. Effect of type of Mycorrhiza and P. concentration on flowering stem length.

3.2. Petal sugar

Petal sugar was affected by mycorrhiza treatment, P, and the combination form of mycorrhiza+ P. the highest petal sugar was obtained by *Glomusmosseae*+ *Glomusintradices*. The highest amount of petal sugar was determined by P100% and the lowest amount was achieved in control. Additionally, the highest sugar content was obtained by *Glomusmosseae*+ *Glomusintradices*+ P 30%. Totally the combination form of mycorrhiza was more efficient than others. It has previously been reported that sugar assimilation in plants suffering salt stress is more than others which are living in normal condition [9].

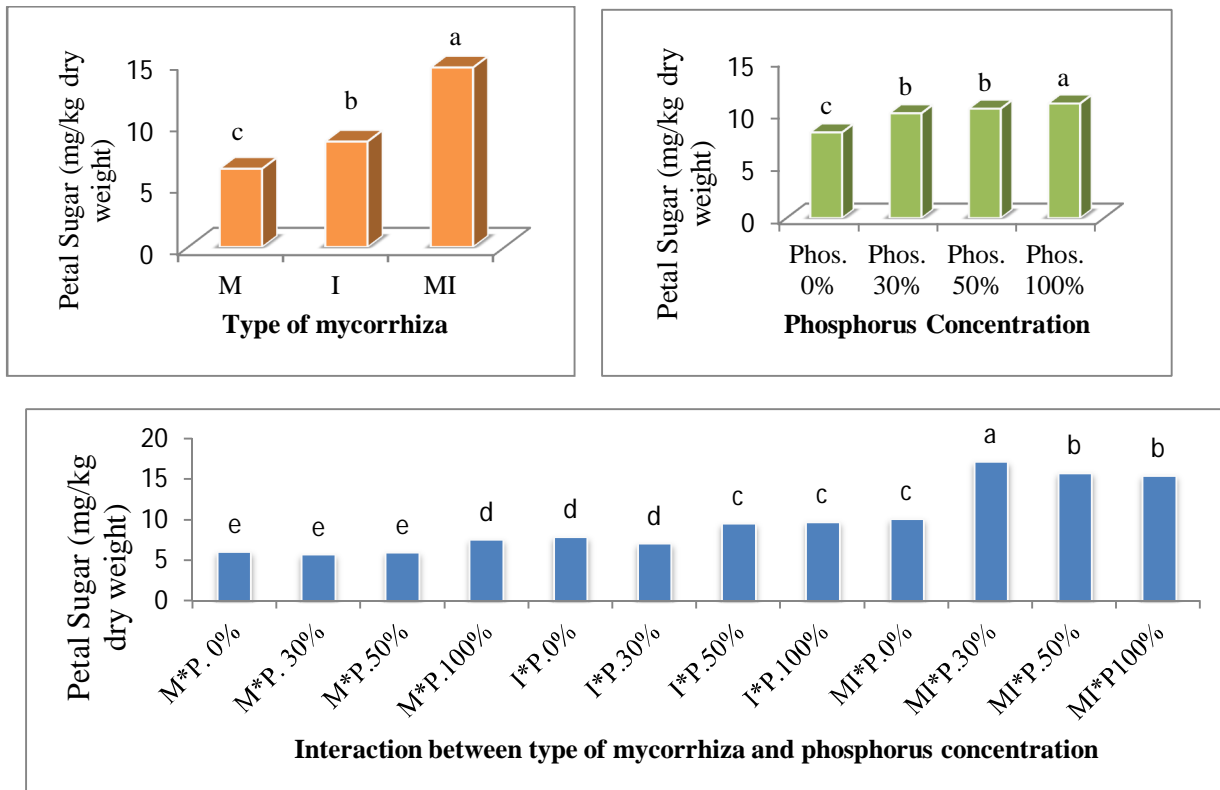


Figure 2. Effect of type of Mycorrhiza and P. concentration on petal sugar.

3.3. Leaf chlorophyll

Mycorrhiza treatment did not significantly affect the leaf chlorophyll but the highest amount of chlorophyll (22.468 Mg/l) was achieved by *Glomusintradices*. P treatment and the combination form of mycorrhiza+ P treatment

had significant effect on the amount of leaf chlorophyll. The best result was obtained by P 100%. By increasing P levels except P 30% the amount of chlorophyll was augmented. The highest chlorophyll content (35.452 Mg/l) was obtained by *Glomusmosseae* + *Glomusintradices* + P 100%. Generally the mycorrhizas which were combined with P 100% had better result. The plants which treated by *Glomusintradices* had more chlorophyll content than *Glomusmosseae*. Ruiz-Lozano et al, (1996) reported that chlorophyll content, photosynthesis rate and water use efficiency in symbiotic plants increase whereas the amount of evaporation and transpiration decrease, so that in the area with salinity stress, symbiotic plants, in addition to photosynthesis increasing and evaporation decreases augments the amount of proline in their tissues.

