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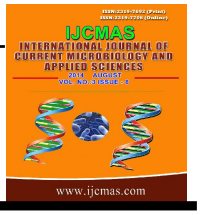

Original Research Article

Effect of phosphorus sources and mycorrhizal inoculation on root colonization and phosphorus uptake of barley (*Hordeum vulgare L.*)

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A B S T R A C T

Phosphorus (P) is the most limiting nutrient after nitrogen (N) for crop growth in many countries. Mycorrhizas (M) have a potential role to increase soil P supply and reduce dependence on expensive fertilisers. During 2010 and 2011, field experiments were carried out on spring barley (cv. Static) at Henfaes Research Centre at Bangor University, Wales, UK. The objective of this study was to further understand the role of mycorrhizae (M), and external P sources including super phosphate (SP), struvite (AMP) and rock phosphate (RP) on phosphorus availability in soil and their effects on P uptake in barley. Field experiments on low P status soils in showed the potential for the use of M in mobilizing P from soil and significantly ($P < 0.05$) enhancing P uptake of barley plant parts. These studies demonstrated the potential of mycorrhiza to mobilise P from low P status soils, enhance colonization and P uptake of plant. However, applications of P fertilizers reduced the colonization of roots by mycorrhiza. The potential role of P uptake enhancing mycorrhizain reducing external inputs in agriculture is discussed.

K e y w o r d s

Mycorrhiza, barley, phosphorus uptake, root colonization

Introduction

The majority of land plants (more than 90% all species) form a symbiotic association with soil fungi called mycorrhiza (myco = fungus, rhiza = root) (Sylvia, 1999). Mycorrhizae are a form of fungi that are able to grow and survive colonized with the host green plants. Both the fungus and the plant benefit from the association. Mycorrhizae take carbohydrates (sugars) from the plant in return for improving the supply of plant nutrients and water from the soil. In this relationship, mycorrhizae take over the role of the plant's root hairs and actions as an extension of the root system (Jakobsen and Abbott, 1992; Pellerin *et al.*, 2007).

Vesicular-arbuscular mycorrhizal (VAM) fungi

Vesicular-arbuscular mycorrhizal (VAM) fungi occur very commonly in an extensive range of plants and soils. VAM consists of three fungal structures: the root, the fungal structure that is found inside the root cells and hyphal mycelia in the soil (Smith &

Read, 1997). Several researchers (Pan et al., 1998;Mehrotra, 2005;Pellerin et al., 2007) have concluded that mycorrhizae are capable of taking up, translocating and transferring water and nutrients (such as nitrogen (N) phosphorus (P), potassium (K), calcium(Ca) magnesium(Mg), manganese (Mn), sulfur (S), iron (Fe), zinc (Zn) and copper (Cu)) from soil to the roots of plants. Likewise mycorrhizae play an important role in absorption of immobile forms of nutrients, especially P, Cu and Zn.

The main mechanism by which VAM are involved in nutrient acquisition is through the development of the depletion zone that forms 10–12 cm from the root surface around the root system through the external hyphae(Abbott & Robson, 1982;Augé, 2001). In general plant root hairs can take up P from a depletion zone extending only to approximately 1-2 mm around the roots (Jakobsen et al., 1992). Thus, the mycorrhizal hyphae can extend in the soil around the root system (rhizosphere) and enhance plant's ability to explore a given volume of soil, thereby increasing the availability of nutrients and water to the roots of the plant and also increasing P transport.(Jakobsen & Abbott, 1992; Dorneles et al. 2001).

Materials and Methods

Experimental site and treatments

The field experiments (1 and 2) were conducted at the Henfaes Research Centre of Bangor University, in Abergwyngregyn, 12 km east of Bangor city, North Wales, United Kingdom (53.14^0 N, 4.01^0 W) with a temperature hyperoceanic climate and a seasonal temperature varying between -3 to 10 ^{0}C in winter and 12 to 25 ^{0}C in summer and the annual rainfall of about 1000 mm). During the first season, 2009-2010, the effects of biological P (M and PSB) and natural sources of phosphorus (RP) on growth and production of barley (Hordeumvulgare L. var. Static) were evaluated. There were seven P-source treatments plus a control (zero P) of P fertilizers treatments of rock phosphorus (RP), phosphorus solubilising bacteria: Pantoea agglomerans and Pseudomonas putida (PSB) from Barvar 2 Iran, vesicular arbuscular mycorrhiza (M) called Bio-root from Glenside Ltd (the details are commercially confidential), RP+PSB, RP+M, PSB+M and RP+PSB+M.

The second experiment conducted during the season 2010-2011 investigated the effects of mycorrhiza on efficiency of inorganic P and RP. PSB could not be sourced in this season. A 2×4 factorial experiment was used with mycorrhiza and P source being the two factors first factor include one with no mycorrhiza or external P (C) and one with just mycorrhiza (M).The three external P sources plus control (non P fertiliser) comprised the second factor and included rock phosphate (RP), triple super-phosphate (TSP), ammonium magnesium phosphate (AMP, Struvite, a waste product from the water industry) and without P fertiliser. .

