DOCTOR OF PHILOSOPHY

The role of bio-inoculants on phosphorous relations of barley

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Award date:
2013

Awarding institution:
Bangor University

*In the Name of **Allah**, Most Gracious, Most Merciful.*

The role of bio-inoculants on phosphorus relations of barley

A thesis submitted to Bangor University by

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In candidature for the degree

Philosophiae Doctor

January2013

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Dedication

This thesis is dedicated toLadies Sayyida Fatima ***Zahra*** (*sa*) and **Zainab** Kubra (*sa*) The role- models of liberality, foresight, intellectual love and bravery

There is no wealth like wisdom, no destitution like ignorance, no inheritance like politeness and no support like consultation. Imam Ali (peace be upon him)

Acknowledgements

Firstly I am grateful to ALLAH for helping me make it through until the end.

I would like to thank my supervisor, Dr Robert Brook for all the feedback, support and advice he gave during my research. Big thanks are owed to Professor Davey Jones, who was my second supervisor, for all his kind advice and technical support over the years. Thanks also to Professor Paul Withers – my third supervisor, for his expert advice.

Thanks are owed to Llinos Hughes, Mark, Kerrin Buckler, Eftikar,at Henfaes and their associated teams for all their help. Thanks are owed to Dr Paul Hill for all his technical support over the lab works. I would like to thank my colleagues and friends for helping and encouraging me over the years. I would also like to thank the lab technicians, Mr Gareth Williams, Gordon and Sara for all the friendly help they offered in the lab.

I would especially like to thank my lovely wife for her kind support to me and my daughters (Zahra &Zeinab) over the years, far from relative family. I would also like to say a particular thank you to my daughters' grandparents, for their prayers and never ending support of my endeavours and studying.

The role of bio-inoculants on phosphorus relations of barley

Abstract

Phosphorus (P) is one of the most limiting and important elements in crop production, yet often has limited availability in the soil. Manufactured inorganic P fertilisers are required to improve soil and crop P supply but their use depletes finite reserves of rock phosphate and impacts on water quality and ecosystem biodiversity. Bio-inoculants have a potential role to increase soil P supply and reduce dependence on expensive fertilisers. The objective of this thesis was to further understand the role of mycorrhizae (M) and P solubilizing bacteria (PSB) bio-inoculants and external P sources (super phosphate (SP), struvite (AMP) and rock phosphate (RP)on phosphorus availability in soil and their effects on the growth, yield and P uptake of barley.

Field experiments on low P status soils in 2010 and 2011 demonstrated the potential for the use of bio-inoculants (PSB and M) in mobilizing P from soil and significantly ($P < 0.05$) enhancing P uptake to increase growth of barley, and to a lesser extent, grain yield. It was postulated that bio-inoculant effects in the field were compromised by the presence of native M and PSB. Glasshouse pot experiments were conducted with a range of growth media: horticultural sand (zero P), field soil (low P status but with native micro-organisms) and heat sterilized field soil. These demonstrated the effects of bio-inoculants without the presence of native M and PSB, and to a lesser extent in the presence of native micro-organisms, in terms of increased plant root and shoot growth, grain yield and tissue P concentration.

Across all experiments bio-inoculants (M and PSB) increased the effectiveness of water soluble SP, partially soluble AMP and insoluble RP. M and PSB were equally effective. In combination with these external P sources, bio-inoculants (M and PSB) significantly ($P < 0.05$) increased yield, P concentration and total P uptake, plant dry matter and concentration of P in the grain compared to P fertilizers without bio-inoculants. However, applications of P fertilizers reduced the colonization of roots by mycorrhizae. The potential role of P uptake enhancing bio-inoculants in reducing external inputs in agriculture is discussed.

