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The Role of Phosphorus Sources on Root Diameter, Root Length and Root Dry Matter of Barley (*Hordeum vulgare* L.)

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Abstract

Roots are the main plant organs that supply nutrients, water, hormones and physical support for the plant. Phosphorus is one of the most limiting and important elements in root growth and crop production. The aims of this study was to investigate the effects of different sources of phosphorus treatments on root growth (root length, diameter and dry matter) of Barley. The two glasshouse pot experiments results showed that under P deficiency, the weight of dry root significantly decreased and the total root length of whole plant significantly increased with decrease of root diameter. Our results suggested that soil fertility and root structure are widely recognized as important role of the soil community and plant growth, the root structure and root extension can directly and indirectly affected by soil fertility and specially P nutrient of the soil. Accordingly, root characteristics can determine the circumstance of plant growth and crop production.

Keywords: Plant root growth, phosphorus deficiency, root characteristics, root morphology

1. Introduction

Soil nutrients and water are taken up by plant roots through the rhizospher, the plant rhizosphere is an important region that replete with rooting depth, distribution and microorganisms, which is the communication zone between plant and soil. Therefore, root development, architecture and rhizosphere processes have great effect on water uptake, soil nutrient uptake, transformation, mobilization, and efficient use by plants (Marschner, 2012; Shen et al., 2013; Jha et al., 2017). Root length per unit of the plant's dry mass is constituted by means of root mass ratio, the distribution component and root excellence and tissued density, the physical components (Ryser, 1998). Plants can produce longer roots either by growing biomass supply or root fineness and/or decreasing root tissue density, leaving biomass distribution unchanged (Manschadi et al., 2014; Abenavoli et al., 2016; Colombi et al., 2017).

Plant root can not only exceedingly control morphological traits to adaption of soil environmental conditions, but also considerably modify rhizospher developments through thir physiological actions, mainly the exudation of phosphatases, organic acids and several signalling substances, redox changes and proton release (Hinsinger et al., 2009; Marschner, 2012; Shen et al., 2013; Zhang et al., 2013; Gul and Whalen, 2016). The effectiveness of root and rhizospher is extremely dependent on natural soil fertility and the amount of soil nutrient supply, which is organized by the response of external nutrients. Therefore root growth and development can be significantly constrained when the available soil nutrient is exceedingly low (Li et al., 2008; Zhang et al., 2010; Manschadi et al., 2014). Roots are able to enhance soil organic matter by contributing to the soil resources of nitrogen, organic carbon and microbial biomass (Xie et al., 2014; Rasmussen et al., 2015; Munda et al., 2016; Maji et al., 2017). Accordingly, an understanding of factors that affect root growth and development are important for improving nutrient cycling and uptake from soil to plant. The growth and development of the fine root system is necessary for good plant growth and development and consequently for satisfactory production (Mollier and Pellerin, 1999; Gao et al., 2010; Gahoonia et al., 1997; Fageria and Moreira, 2011; Shi et al., 2013).

The capability of plant roots for P achievement is one of the main factor for root and plant growth. The major characters and processes that lead to improved P acquisition include greater root growth and higher root to shoot biomass ratio, improved root architecture, development of root architecture, development of root hairs, increased production and exudation of phosphatases, secretion of organic acids, increased expression of P transporters, and symbiotic relations with mycorrhizal fungi and bacteria (Vanceet at al., 2003; Ramaekers et al., 2010; Richardson et al., 2011; Meyer et al., 2017). It is widely documented that succsesful P acquisition on efficiency will consequence in laeg gains in P use effective and crop yield, subsequently on average only 15-30% of applied P fertilizer is uptake by crops in the year of fertilizer application (Tilman et al., 2002; Syers et al., 2008). Therefore, more than 30% of soils cultivated in the world suffer from P deficiency (Hinsinger, 2001). Resources of mineral phosphate are becoming limited, and recent global reserves may be depleted in 50-100 years (Cordell et al., 2009). Since the main organ for water and nutrient uptake, root play an important role in P uptake from soil. Plant able to adapt to low P stress by exchanging root physiology and morphology (Lynch and Brown, 2008; Lambers et al., 2006). Charasteristic root morphology reactions to low P stress contain a higher root to shoot ratio (Hermans et al., 2006; Ramaekers et al., 2010), a greater number of lateral root (Liu et al., 2004; Zhu and Lynch,

2004), extended total root length (Liu et al., 2004; Shen et al., 2013), additional root hairs (Gahoonia et al., 2001; Ma et al., 2001), finer roots (Wissuwa, 2003; Marschner, 2012), expansion of root cortical aerenchyma (Hinsinger et al., 2009; Postma and Lynch, 2010), activity of microorganisms (P soilobilizing bacterias) in the soil (Gyaneshwar et al., 2002; Patel et al., 2011), and mycorrhizal symbiosis (Bolduc and Hijri, 2011; Doubková et al., 2012; Rodríguez-caballero et al., 2017).

Bio-inoculants can be considered as a type of Bio-fertilizer. Bio-inoculants include living organisms that enhance the nutrient acquisition of the host plant through their continuous presence within the plant's rhizosphere (Chen, 2006; Mirzaei Heydari, 2013). Many plants have benefited from an association with micro-organisms under P-deficient conditions. These associations can result either in better uptake of the available P in the soil, or in rendering unavailable P sources accessible to the plant. Bio-inoculants utilize single or multiple strains of naturally occurring microorganisms to change essential elements, such as P, from unavailable to available forms via biological and chemical processes (Richardson, 1994: Zheng, et al., 2011; Bokhorst et al., 2017). Bio-inoculants can be beneficial, and various claims have often been made about their ability to promote plant and root growth and decrease the need for chemical fertilizers (Rai, 2006; Magadlela et al., 2017; Sihi et al., 2017).