Experimental soil

The soil at the field study site was sandy loam, which had received no P fertilizer for years. Soil samples were taken at depths of 0-30 cm after removing 3 cm of the soil surface and used for soil analysis. Initial soil analysis showed as being a low-P containing soil (soil P index = 1; rated according to DEFRA (2010); available P 10.6 mg L-1; (analysed according DEFRA (1994), available K at 90 mg L-1 and available Mg at 42 mg L-1 and pH was 6.4. The experimental site has been sheep grazed and

without being cultivated for a considerable number of years, previously.

Experimental design

The first experimental design was a randomized complete block design with three replications. The plot size was 19.2 m^2 (10×1.92 m). Seeds were drilled in rows 12 cm apart and density of plants was intended to be 350 plant/m^2. The seeds were inoculated with M and PSB in the appropriate treatments before sowing and RP was applied with the seeds. Barley seeds were sown on 26th March, 2010 and harvested on 22th July.

The second experiment was a 2×4 factorial design with a randomized complete block layout replicated four times. The plot size was 5.76 m^2 (3×1.92 m) in rows 12 cm apart and density of plants calibrated for 600 plant/m^2then after seeds emergence the plants were thinned to 400 plant/m^2. Barley seeds were sown on 12th May, 2010 and harvested on 22th August. The seeds were inoculated with M in the appropriate treatments before sowing (A suspension of the biofertilizer of desired concentrations was prepared in 10% solution of sugar) and RP (rock phosphate was milled before addition). The recommended basal doses of N (100 kg N ha-1) as urea and K (50 kg ha-1) as K_2SO_4. All TSP, RP, AMP and half of N was applied at sowing (including N from ammonium phosphate) while the other half was top-dressed at 40 days after sowing.

Measurements and data recorded

In the first experiment samples were randomly taken at 43, 53, 64, 73, 83, 94 and 104 days after sowing in 50 cm row length of each plot to determine P uptake. The sampling procedure of the second experiment was similar to the first experiment but with five samples (at 44, 76, 91, 107 and 133 days after sowing). Stems and leaves were not separated, but recorded together as straw.

Staining and colonization of the roots

For measurement of the mycorrhiza colonization level, barley roots were washed in water to remove soil particles then cut into 1-2 cm length from five different sites around the roots randomly, and stored at 5 ºC after being fixed in 50 % ethanol for at least 24 h. The roots were cleared and stained for microscopic observation using the method of Phillips & Hayman (1970).The degrees of colonisation being determined by the grid-line intersect method (Giovanetti and Mosse, 1980).

Phosphorus analyses

The roots, leaves, stems and grains were dried (75°C for 24 h) separately for dry weight measurement. P concentration was determined after dry ashing (450°C for 24 h) using the vanadate-molybdate method (Page *et al.*, 1982).

Statistical analyses

Data were analysed by one-way and two-way analysis of variance (ANOVA) to determine the main factor and their interaction effects. Mean comparisons were conducted using Tukey test by GenStat 14th Edition, SPSS version 19, and Sigma Plot version 12 at $P = 0.05$.

Results and Discussion

First Field Experiment results (year 2010)

Root colonization

The root colonization was significantly affected by P sources in the all of eight samples (Table 1.). The highest root

colonization of the samples 2, 3, 4, 5 and 6 were most affected by M and also the highest root colonization of the samples 1, 7 and 8 were most affected by RP+M (Fig 1.).

Total P uptake

Analysis showed that the total P uptake in root, stem, leaf and ear were significantly affected by RP+PSB+M at all eight sample stages (Tables 2, 3, 4, 5 and 6). The highest amount of P uptake of all samples in root, stem, leaf and ear were affected by RP+PSB+M treatment. The highest P uptake in the root, stem and leaf were in samples 3 (64 das) and 4 (73 das) (Fig. 2, 3, 4, 5, and 6).

Second field experiment (year 2011) results

Root colonization

Analysis showed that the root colonization by mycorrhiza was significantly affected by non-biological P (NBP) and biological P (BP) and their interaction in the all of five samples (Table 7). The highest root colonization of the all fifth samples was found in M+RP (Fig. 7).

Total P uptake

Analysis showed that the total P uptake in root, straw and ear were significantly affected by non-biological P (NBP) and biological P (BP) in the all of the five samples (Tables 7, 8, 9, 10 and 11). The highest root, straw and ear P concentration of the samples and final sample were in the M+TSP and M+AMP treatments (Figs 7, 8, 9, 10 and 11).

The results of two field experiments (Fig 2.5.1) showed that M applied in combination with P sources significantly increased P uptake in the plant parts.Application of M (mycorrhiza colonization) are playing significant roles in the optimization of P solubilization (solubilisation of inorganic phosphate), increase of nutrient levels and mineralization of organic phosphate (RP) (Adesemoye & Kloepper, 2009;Heydari et al. 2009; Singh et al., 2011;Groppa et al., 2012;Fernández Bidondo et al., 2012).