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Abbreviations

P	Phosphorus
VAM	Vesicular arbuscularmycorrhizae
PSB	Phosphorus solubilizing bacteria
CF	Chemical fertilizers
M	Mycorrhiza
OF	Organic fertilizers
PGPR	Plant growth promoting rhizobacteria
CPF	Chemical phosphorus fertilizer
TSP	triple superphosphate
SSP	Single superphosphate
DAP	Diammonium phosphate
MAP	Monoammonium phosphate
AMP	Ammonium magnesium phosphate
RP	Rock phosphate
P_o	Organic phosphorus
P_i	Inorganic phosphorus
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
DNA	Deoxyribonucleic acids
RNA	Ribonucleic acids
MF	Mycorrhizal fungi
AM	Arbuscular mycorrhiza
C_2H_5OH	Ethanol
µL	Microliter
g^{-1}	Per gram(s)
g	Gram(s)
mL	Millilitre
v:v	Volume to volume
w:w	Weight to weight
m^{-2}	Per meter square

ppm	Part per million
ANOVA	Analysis of variance
SPSS	Statistical Package for the Social Sciences
mg	Milligram(s)
kg	Kilogram(s)
HGCA	Home Grown Cereals Authority
AHDB	Agriculture and Horticulture Development Board
LAI	Leaf area index
NBP	Non-biological phosphorus
NBP	Biological phosphorus
N	Nitrogen
P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
Mn	Manganese
S	Sulphur
Fe	Iron
Zn	Zinc
Cu	Copper
PSM	Phosphorus solubilizing microorganisms
mm	Millimetre(s)
cm	Centimetre(s)
FAO	Food and agriculture organisation
HCL	Hydrochloric acid
L	Litre(s)
Ha^{-1}	Per hectare
ha	Hectare(s)
das	Days after sowing
SED	Standard error differences
USS	Unsterilized soil
SS	Sterilized soil

Title: The role of bio-inoculants on phosphorus relations of barley (*Hordeum vulgare L.*)

Chapter 1: Introduction and Literature Review

1.1. Introduction

World population is projected to grow from the current 7 billion to over 9 billion in the next four decades, an increase of approximate 40 percent (Badgley*et al*., 2007;Bacci 2012). To meet this increase in demand, crop production must be enhanced. One important way to increase crop production per unit area is to improve the efficiency of fertilizer use. This is achieved through scientific advances in our understanding of soil fertility and plant nutrient acquisition. The expansion of fertilizer responsive varieties in the Green Revolution, together with the realization by farmers of the importance of fertilizers, has led to high levels of fertilizer use in agriculture (Cassman, 1999; Tilman*et al.*, 2002). Enhanced future demand for food may require even greater fertilizer inputs, but the increase in agricultural production required for our future food security must be achieved without endangering human health or the wider environment.

Knowledge of soil fertility is vital for the development of soil management systems that produce profitable crop yields while maintaining soil sustainability and environmental quality (Foth and Ellis, 1996). Soil fertility is a measure of the ability of a soil to provide elements necessary for plant growth without a toxic concentration of any element. Soils are classed as fertile when they are balanced providers of elements in a sufficiently labile or usable form to satisfy the needs of plants (Foth, 1996). Phosphorus (P) is one of the essential nutrients for crop growth and maximizing the utilization of other nutrients such as nitrogen (Brady and Weil, 2002; Mehrvarz*et al*., 2008) and is the topic of this thesis. P is inherently one of the

least available and least mobile elements in soils (Hinsinger, 2001). Soil amendment with soluble chemical P fertilizers is a costly and potentially contaminating practice, especially if one considers the highly polluting mode of production of these fertilizers (Sharply *et al*., 2000). Natural reserves of phosphate rock required for P fertilizer manufacture are also becoming rapidly depleted (Fig.1.1). There is no alternative to phosphate rock as a P source so it is important to help preserve these reserves for future generation (Cordell *et al.*, 2009).