Root growth and development are under genetic control, but environmental factors such as mineral nutrition and soil physical conditions can also effect on root growth. Root growth and development are very important for early P uptake of plants. P is therefore hormone regulated according to the phosphorus status of the plant (Romer et al., 1986; Zhu et al., 2003; Fageria and Moreira, 2011; Zeng et al., 2014). Because roots are out of sight in the soil and difficulties associated with extracting whole root systems from the soil, information on total root length of crops and phosphorus effects are limited. Therefore measurement and analysis of relations between phosphorus, root diameter, total root length and root dry matter may be useful for finding their relations to crop yield. Little is known about the effect of P fertilization on roots diameter and total root length in barley which this study attempted to elucidate.

The aim of the present study was to clarify the root architecture and the comparison between root diameters, total dry root weight of plant and total root length of whole plant in barley under different P treatments.

2. Materials and methods

2.1. Experiment 1: Horticultural sand experiment

2.1.1. Experimental design and culture practices

The first pot experiments were conducted in a horticultural fine, silver sand (easy extracting whole root systems from the horticultural sand and able to the nutrition) (Table 1.) with nutrient supplied by Long Ashton nutrient solution (Hewitt, 1966). Plant were grown in a glasshouse (12/25 C night/day mean air temperature; additional light, approximately 120 µE s^{-1} m^{-2} was supplied by high pressure sodium lamps for 16 hd^{-1}), watered with water distilled reverse to 10% w:w by osmosis every 2 days.

((Table 1))

The experiment was a completely randomized 3×4 factorial design with 9 replicates per treatment with 3 replications each being harvested at three sampling date. There were three biological P treatments (vesicular arbuscular mycorrhizal inoculum (M) called Biagro from Glenside Company Starling that located in Scotland), phosphorus solubilising bacteria: Bacillus Spez (PSB) from Green Max Agro Tech Company that located in Germany and no M & PSB (Control)), four fertilizer P treatments (no P (control), sodium dihydrogen orthophosphate = $NaH_2PO_4.2H_2O$ (SP), ammonium magnesium phosphate = Struvite (AMP), and powdered rock phosphate with 10% P (RP).

Fertilizers were mixed with horticultural sand and applied to each pot in the horticultural sand before sowing in plastic cylindrical pipes (height 30 cm, diameter 11 cm, volume 2.55 L with a 100 µm nylon mesh in the bottom to prevent mycorrhiza emergence). Following the manufacterer's instructions barley (Hordeum vulgare L. cv. Static) seeds were inoculated with M (1 kg ha^{-1}) and PSB (2 L ha^{-1}) applied before sowing two seeds at 7-8 cm depth per pot. After germination the seedling were thinned to one plant per pot and the experiment was in 126 days (from 26/09/2011 to 30/01/2012

2.1.2. Measurements and data recorded

At 22 days (early vegetative stage), 57 days (ear emergence) and 126 days (almost Ripe stage stage) after sowing 3 pots per treatments were harvested to determine root length, leaf area, root dry matter, stem dry matter, leaf dry matter and straw dry matter and yield components of barley (as appropriate for stage of growth).

2.2. Experiment 2: (Non Sterilized Field Soil & Sterilized Field Soil experiment)

2.2.1. Soil characteristics and experimental site

The soil used for the second pot experiment in the glasshouse was sandy loam (Table 2) from the same site as the field experiment at Henfaes Research Centre of Bangor University, which had received no P fertilizers for many years. Soil samples were taken at depths of 0-25 cm after removing 3 cm of the soil surface, in the winter of 2011.

((Table 2))

2.2.2. Soil sterilization procedures

Sterilization and non-sterilization of the soil were the main factors in this experiment. The soil (0-20 cm) was collected from the field at the Henfaes Research Centre of Bangor University. The soil properties were analysed before sterilization. The sterilization of soil was achieved by incubation of the wet soil for three days to allow spores to germinate then heating soil at 90˚C for 1 h (Trevors, 1996) to avoid oxidation of any organic matter. Sterilised soil could be useful for understanding the effects of dead/life native microorganisms on plant growth.

2.2.3. Experimental design and culture practices

In the second pot experiment horticultural sand changed to the field soil. Also for control of interaction between native microorganisms and M and PSB treatments, second experiment was split in to sterilized and non-sterilized soil.

The pot experiment was set out as a randomized complete block design 2×3×4 factorial design with six replicates per treatment with three replications each being harvested at two sampling dates. Two seeds were initially sown in each pot. After germination the seedling were thinned to one plant per pot. Barley seedswere sown on 6th January, and harvested on 6th June, 2012 (total 120 days). There were two soil factors (unsterilized soil and sterilized soil), three biological P treatments (M, PSB and no M & PSB), four non-biological P treatments (no P (control), super phosphate (SP), ammonium magnesium phosphate = Struvite (AMP) and RP. Non-biological P fertilizers were applied to each pot and mixed with soil before sowing in the plastic cylindrical pots in the appropriate treatments for each pot before sowing in the sandy loam soil.

2.3. Determination of root diameter, root length and root dry matter

Roots were scanned and analyzed using the WinRHIZO software (WinRhizo 5.0a, Regent Instruments Inc., Canada) for determine of root diameter and total root length of plant. After root scanned the roots were dried (75 C˚ for 48 h) separately for dry root weight measurement.

2.4. 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.

3. Results

3.1. First Pot Experiment (Horticultural sand) results

3.1.1. Compare of root length and dry root weight

The comparison between total dry root weight of plant and total root length of plant showed that the root length was significantly increased by RP treatment in the third sample (ripe stage) and also the root dry weight was significantly increased by AMP treatment in the second (ear emergence) sample (Figure 1).