The results of first field experiment showed that the combination of M, PSB and RP significantly increased P uptake in the plant parts but decreased the root mycorrhizal colonization. However RP, M and PSB treatments alone did not produce significant effects. Results indicating that mycorrhiza and P-solubilizing organisms were necessary for the plant to maximize P uptake. A secondary effect might have been development of a more extensive root system and thus enabling plants to extract water and nutrients from deeper depth, but it needed more data collection in the pot experiments to prove this.

Plants suffering from nutrient deficiency during reproductive development may totally rely on reserves within the roots, stem and leaves for nutrient concentration in seeds (Grusak*et al.,* 1999). Higher P concentration in the M+PSB+RP treatment compared to other fertilizing treatments indicated the efficiency of the bio fertilizers (M and PSB) for insoluble soil P to be released for uptake by the roots.

The results of second field experiment showed application of non-biological P fertilizers (RP, TSP and AMP) combined with M significantly increased P concentration and total P uptake. The results may be related to ability of mycorrhiza to increase nutrient uptake, especially P uptake, via mycorrhizal hyphae and extension of the root system.

Table.1 Table ofmean values,standard errors of differences and P values for data presented in Figure 1. Significant levels of P are in bold script.

Treatments	**Root colonization (%)**							
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**
C	8.2	24.2	37.2	36.8	37.8	30.3	26.8	18.4
RP	9.7	24.7	34.6	36.1	39.0	34.2	32.8	20.8
RP+PSB	12.5	23.2	29.2	34.5	34.8	35.0	33.5	19.2
RP+PSB+M	13.0	22.0	26.7	35.2	29.6	34.1	31.8	16.5
PSB+M	14.8	27.8	42.0	47.2	47.0	36.3	35.6	17.4
PSB	12.1	28.4	39.2	36.2	38.9	36.4	34.7	17.8
RP+M	16.7	33.4	45.8	49.4	48.5	40.0	35.5	22.4
M	13.5	34.2	48.0	52.3	52.3	41.3	32.1	17.1
SED	2.763	4.501	7.305	7.170	7.352	3.627	3.067	2.484
P	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**0.019**

Table.2 Table ofmean values,standard errors ofdifferences and P values for data presented in Figure 2. Significant levels of P are in bold script.

Treatments	Total P uptake in root (mg m^{-2})							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
C	10.0	41.3	78.7	115.0	136.3	86.0	56.0	56.0
RP	16.0	59.3	109.0	143.3	166.0	112.7	75.0	75.7
RP+PSB	18.3	88.0	139.7	156.7	182.3	126.0	83.7	89.0
RP+PSB+M	19.7	95.0	151.7	186.0	198.0	143.0	117.0	115.3
PSB+M	17.0	67.7	122.3	153.0	159.7	110.3	81.0	74.7
PSB	15.3	69.7	101.3	128.0	163.0	107.0	75.3	72.3
RP+M	18.0	83.3	133.0	158.0	187.7	123.0	115.0	111.3
M	14.7	65.0	97.7	146.3	165.3	111.0	79.0	74.0
SED	3.468	19.379	24.158	23.905	20.820	18.054	20.592	20.276
P	**0.005**	**0.002**	**<.001**	**0.002**	**<.001**	**<.001**	**<.001**	**<.001**

Table.3 Table ofmean values,standard errors ofdifferences and P values for data presented in Figure 3. Significant levels of P are in bold script.

Treatments	Total P uptake in stem (mg m^{-2})							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
C	4.7	36.7	122.3	498.0	526.0	430.7	293.3	96.0
RP	5.3	44.0	137.3	592.7	587.3	485.7	346.3	111.7
RP+PSB	5.3	48.3	197.3	618.0	682.3	550.0	385.7	137.3
RP+PSB+M	7.0	50.7	219.0	709.0	725.0	630.3	440.7	190.7
PSB+M	5.7	49.3	192.7	613.7	635.7	555.7	349.0	104.7
PSB	4.7	38.0	173.3	565.3	592.7	520.0	323.7	110.7
RP+M	6.3	46.0	193.7	628.0	686.7	572.0	427.0	179.0
M	5.0	41.0	136.0	592.3	582.0	527.7	334.7	104.3
SED	0.88	5.57	38.66	68.38	70.16	66.72	52.75	35.85
P	**<.001**	**<.001**	**0.001**	**0.003**	**<.001**	**0.001**	**<.001**	**<.001**

Table.4 Table ofmean values,standard errors ofdifferences and P values for data presented in Figure 4. Significant levels of P are in bold script

Treatments	Total P uptake in leaf (mg m^{-2})							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
C	42.0	133.9	189.0	177.2	140.7	92.6	72.5	40.0
RP	47.5	181.2	211.7	245.3	181.0	109.7	86.8	55.9
RP+PSB	63.1	213.8	255.0	256.5	198.5	121.5	90.3	58.7
RP+PSB+M	69.1	220.9	299.8	279.3	229.9	147.4	124.0	90.2
PSB+M	46.9	191.3	258.0	261.4	191.7	122.1	87.6	62.9
PSB	47.7	138.5	215.3	233.9	178.4	99.7	78.1	51.6
RP+M	43.8	196.2	257.6	255.8	198.8	117.5	119.0	79.7
M	56.5	176.9	224.8	246.3	176.3	111.3	86.2	57.1
SED	10.0	32.7	38.6	31.9	27.8	17.8	19.3	16.1
P	**<.001**	**<.001**	**0.001**	**<.001**	**0.001**	**<.001**	**<.001**	**<.001**

Table.5 Table ofmean values,standard errors ofdifferences and P values for data presented in Figure 5. Significant levels of P are in bold script.