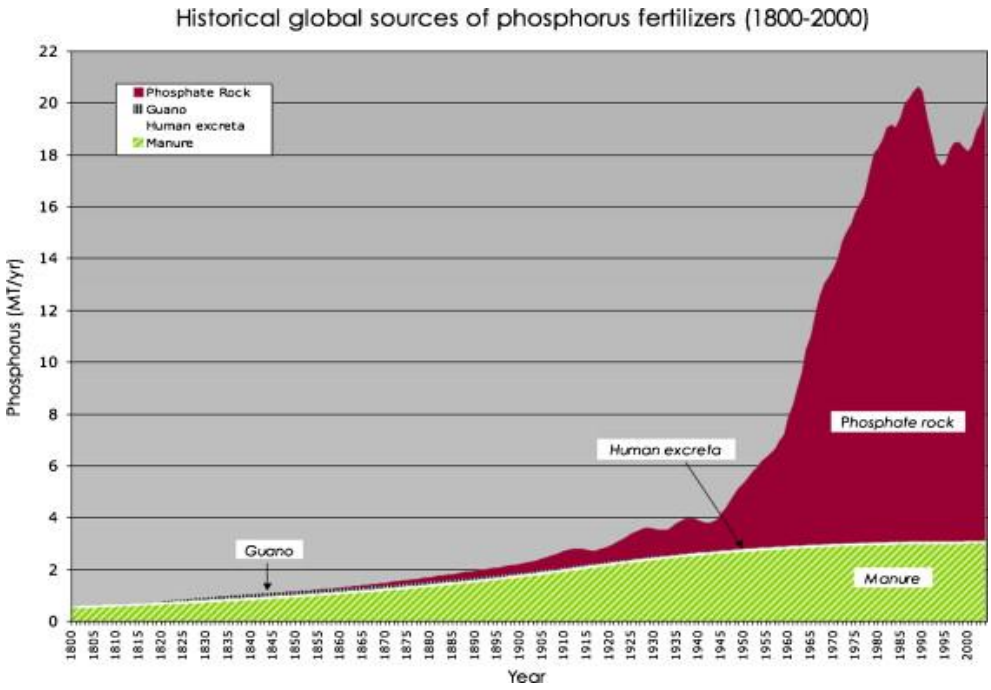

Fig.1.1. Historical consumption of phosphorus sources for use as fertilizers,(1800–2000) (Reliability of data sources vary, hence data points for human excreta, guano and manure should be interpreted as indicative rather than precise) (Cordell et al., 2009).

One potential option to decrease the environmental impact of current fertilizer P use, whilst maintaining high crop yields and farmer incomes, is to replace expensive soluble chemical P fertilizers with new, cheaper, more efficient bio-inoculants. Bio-inoculants offer a

potentially useful means of increasing nutrient acquisition from soils and reducing our dependence on chemical inputs (Buckingham and Jasinski, 2004).

1.1.1. Justification for the study

- Phosphorus is one of the most widely used materials in the agricultural world and also it is inherently one of the least available and least mobile elements in soils but is necessary for efficient crop production (Ramaekers*et al.*, 2010).
- In recent times, there has been increasing interest in developing alternatives/reducing to chemical fertilizers, so bio-inoculants offer a potentially useful means of increasing nutrient acquisition from soils and reducing our dependence on chemical inputs.
- Different effects of P fertilizers and bio-inoculants on root and shoot growth of cereal crops and consequently different levels of production.
- Recovery of P from wastewater sources offers a potential means of closing the P cycle and there is potential to substitute fertiliser P by recycling these products (e.g. struvite).
- Mycorrhiza colonization of crops can be affected by P content in soil and P fertilizers.

1.1.2. Objectives of Study & Research Questions

The aim of this study is to investigate the response of barley plants to P fertilizers and bio-inoculants. Barley is an important crop for animal feed, malt, and human food and potentially important means to achieve the goals of sustainable agriculture is to expand the application of bio-inoculants if it can be demonstrated that they increase soil P acquisition at

the expense of fertiliser P and benefit yield. In addition, because of the potentially harmful effects of chemical fertilizers in soils and the wider environment.

General questions:

- How can we help to increase agricultural production in order to ensure future food security without endangering human health and creating adverse environmental impacts?
- Can bio-inoculants increase cereal (particularly barley) growth parameters and the quality and quantity of yield parameters?
- To what extent can bio-inoculants act as an adjunct to chemical fertilizers to enhance the productivity of cereal crops?
- How can recovered wastewater products (e.g. struvite) be effectively utilized in agricultural systems?
- Relation of mycorrhiza colonization and bacterial activity with P in the soil and P fertilizers?