((Figure 1))

3.1.2. Total root length and root dry matter

The comparison between total dry root weight of plant and total root length of plant showed that under P deficiency, the weight of dry root decreased and the total root length increased (could be with a decrease of root diameter) (Figure 1). It seems that under lack of P, plants increase of root length and decrease root diameter thereby may be achieving a greater surface area and increasing the volume of soil that is explored by the roots (Division and Ridge, 2000; Toro et al., 1997a; Wang, et al., 2010).

((Figure 2))

3.2. Second pot experiment (Non Sterilized & Sterilized Field Soil) results

3.2.1. Root diameter

The root diameter was significantly increased by soil treatments in the first and second samples. The lowest root diameter of the first and second sample were observed in the C (no P fertilizer)

treatments in the unsterilized and sterilized soil and also the highest root diameter of the first and second sample were in the SP treatment in the unsterilized and sterilized soil (Figure 2,4).

3.2.2. Total root length

The root length was significantly increased by soil treatments in the first and second samples. The highest root length of the first and second sample were observed in the C (no P fertilizer) treatments in the unsterilized and sterilized soil and also the lowest root length of the first and second sample were in the SP treatments in the unsterilized and sterilized soil (Figure 3,5).

3.2.3. Root dry matter

The root dry weight was significantly increased by soil treatments in the first and second samples. The highest dry root weight of the first and second sample were observed in the SP treatments in the unsterilized and sterilized soil and also the lowest dry root weight of the first and second sample were in the C (no P fertilizer) treatments in the unsterilized and sterilized soil (Figure 3,5).

3.2.4. Compare of roots diameter, root length and dry root weight

The comparison between roots diameter, root length and dry root weight of plant showed that the root length was significantly increased by C (no P fertilizer) treatment in the first and second samples and also the root dry weight was significantly increased by SP and AMP treatments in the first and second samples.

4. Discussion

It is well known that root growth and characteristics plays a key role in plant adaptation to low P stress (Liu et al., 2004; Lynch, 2011; Hamadoun, et al., 2016). The aim of the present study was to clarify the comparison between root diameters, total dry root weight of plant and total root length of whole plant in barley under different P fertilizer treatments, mainly focusing on the responses of p deficiency and root growth in barley.

In the present study, showed that root architecture of barley (root diameters, total dry root weight of plant and total root length of whole plant) was significantly correlated with amount of P concentration in the soil. This suggests that the root diameter and total root length of plant is particularly important in the barley plant. In the low P treatment, the root capacity to uptake and mobilise P in the soil becomes more important. The results, showed that under P

deficiency, the weight of dry root (Figure 1, 3, 5) decreased and the total whole root length (Figure 1, 5) significantly increased with decrease of root diameter (Figure 2, 4). The greatest root dry weight was obtained in M+SP treatment under sterilized soil and the smallest root dry weight was achieved in M treatment under unsterilized soil (Figure 3, 5). The associated this effect with P deficiency improved root characteristic, which seemed to be relatively more effective at low P in the soil. It seems that under lack of P, plants increase of root length and decrease root diameter thereby achieving a greater surface area and increasing the volume of soil that is explored by the roots (Toro et al., 1997b; Vierheilig et al., 2000; Wamberg et al., 2003; Herdler et al., 2008; Fernandes et al., 2014).

Improvement of root architecture may increase the rhizosphere microbial communities (Hao et al., 2008; Trabelsi, et al., 2017), release more organic acid and increase the utilization of P sources with sustainability (Haynes, 1982; Feng et al., 2016; Mundaa, et al., 2016), changing some of chemical, biological and physical properties in the rhizosphere (Walker et al., 2003; Shen et al., 2013; Maji, et al., 2017) and Shoot P uptake, rhizosphere properties and growth plants (Marschner et al., 2007). Zhu and Lynch (2004) resulted that sustained lateral rooting encourages maize seedling growth under P-limiting conditions. The benefit of a large root system in T149 in higher low-P tolerance was best in the calcareous soil. The hybrid T149 took up more P and produced more shoot biomass and leaf area than T222, results of this research showed that increasing root can increase P uptake under low P stress (Feng et al., 2016; Imtiaz et al., 2016). Lynch (2011) has suggested that root architecture may be more effective for P acquisition. 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. This result is in agreement with that of other researchers (Pellerin et al., 2007; Maji et al., 2017), who concluded that mycorrhiza are capable of taking up, translocating and transferring water and nutrients from soil to the roots of plants. Likewise mycorrhiza play an important role in absorption of poorly available forms of nutrients, and increase the nutrient bioavailability through mobilization of key nutrients (especially P) by mechanisms of development of the depletion zone that from the root surface around the root system through the hyphae to the crop plants (Bolan, 1991; Imtiaz et al., 2016; Munda et al., 2016; Rodríguez-caballero et al., 2017).
The previous studies indicated that the development of the root architecture system and the capability to release organic acid anions can be important to enhance P acquisition in the plant (Shen et al., 2013). In cropping cultivation, the rhizospheres can intersection each other and form an enormous continuum with growth of root systems in the complete root area, where

root/rhizospher interface occur between plants, soils, and even among different plant species in intercropping cultivation (Shen et al., 2011; Shen et al., 2013; Rodríguez-caballero et al., 2017). The improvement of plant growth and nutrient uptake after the bio-inoculation has already been informed in several studies with related environmental characteristics (Alguacil et al., 2011).

The results of two pots experiments have shown that under P deficient of soil the barley root architecture is an effective approach for increasing P uptake and nutrient use efficiency, which thinner roots with longer roots can develop rhisospher area with low use of assimilation. This effect has not previously been recorded in the literature.

((Figure 3, 4, 5, 5, 7))

Conclusion

The present research concludes the importance of bio-inoculates in improving P availability, growth root and shoot and mobilization of soil P to barley crop. In conclusion, tolerance to low P in barley can be improved by transforming root morphology. Increasing root length (especially decreasing root diameter) may enhanced the ability of barley plants to take up P and nutrition from the deficient soil and increase the growing of barley plants.