Treatments	Total P uptake in ear (mg m^{-2})			
	Sample 5	Sample 6	Sample 7	Sample 8
C	197.9	583.8	897.5	1278.9
RP	262.3	682.5	1049.9	1436.1
RP+PSB	318.4	751.0	1088.1	1560.8
RP+PSB+M	368.2	835.8	1253.2	1630.3
PSB+M	321.1	748.9	1112.4	1478.6
PSB	268.0	686.3	1017.0	1386.6
RP+M	344.1	792.6	1106.6	1528.5
M	282.2	703.0	1069.6	1449.6
SED	65.1	81.2	114.4	121.8
P	**0.012**	**<.001**	**0.003**	**0.001**

Table.6 Table ofmean values,standard errors ofdifferences and P values for data presented in Figure 6. Significant levels of P are in bold script

Treatments	Final P uptake (mg m^{-2})			
	Root	Stem	Leaf	Ear
C	56.0	96.0	40.0	1278.9
RP	75.7	111.7	55.9	1436.1
RP+PSB	89.0	137.3	58.7	1560.8
RP+PSB+M	115.3	190.7	90.2	1630.3
PSB+M	74.7	104.7	62.9	1478.6
PSB	72.3	110.7	51.6	1386.6
RP+M	111.3	179.0	79.7	1528.5
M	74.0	104.3	57.1	1449.6
SED	20.28	35.85	16.09	121.85
P	**<.001**	**<.001**	**<.001**	**0.001**

Table.7 Table ofstandard errors ofdifference and P values for data presented in Figure 7. Significant levels of P are in bold script.

Treatments	Root colonization (%)									
	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
	SED	P	SED	P	SED	P	SED	P	SED	P
Non-Biological P	0.571	**<.001**	2.579	**<.001**	1.630	**<.001**	1.978	**<.001**	1.368	**<.001**
Biological P	0.403	**<.001**	1.824	**<.001**	1.152	**<.001**	1.398	**<.001**	0.967	**<.001**
Non-Biological P* Biological P	0.807	**<.001**	3.648	**0.001**	2.305	**<.001**	2.797	**0.017**	1.934	**<.001**

Table.8 Table ofstandard errors ofdifference and P values for data presented in Figure 8. Significant levels of P are in bold script

Treatments	Total P uptake in root (mg m^{-2})									
	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
	SED	P	SED	P	SED	P	SED	P	SED	P
Non-Biological P	6.470	**<.001**	5.820	**<.001**	6.990	**<.001**	7.290	**0.001**	2.930	**<.001**
Biological P	4.570	**<.001**	4.120	**<.001**	4.940	**0.008**	5.150	**0.013**	2.070	**<.001**
Non-Biological P* Biological P	9.150	0.283	8.240	**0.007**	9.880	0.276	10.300	0.173	4.150	0.590

Table.9 Table ofstandard errors ofdifference and P values for data presented in Figure 9. Significant levels of P are in bold script

Treatments	Total P uptake in straw (mg m^{-2})									
	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
	SED	P	SED	P	SED	P	SED	P	SED	P
Non-Biological P	37.3	**<.001**	34.8	**<.001**	48.6	**<.001**	26.4	**<.001**	24.2	**<.001**
Biological P	26.4	**<.001**	24.6	**<.001**	34.3	**<.001**	18.6	**<.001**	17.1	**0.046**
Non-Biological P* Biological P	52.7	0.305	49.1	0.246	68.7	0.557	37.3	0.548	34.3	0.493

Table.10 Table ofstandard errors ofdifference and P values for data presented in Figure 10. Significant levels of P are in bold script

Treatments	Total P uptake in ear (mg m^{-2})					
	Sample 3		Sample 4		Sample 5	
	SED	P	SED	P	SED	P
Non-Biological P	15.500	**<.001**	32.300	**<.001**	43.400	**<.001**
Biological P	10.960	**<.001**	22.800	**0.017**	30.700	**<.001**
Non-Biological P* Biological P	21.930	0.901	45.600	0.679	61.400	0.502

Table.11 Table ofstandard errors ofdifference and P values for data presented in Figure 11. Significant levels of P are in bold script.

Treatments	Final P uptake (mg m^{-2})					
	Root		Straw		Ear	
	SED	P	SED	P	SED	P
Non-Biological P	2.930	**<.001**	24.23	**<.001**	43.400	**<.001**
Biological P	2.070	**<.001**	17.13	**0.046**	30.700	**<.001**
Non-Biological P* Biological P	4.150	0.59	34.26	0.493	61.400	0.502

Fig.1 Mean values of root colonization by P source at eight samples (43, 53, 64, 73, 83, 94, 104 and 119 days after sowing). Error bars show standard error of means (n=3).