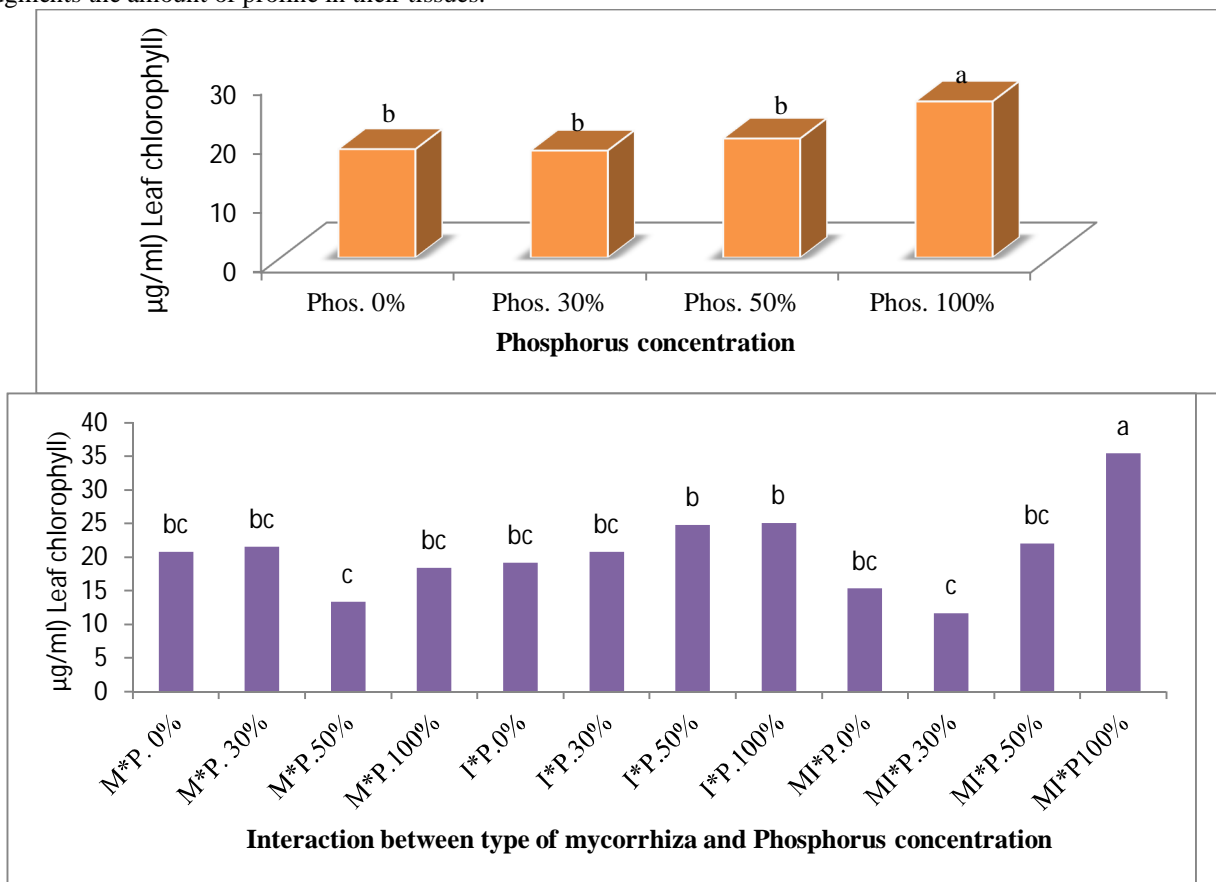


Figure 3. Effect of type of Mycorrhiza and P. concentration on leaf chlorophyll.