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Table 1- Analysis of Variance of the Effect of biological and non-biological P fertilizers and in unsterilized soil and sterilized on growth indices and P uptake

		Mean Squares									
SOV	DF	Total Root Lebgth (S1)	Total Root Lebgth (S2)	Root DW (S1)	Root DW (S2)	Shoot DW (S1)	Shoot DW (S2)	R/S (S1)	RR/S (S2)	Total P Uptake (S1)	Total P Uptake (S2)
Replication	2	175372**	394463**	0.05*	0.002ns	3.86**	0.27ns	0.001**	0.00ns	24.8**	1.6ns
Soil (S)	1	1443300**	4110144**	25.20**	30.22**	174.6**	915.7**	0.05**	0.03**	1999**	4776**
Biological P (B)	2	114056ns	182371**	0.13**	0.01**	2.50**	16.06**	0.001**	0.00**	7.16*	135**
S*B	2	567928ns	948738ns	0.01ns	0.01**	2.12**	0.30ns	0.00ns	0.00**	36.05**	1.8*
Non-Biological P (N)	3	532852ns	8416349ns	0.54**	0.06**	19.56**	11.47**	0.001**	0.00**	129.1**	91.1**
S*N	3	3310592**	1168373**	0.13**	0.05**	0.61*	0.66*	0.001**	0.00**	5.00*	6.9**
B*N	6	817947ns	1239210ns	0.05**	0.005*	0.69**	0.14ns	0.00ns	0.00*	5.5**	1.07ns
S*B*N	6	79097ns	1288239ns	0.03*	0.007**	0.32ns	0.31ns	0.00ns	0.00*	8.8**	1.51*
Error	46	560207	1387370	0.01	0.002	0.15	0.16	0.00	0.00	1.52	0.52
C.V.	-	5.36	4.69	5.52	3.09	2.83	2.03	6.04	3.69	5.08	2.16

***, ** and ns denotes significant effect at 5 and 1 percent and non-significant effect, respectively**

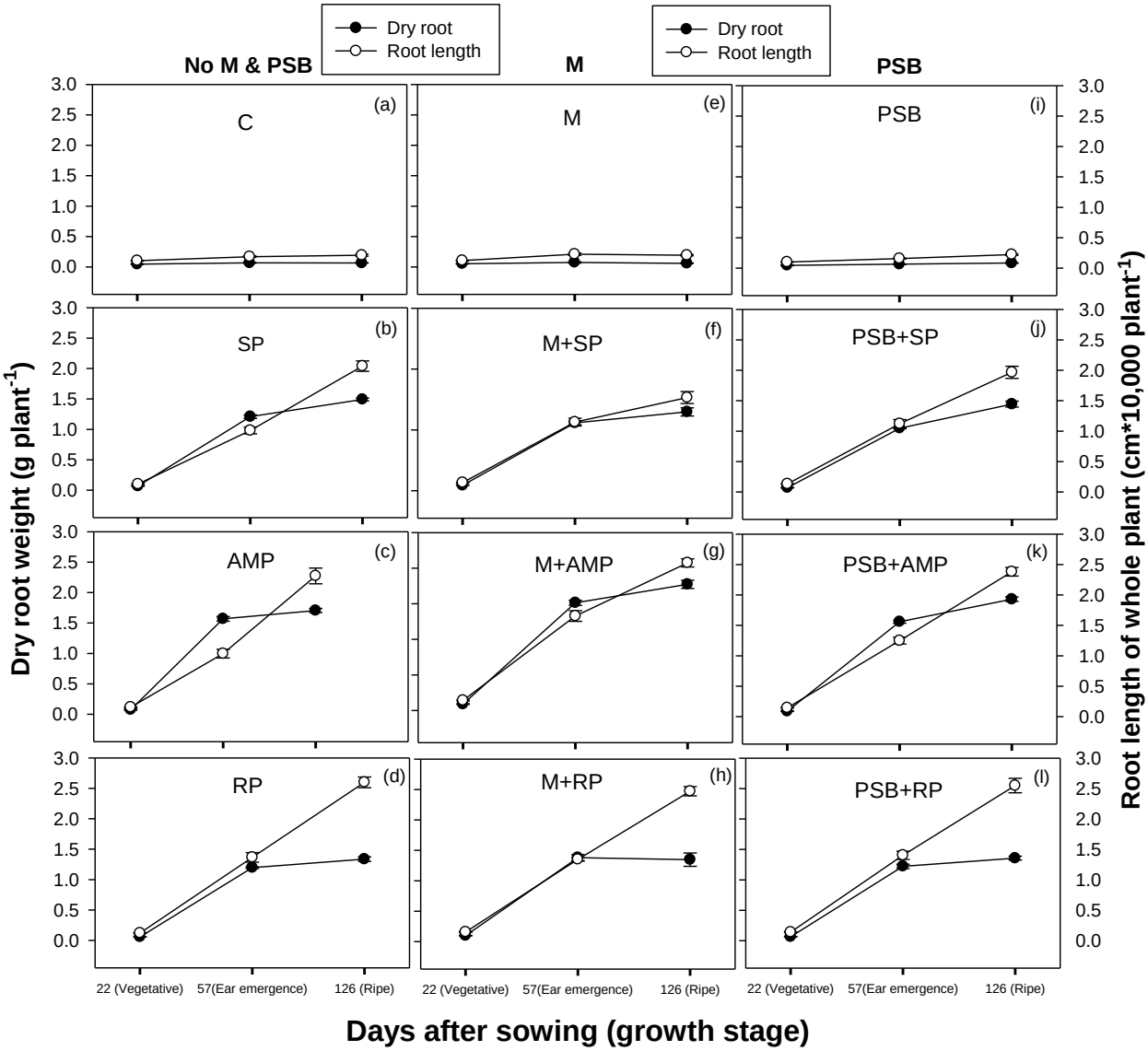


Figure 1: Mean values of total dry root weight and total root length of plant at vegetative stage (22 das), ear emergence (57 das), and ripe stage (126 days). (a) Effect of no P on dry root weight and root length; (b) Effect of SP on dry root weight and root length; (c) Effect of AMP on dry root weight and root length; (d) Effect RP on dry root weight and root length; (e) Effect of M on dry root weight and root length; (f) Effect of M+SP on **dry root weight** and **root length**; (g) Effect M+AMP on dry root weight and root length; (h) Effect M+RP on dry root weight and root length; (i) Effect of PSB on dry root weight and root length; (j) Effect of PSB+SP on dry root weight and root length; (k) Effect PSB+AMP on dry root weight and root length; (l) Effect PSB+RP on dry root weight and root length. Error bars show standard error of means (n=3).