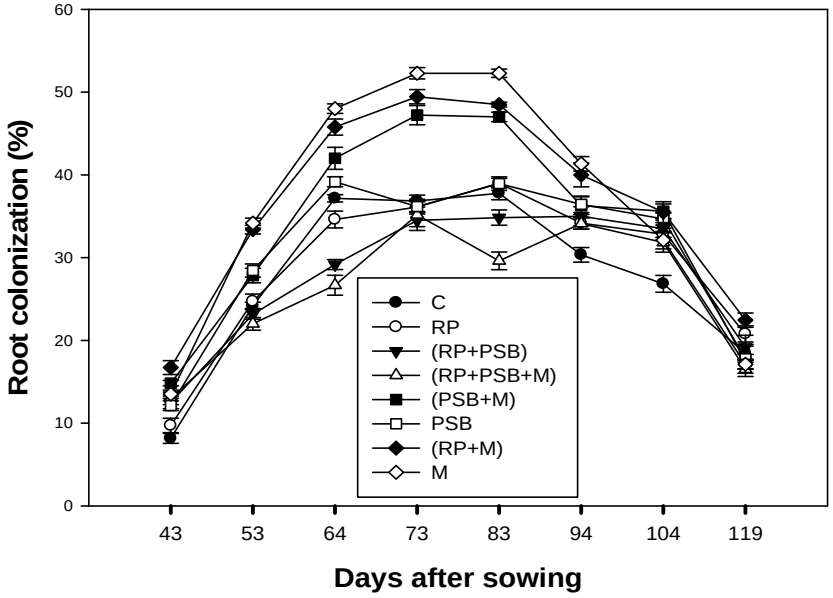


Fig.2 Mean values of P uptake in root by P source at eight samples(43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3)

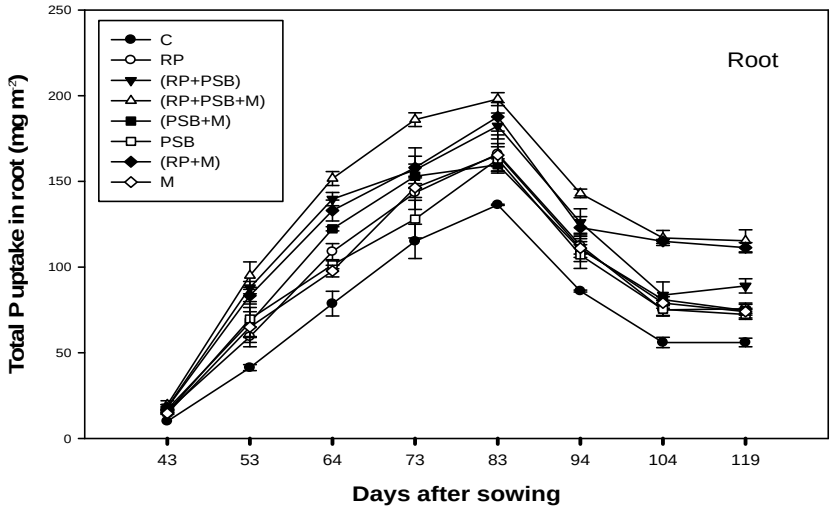


Fig.3 Mean values of P uptake in stem by P source at eight samples (43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3)

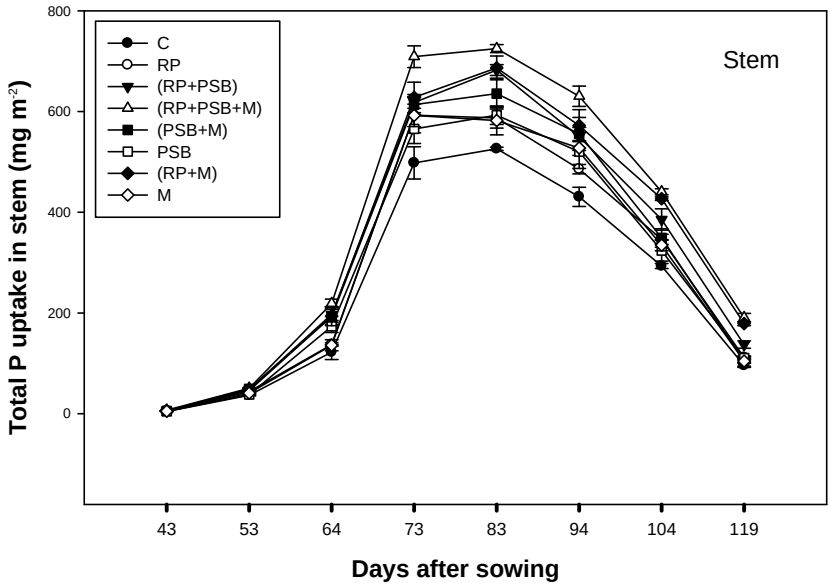


Fig.4 Mean values of P uptake in leaf by P source at eight samples(43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