1.1.3. The null hypotheses to be tested are:

- Bio-inoculants have no effect on yield and yield components of barley (*Hordeum vulgare L.*).
- Bio-inoculants have no effect on P uptake of grain and straw.
- There are no responses of barley to various forms of phosphorus supply.

- The application of bio-inoculants and integration/with different fertilizer sources do not have a promoting effect on the morphology, growth and yield of barley.
- The application of P fertilizers and bio-inoculants and integration do not have an encouraging effect on the root and shoot growth and yield of barley.
- Struvite is not a suitable source of P for barley because of its low water-solubility

This research will:

- Compare the effects of different bio-inoculants (micorrhizal and phosphorus solubilizing bacteria) on yield and yield components of barley.
- Evaluate various inorganic P sources (fertiliser and struvite) to optimize crop production.
- Measure the difference in growth of root, straw, and yield and yield components of barley under different bio-inoculants and P fertilizers regimes in the field and greenhouse studies.
- Assess the efficiency of phosphorus uptake by barley and assess changes in the quality (P content in root, straw and grain) and quantity of crop (barley) production.
- Determine the colonization of mycorrhiza and P analysis of the root, stem, leaf and grain of barley.

1.2Literature Review

1.2.1 Soil Fertility and Plant Nutrition

Soil fertility and plant nutrition are two strongly related topics that emphasize the configuration and availability of nutrients in soils, their transportation and their uptake by roots, and use of nutrients in the plants (Foth and Ellis, 1996).

1.2.1.1 Soil Fertility

Soil fertility depends on physical, biological, and chemical factors. Soil is an active and complex system which includes living organisms interacting with particles of inorganic mineral and organic substances. Microbial activity is strongly connected with nutrient availability and soil fertility (Garcia *et al.*, 1994). A diverse range of roles is executed by soil that directly or indirectly sustain the world's human population. In food production, soil performs a vital function; it acts as a reservoir for water; a store of nutrients, a medium for root anchorage, and also as a filter for pollutants. Additionally, soils store approximately twice as much carbon as the atmosphere and are significant links in the natural cycle to determine the level of atmospheric carbon dioxide (O'Donnell and Gorres, 1999).

1.2.1.2 Plant Nutrition

Plants can absorb nutrients, along with water, through their root hairs, from a zone of approximately one centimetre around the root. For some nutrients the mechanism of root uptake of the soil solution nutrients is transportation from more saturated areas to less saturated areas by mass flow (Chen *et al.*, 2009). The efficiency of uptake of applied

fertilizers by crops is important in determining the efficacy of and utilization of the fertilizer. Plants that possess an enhanced absorption and utilization capacity for nutrients may enhance the efficiency of applied fertilizers (Graham and Welch, 1996). Anatomical and physiological characteristics are able to create a large variation in optimal and critical nutrient values in support of different species or varieties, even under equal growing conditions (Drechsel and Zech, 1993). Crop roots are able to increase the radius of the zone from which nutrients can be obtained through elongation (Ge*et al.,* 2000). Phosphorus however is relatively immobile and reaches the root surface by diffusion, often moving only a few millimetres in one season.

1.2.2Fertilizers

Fertilizers are the widely used material input into agricultural systems on a global scale. Fertilizers can be classified to four types according to their production process:

1. Manufactured inorganic fertilizers (chemical fertilizers (CF))
2. Organic fertilizers (animal manures, biosolids, crop residues, compost and green manures (OF)).
3. Natural mineral fertilizers (rock phosphate).
4. Bio-inoculants (azotobacters, rhizobia, phosphate-solubilizing bacteria, mycorrhiza, and plant growth promoting rhizobacteria) (Chand *et al.*, 2006).