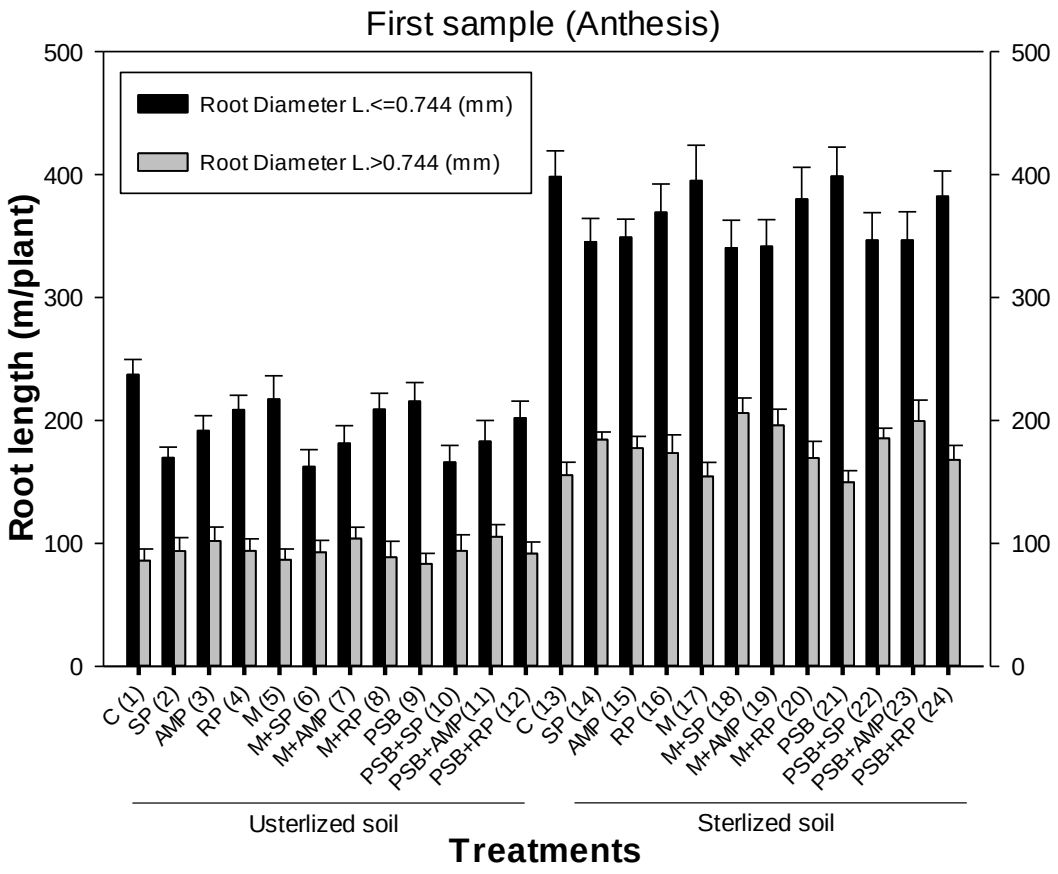


Figure 2: Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no P, M & PSB (C) on **root length** in unsterilized soil and sterilized soil in the anthesis and ripe stage. Error bars show standard error of means (n=3).