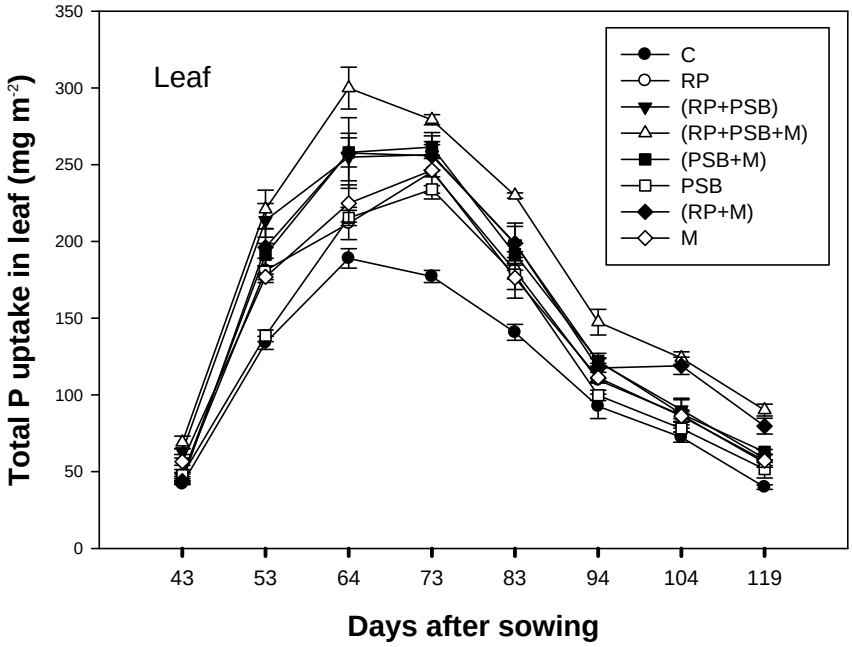


Fig.5 Mean values of P uptake in ear by P source at four samples(83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

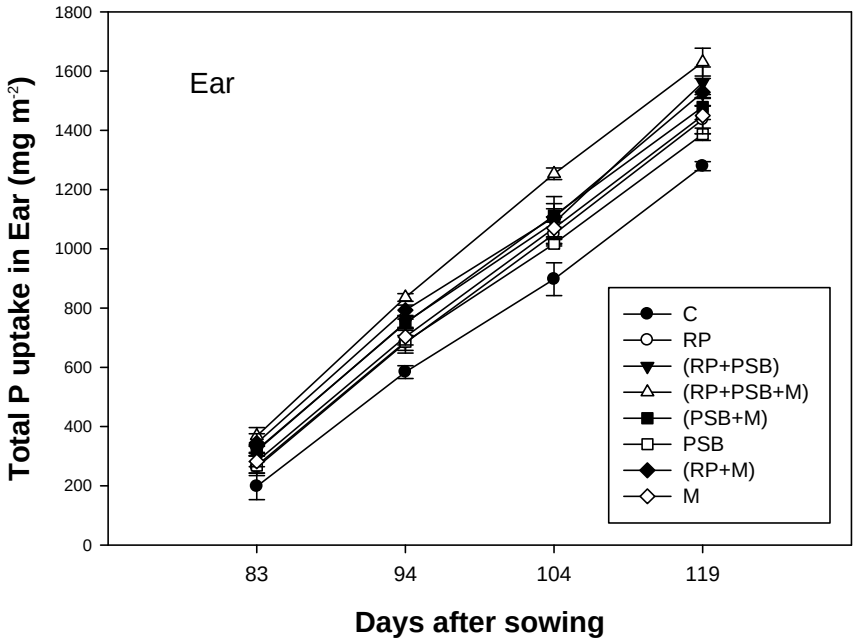


Fig.6 Mean values of total P uptake in root, stem, leaf and ear by P source at the final sample.Error bars show standard error of means (n=3).

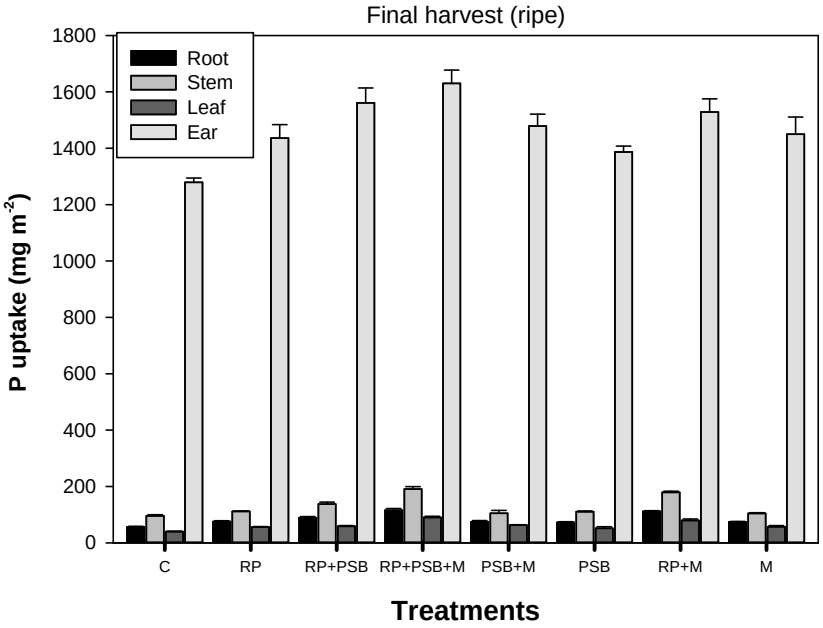


Fig.7 Mean values of root colonization by P source at five samples (44, 76, 91, 107 and 118 days after sowing). Error bars show standard error of means (n=4).