Nutrients should be obtainable in sufficient and balanced quantity for optimum plant growth. Soils include natural reserves of plant nutrients, nevertheless these natural reserves are mostly in forms unavailable to plants, and therefore only a small portion is released each growing season through biological activity and chemical processes. The release of natural reserves is also too slow to compensate for the nutrients which have been absorbed by crops

and removed by harvest. Consequently, fertilizers are designed to increase the available nutrients in soil and uptake by the crop. The use of inorganic fertilizer (chemical fertilizers), organic fertilizers or bio-inoculants have some advantages and disadvantages in the context of nutrient provision, crop growth, their effect on the environment, human health and animal health (Xie, *et al.,* 2011). The advantages and disadvantages both need to be considered to optimize the use of each form of fertilizer and achieve balanced nutrient management for plant growth and crop production.

1.2.2.1 Bio-inoculants

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). 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). Bio-inoculants can be beneficial, and various claims have often been made about their ability to promote plant growth and decrease the need for chemical fertilizers (Rai, 2006).

Bio-inoculants consist of five main groups:

1. Vesicular arbuscularmycorrhizae (VAM)
2. Phosphorus solubilizing bacteria (PSB)
3. Plant growth promoting rhizobacteria (PGPR)

4. Azotobacters and azospirillum
5. Rhizobium complex

1.2.2.1.1 Advantages of bio-inoculants

Various authors have reported beneficial effects as a result of bio-inoculant usage. For example, Rai (2006) noted that they promote plant growth and reduce chemical inputs, and are easy to transport and apply. In a field study utilizing PSB and VAM, Kaushik (1993) and Heydari*et al.* (2010) reported that they are cheaper than chemical phosphorus, increase phosphorus uptake by the plant, promote increased yield and yield components, help to decrease chemical fertilizer use. Buckingham and Jasinki (2004) have described bio-fertilizers as being relatively easy to produce. The equipment and cost of bio-inoculant production is very low compared to chemical fertilizer plants. Sinha (1998) and Ahmed (1995) described bio-fertilizers as being efficient at improving soil fertility, nutrient absorption and helping plants absorb trace elements from around the root zones. They also noted that they are ecologically safer than chemical fertilizer. Weller (1988) and Singh and Ram (2005) examined and reported the effects of bio-fertilizers alongside a reduction of artificial compounds, and thus decreased environmental impacts and enhanced, or maintained, yields at a sustainable level by assisting plant growth, promoting bacteria and optimising fertilizer application rates. Finally an integrated plant nutrient-management system represents an alternative and more sustainable strategy for crop nutrition that consideres decreased inputs of chemical fertilizers combined with the application of organic inputs and/or bio-fertilizers (Obersonet *al.*, 1996; Gunapala and Scow, 1998).

1.2.2.2 Organic fertilizers

Organic fertilizers (OF) can function as an alternative to mineral fertilizers or bio-fertilizers to improve soil structure and microbial biomass. Hence, the use of OF may raise crop yields with minimum of CF use. Recently, consumers demanding good quality and safer food have become very interested in so-called "organic" products (GargBahl, 2008), that is they are grown without the use of manufactured chemical inputs. However the use of OF alone may cause problems for human health and the environment (Arisha and Bardisi, 1999). OFs also have some disadvantages such as; low nutrient contents per unit volume or mass meaning that large amounts are required to provide crop sufficient nutrition, the rate of nutrient release is rather slow for short term production and some plant nutrients are not available in OF for high crop yield (Mandal*et al.*, 2007). Also, it is sometimes difficult to obtain the large quantities required. Organic farming production relies on crop rotations, compost, animal manure and plant residues to preserve soil fertility without the use of mineral fertilizers (Elstrand*et al.*, 2007). Organic matter has significant effects on soil such as improved physical, chemical and biological properties and enhanced functional stability of the microbial community (Toyota and Kuninaga, 2006). Thus, organic manure can be used as a replacement for mineral fertilizers (Gupta *et al.*, 1988; Wong et al., Naeem*et al.*, 2006) to improve soil structure (Bin, 1983; Dauda*et al.*, 2008) and microbial biomass (Suresh *et al.*, 2004).