3.4. Membrane permeability

Mycorrhiza was not significantly effective in improving membrane permeability but the highest yield was achieved by *Glomusintradice* and the lowest yield was by the combination form of two mycorrhiza. P treatment and the combination form of P and mycorrhiza had a significant effect on membrane permeability. The highest result (86%) was obtained by P50% and by applying P0% + *Glomusintradices*. In *Glomusmosseae* the lowest yield was obtained by P100%. Jose Beltrano et al (2007) reported that plants which are treated by mycorrhiza had the highest membrane permeability than control. mycorrhiza cause a significant increase in P adsorption and by this the plants which are treated by mycorrhiza has the improved membrane permeability (H Souzu, 1986). Fujita et al (1990) reported that the plants in which there is not P deficiency, membrane permeability is increased.

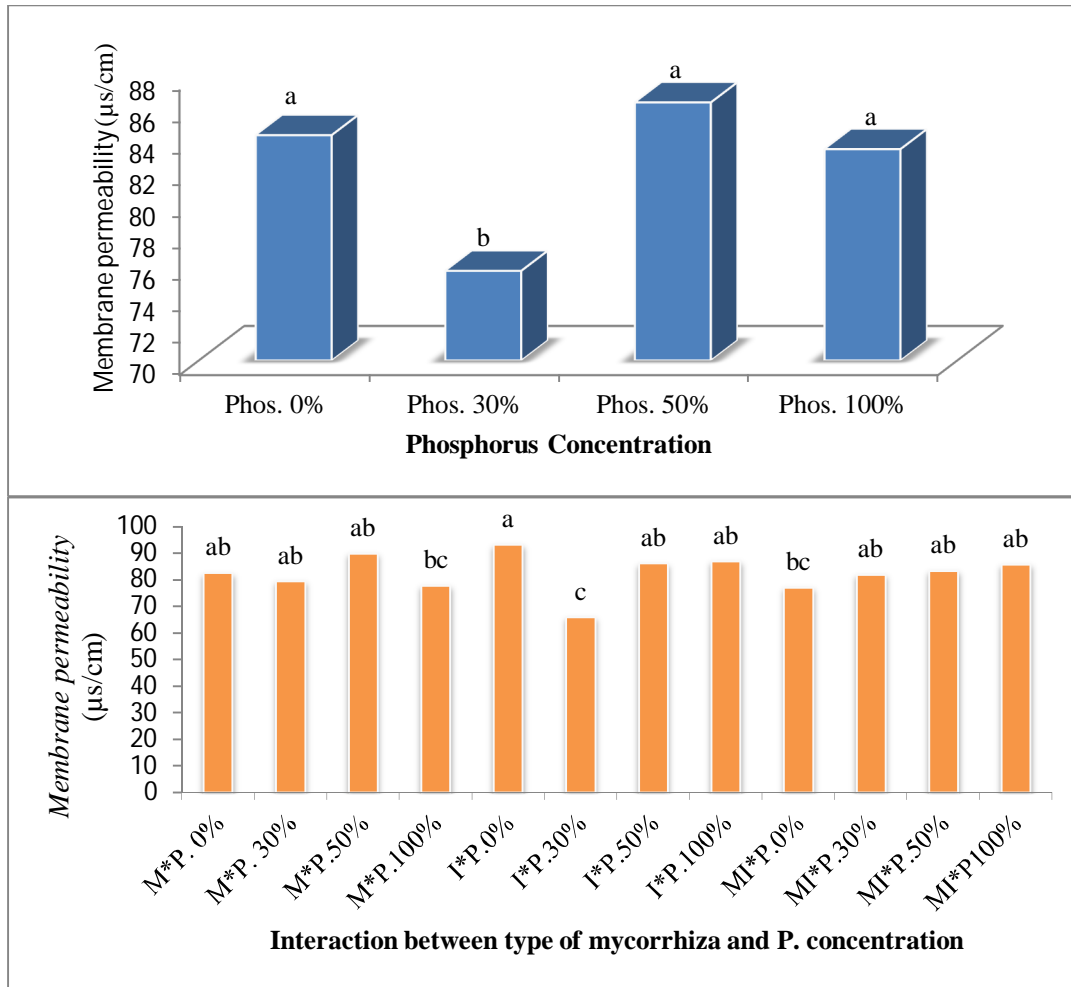


Figure 4. Effect of type of Mycorrhiza and P. concentration on membrane permeability.

3.5. Phosphorus amount

P amount was significantly affected by all treatments. The highest P amount (142.44 mg) in leaf was by *Glomus intradices* treatment and also by P 50%. By increasing P level, the amount of P in leaf increased but there was a significant decrease by P 100% that it must be due to toxic concentrations of P. The highest amount of P was obtained by *Glomus intradices*+ P50%. *Glomus intradices* by stimulation of a gene which is responsible for P absorption cause a significant increase in P absorption [10]. It seems that membrane transmission systems in fungi is more demanded to be combined with P than plant roots [11].