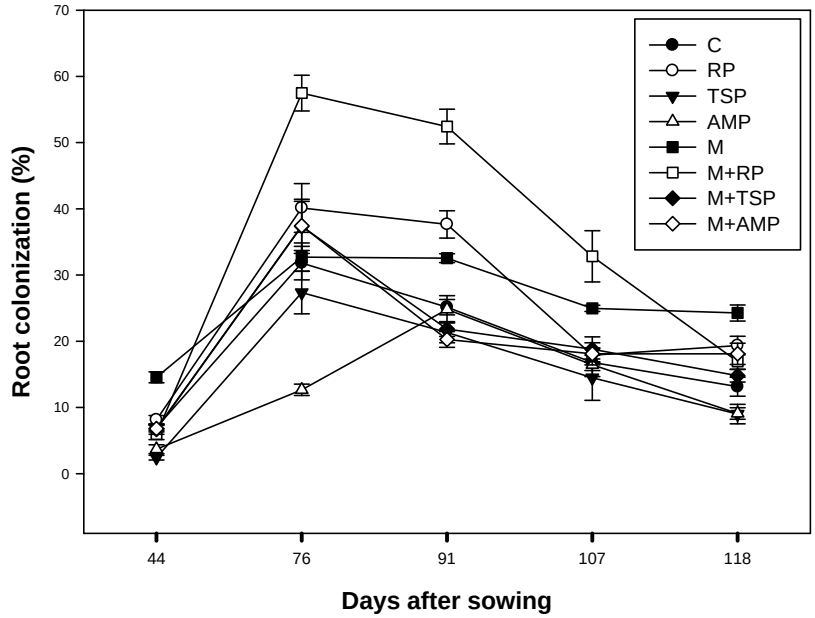


Fig.8 Mean values of P uptake in root by P source at five samples(44, 76, 91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

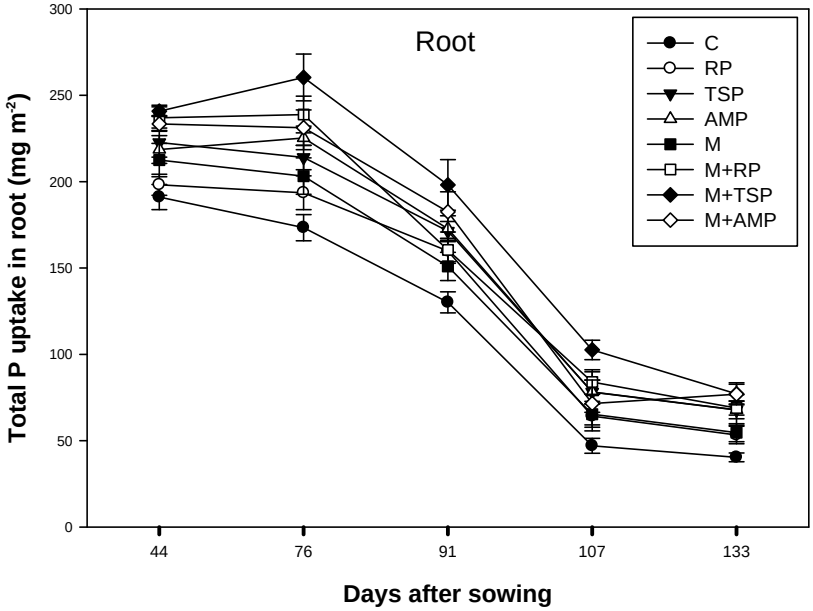


Fig.9 Mean values of P uptake in straw by P source at five samples(44, 76, 91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