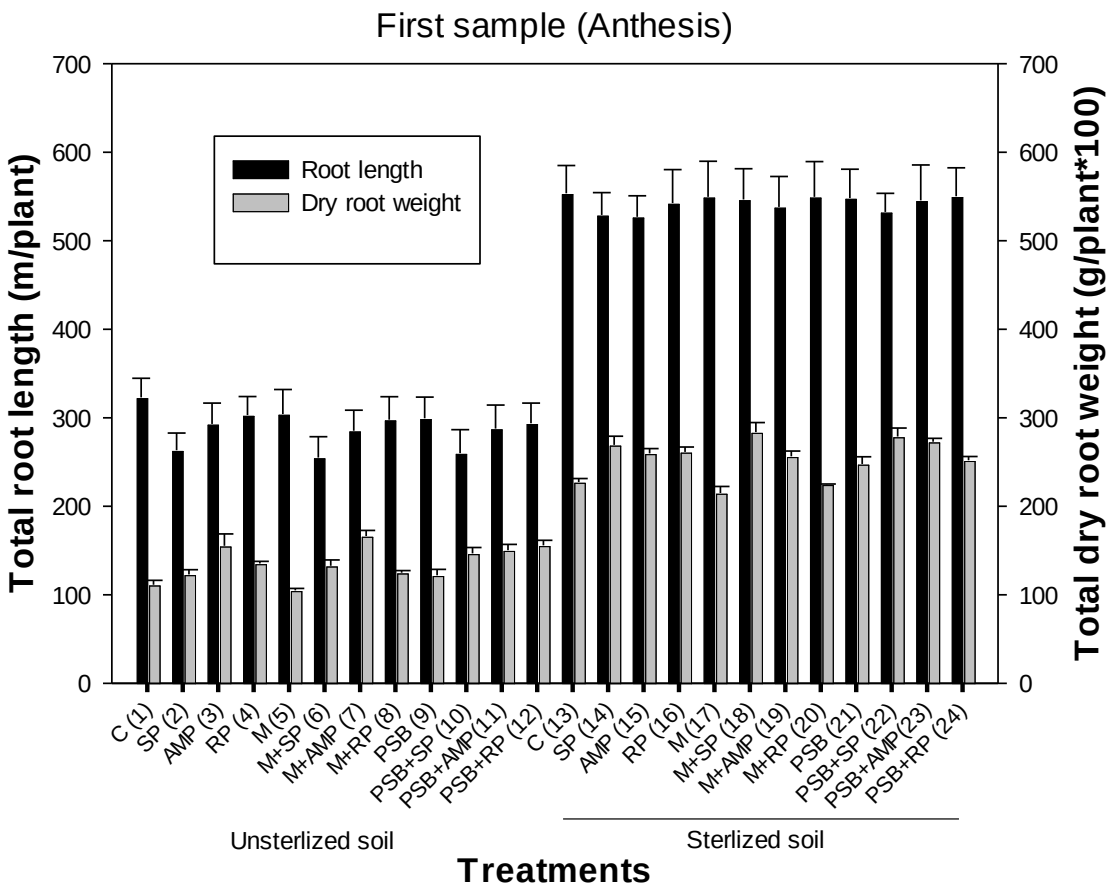


Figure 3: Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no P, M & PSB (C) on **root length** and **dry root weight** in unsterilized soil