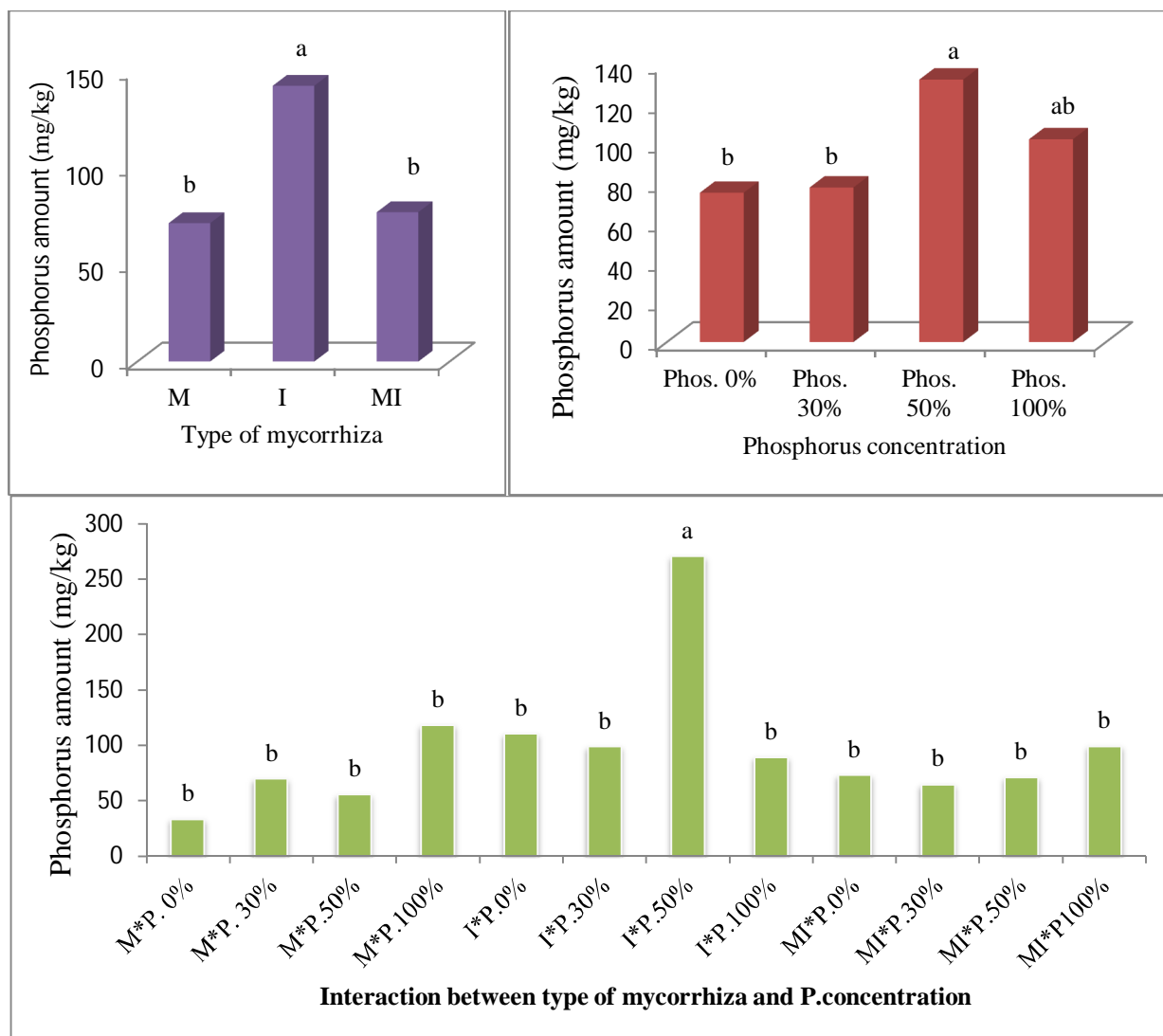


Figure 5. Effect of type of Mycorrhiza and P. concentration on leaf phosphorus amount.