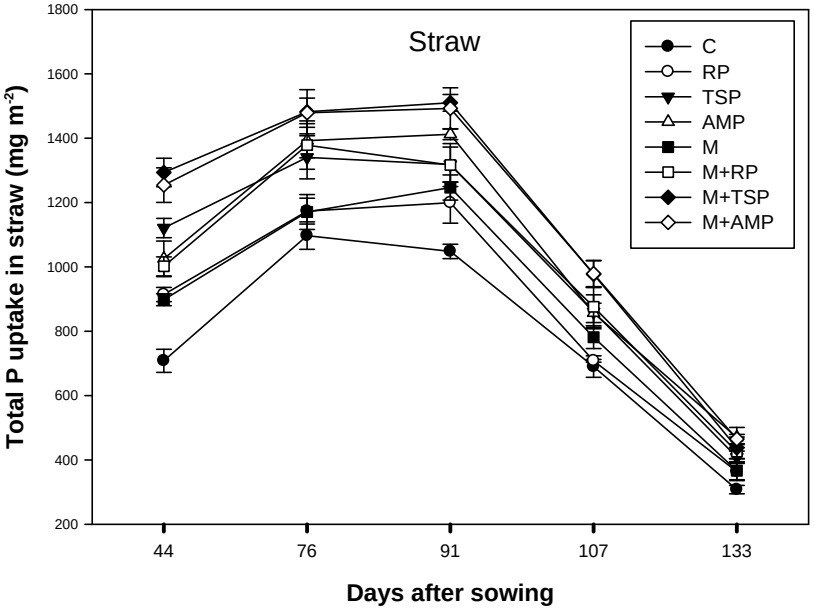


Fig.10 Mean values of P uptake in ear by P source at 3 samples(91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

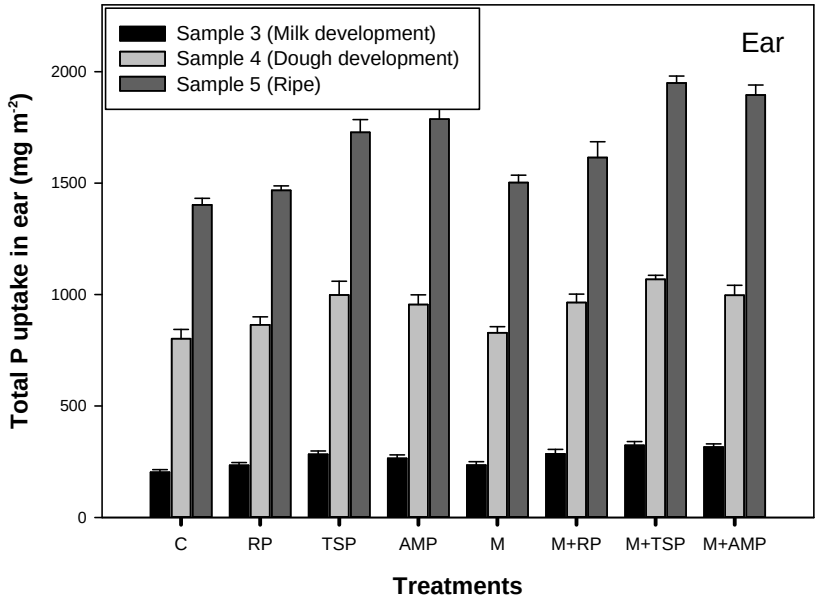
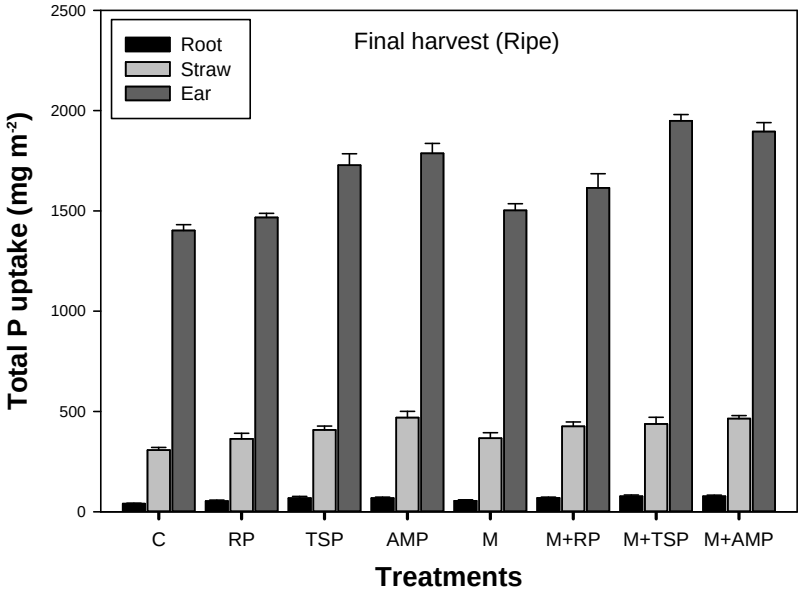


Fig.11 Mean values of P uptake in root, straw and ear by P source at final sample (133 days after sowing).Error bars show standard error of means (n=4).



Several researchers (Mehrotra, 2005;Pellerin et al., 2007; Li et al., 2011; Bongard, 2012) have concluded that mycorrhizaeare capable of taking up, translocating and transferring water and nutrients from soil to the roots of plants. Likewise mycorrhizae play an important role in absorption of immobile forms and limited forms of nutrients, especially P by mechanisms of release of organic acid and development of the depletion zone that from the root surface around the root system through the hyphae.

Thus, the use of mycorrhiza could allow the achievement of satisfactory crop growth and P uptake with reduced amounts of non-biological phosphorus fertilizers, and so decrease of fertilization costs and environmental pollution. For these reasons the application of M with non-biological phosphorus fertilizers (organic and inorganic p fertilizers) could be encouraged in barley growing and P uptake.

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