and sterilized soil in the anthesis and ripe stage. Error bars show standard error of means (n=3).

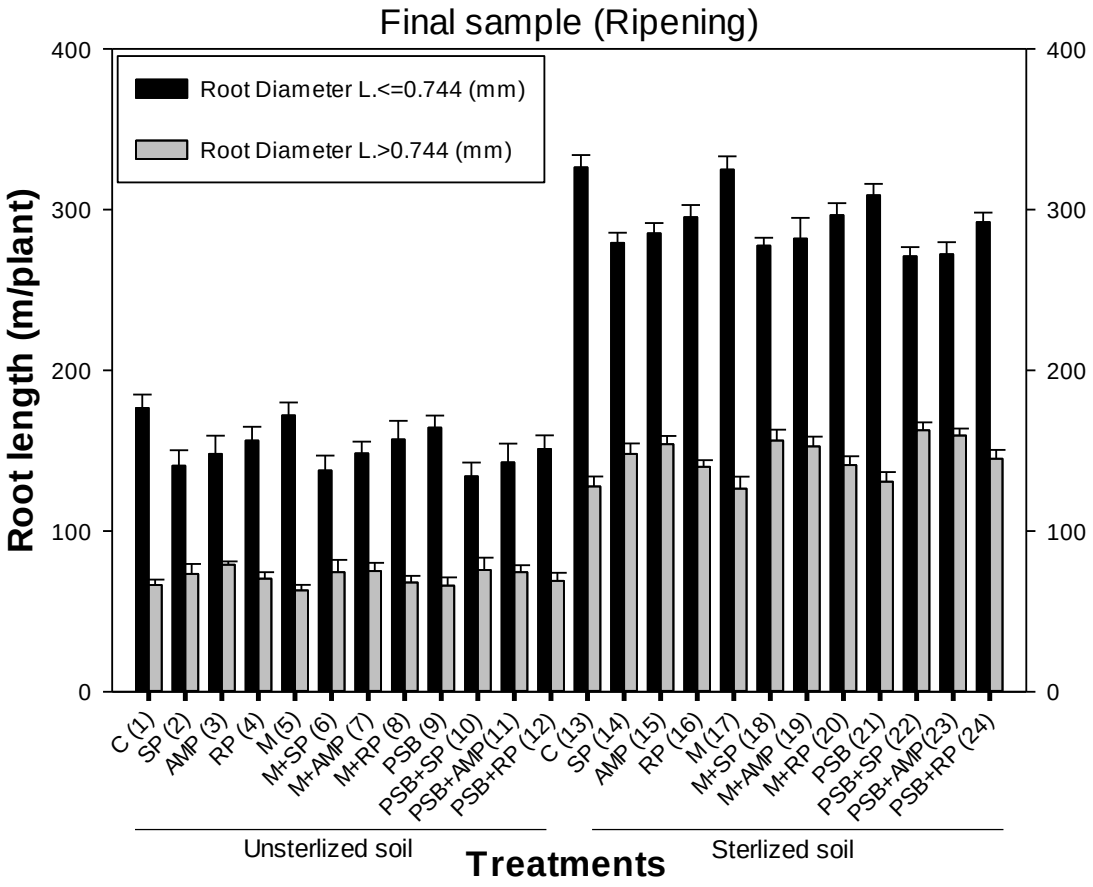
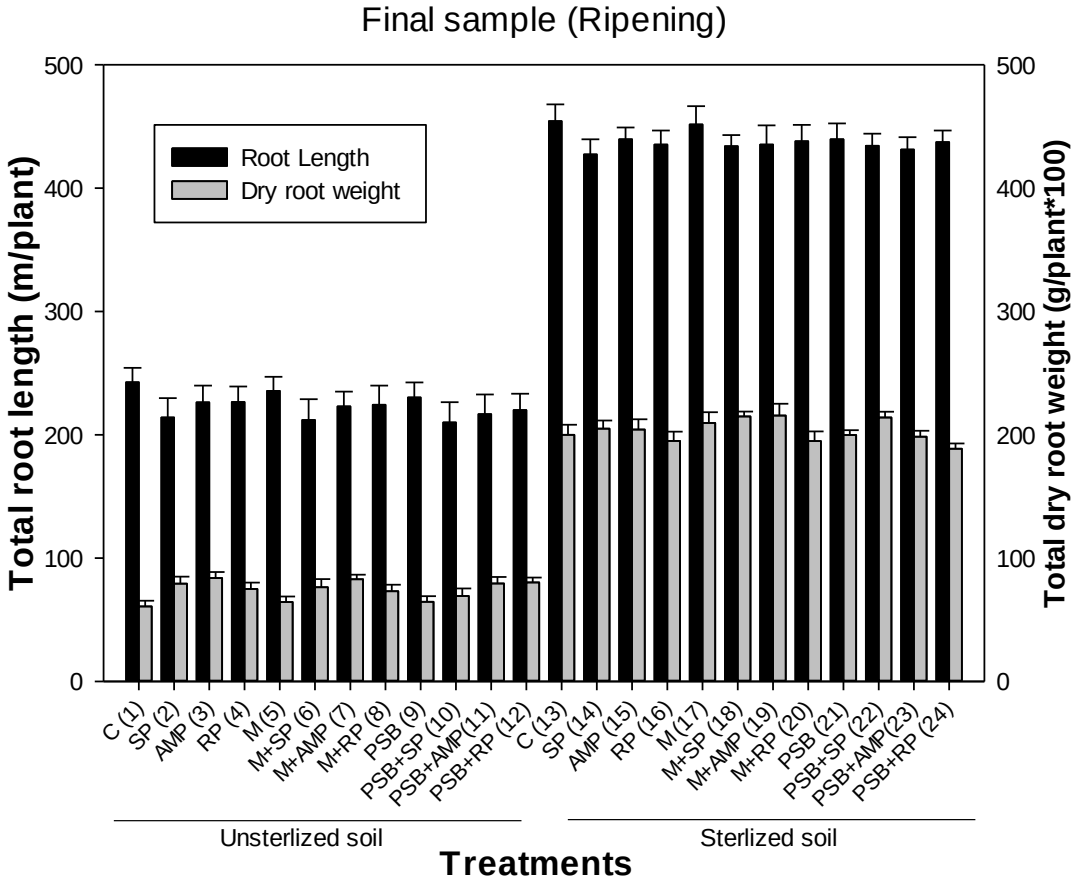