3.6. Viability of flower on plant

Viability of flower on plant was not significantly affected by mycorrhiza treatment but generally, *Glomus intradices* had the best result and similarly the combination form of mycorrhiza and *Glomus mosseae* were same. P treatment was significantly efficient in viability of flower on plant and P 100% was more efficient than others. The lowest viability of flower on plant was determined in control. By increasing P levels, the viability of flower on plant augmented. By application of mycorrhiza and P treatment, *Glomus mosseae*+ P 50% had the best result.

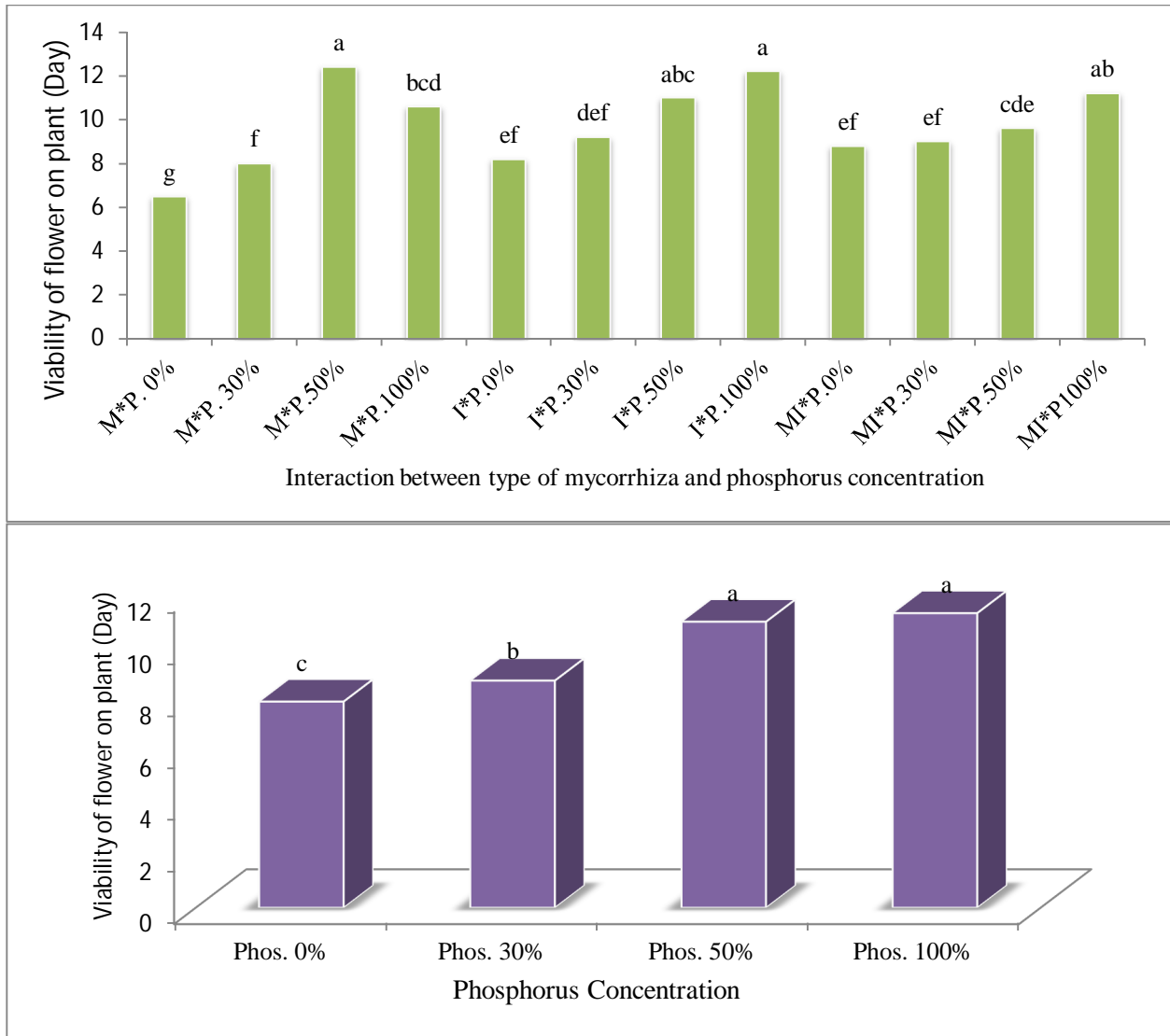


Figure 6. Effect of type of Mycorrhiza and P. concentration on viability of flower on plant.

3.7. Solution absorbtion

Solution absorbtion was affected by all the treatments. The highest solution absorbtion was obtained by the combination of two mycorrhiza treatment. the solution absorbtion was not significantly affected by P treatment but The highest solution absorbtion was obtained by P 100% . By applying mycorrhiza and P treatment, the highest solution absorbtion was determined by the combination of two mycorrhiza + P 30%. Totally, the results show that mycorrhiza especially in combined form is more effective than P in the process of increasing solution absorption. Marschener (1995) reported that mycorrhiza fungi cause a significant physical, chemical and microbiological changes in rhizosphere area that result in macro and micro elements absorbtion by plant.

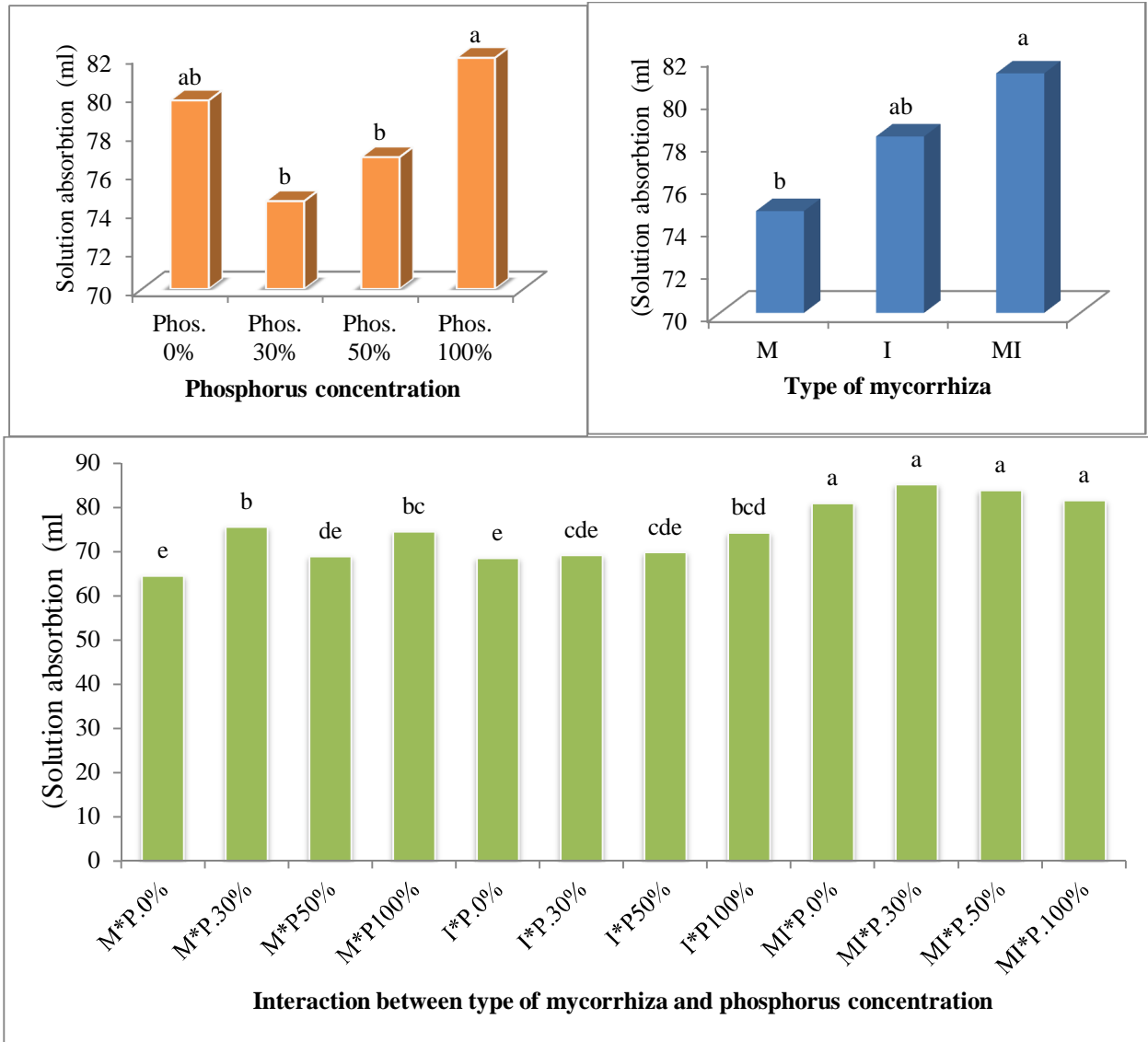


Figure 7. Effect of type of Mycorrhiza and P. concentration on solution absorption.

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