Figure 4: Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no P, M & PSB (C) on **root length** in unsterilized soil and sterilized soil in the anthesis and ripe stage. Error bars show standard error of means (n=3).



459

460 **Figure 5:** Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium
461 magnesium phosphate (AMP) and powdered rock phosphate (RP) and biological phosphorus fertilizers
462 (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no P, M & PSB (C)
463 on **root length** and **dry root weight** in unsterilized soil and sterilized soil in the anthesis and ripe stage.
464 Error bars show standard error of means (n=3).

465

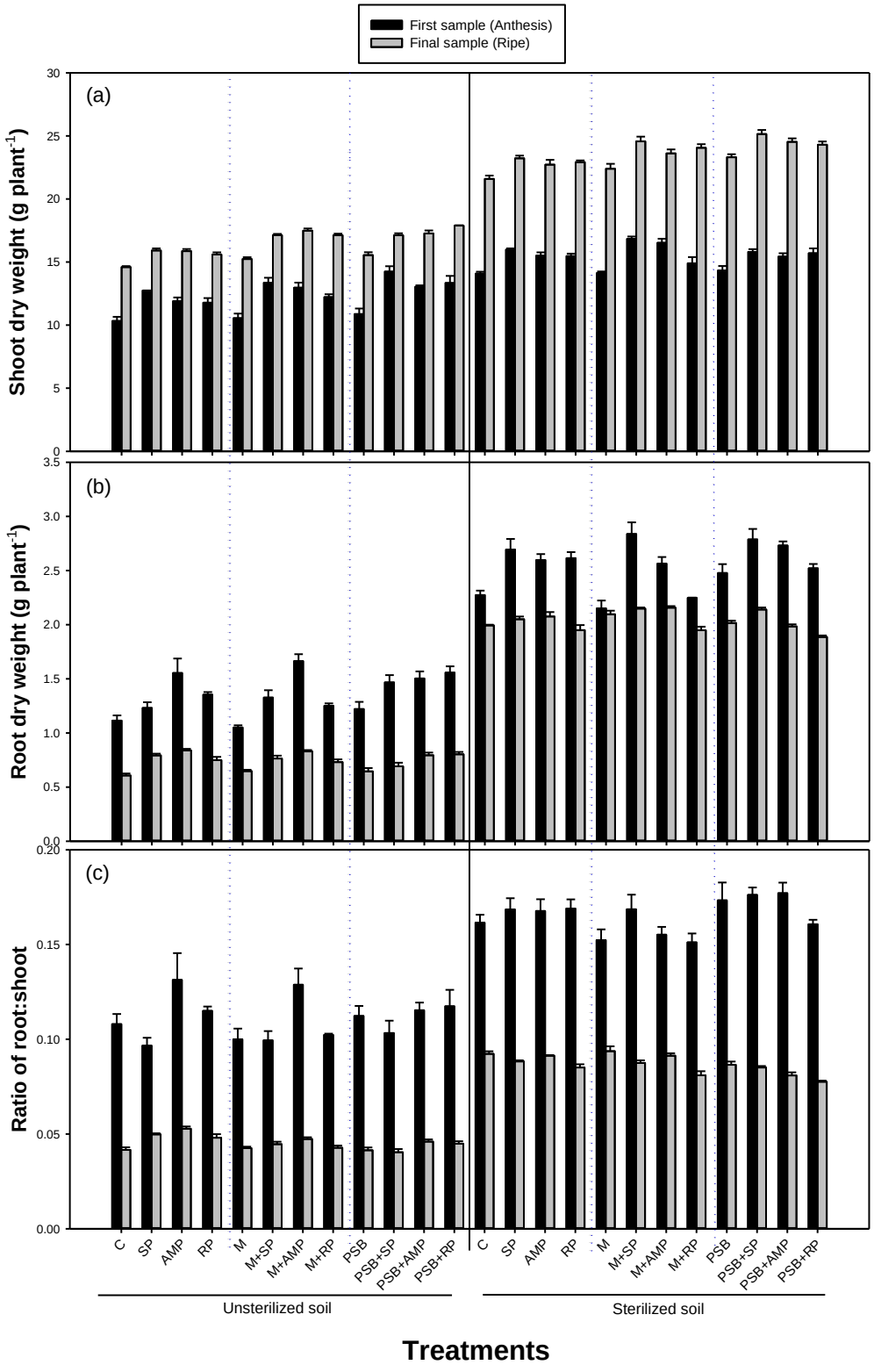


Fig. 6. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on shoot dry weight (a), root dry weight(b) and ratio of root:shoot (b) in unsterilized soil and sterilized soil in the an thesis and ripe stage. Error bars show standard error of means (n=3).

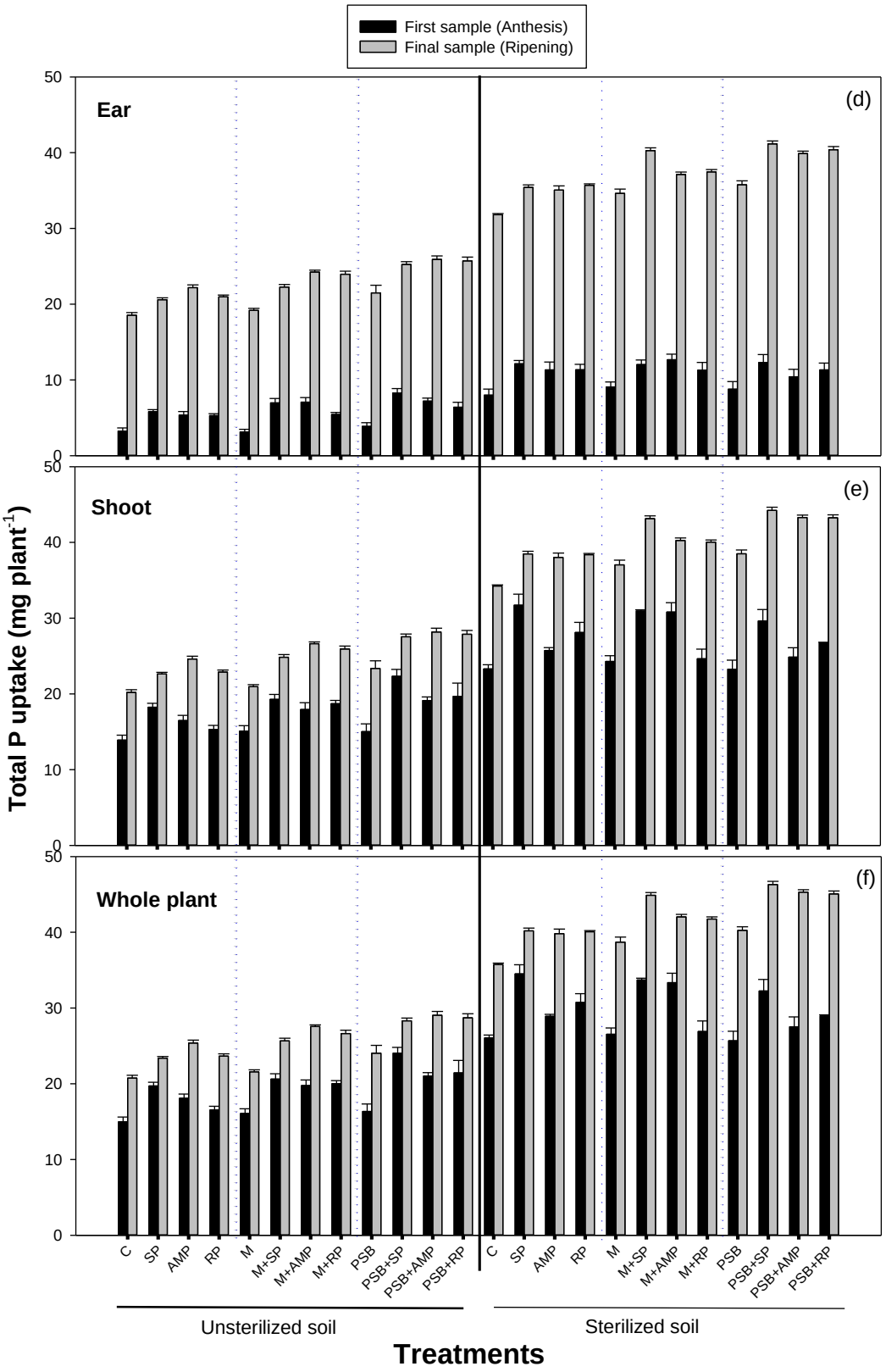


Fig. 7. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on total P uptake of ear(a), shoot(b), whole plant(c) in unsterilized soil and sterilized soil in the anthesis and ripe stage. Error bars show standard error of means (n=3).

Supplementary

Table 1: Properties of the used in the first pot experiment.

Sand (%)	10
pH	5.7
Available P (Olsen P) (mg kg^{-1})	4.6
P index	0
Available K (mg kg^{-1})	53
K index	0
Available Mg (mg kg^{-1})	165
Mg index	3
Total organic C (%)	0

Table 2: Properties of the used in the 2nd pot experiment.

Particle size distribution	**(%)**
Sand	83.2
Silt	13.5
Clay	3.3
Textural class	Sandy loam
pH	5.9
Available P (Olsen P) (mg kg^{-1})	11.5
P index	1
Available K (mg kg^{-1})	78
K index	1
Available Mg (mg kg^{-1})	55
Mg index	2
Total organic C (%)	0.65