1.2.2.3 Chemical fertilizers

Human and animals excreta were the initial empirically acknowledged fertilizers. Though, only the Chinese applied some residues in an organized way, until about 300 years

ago (Igual, and Rodrigues-Barrueco, 2003). The foundations of plant nutrition knowledge were the experiments and research of the French scientists Antonie Lavoisier in 1774 and Boussingault in 1834, and also the German chemist Liebig in 1840, who undertook chemical analyses of plants and soils and succeeded in proving that chemical elements in plants came from soil and air. Von Liebig considered this theoretical base the main advance within the fertilizer industry that led to the importance of mineral element in plant nutrition (Igual and Rodrigues-Barrueco, 2003).

Chemical fertilizers have played an important role in the Green Revolution for global food security and transforming developing countries (Ahmed, 1995). However the intensive cropping and incessant use of high levels of chemical fertilizers frequently leads to nutritional imbalance in soil and ultimately a decline in crop productivity (Nambiar, 1994).

1.2.2.3.1 Chemical phosphorus fertilizers

In 1842 Lawes and Gilbert produced superphosphate for the first time from compressed bones and mined phosphate by treating with sulphuric acid (Igual and Rodrigues-Barrueco, 2007). This has become the major form of mineral fertilizer supplying soluble P to agricultural systems.

As a consequence of the need to increase crop production and the limited availability of P for plant roots in two thirds of the agricultural soils of the world, chemical phosphorus fertilizer (CPF) application is necessary. The main active component of P fertilizers is phosphorus that has been obtained from the RP. The common phosphorus fertilizers that generally applied in agricultural systems are triple superphosphate (TSP) (17-23% P; 44-52% P2O5), single superphosphate (SSP) (7-9.5% P; 16-22% P2O5), diammonium phosphate

(DAP) (20% P; 46% P2O5) and mono-ammonium phosphate (MAP) (48-61% P2O5). In the chemical process, RP is the initial material for the production of P fertilizers. The manufacture of P fertilizers involves milling of the rock phosphate, treatment with acid to produce phosphoric acid followed by heating to drive off water (Batjes, 1997; Tunney et al., 2003; Chen et al., 2006; Roselli et al., 2009).

1.2.2.4 Struvite

Struvite (ammonium magnesium phosphate = AMP) recovered from wastewater sources as fertilizer that recently has advanced considerably. It has a N-Mg-P composition of 5:10:28 with low water solubility and low P availability (the same or higher than triple-super phosphate) for crop growth and production (Johnston & Richards, 2003; CabezaPerez *et al*., 2009).

1.2.2.5 Rock Phosphate

Rock phosphate (RP) is a valuable option as a source for P fertilizer. In recent years the prospect of RP as fertilizers has received major interest (Bhatti and Yawar, 2010). RP is a natural, low cost, raw material that has been accepted as a valuable P fertilizer, particularly for acid soils (Goenadi*et al*., 2000; Stamford et al., 2007). However solubility of RP is low in non-acidic soils (calcareous soils) and the main problem for the direct application of RPs in the soils is the low rate of release of P into the soil solution (Khasawneh and Doll, 1978; McLaughlin *et al*., 1990; Kpomblekou and Tabatabai, 2003; Rajan and Watkinson, 1992). Nevertheless, several researchers have reported that plants can solubilize P from RP through

root acid exudation (Bolan *et al*., 1990; Hinsinger, 1998; Liu *et al*., 2004) and differing effects of crop varieties on the acidification of their rhizospheres (Flach*et al*. 1987). It is possible to increase the effect of PR on plant growth by manipulation of it, such as decreasing the particle size, acidulating RP by artificial or natural organic acids, mixing with chemical materials to produce acids (e.g. elemental sulphur, pyrites, $(NH_4)_2SO_4$, $NH4NO_3$) in non-acidic soils, and sulphur oxidation. Calcareous and alkaline soils are able to increase phosphorus availability from RP by reducing the soil reaction (Singh and Amberger, 1998; Stamford *et al*., 2003; Babare*et al*., 1997; Sagoe*et al*., 1998; Hammond *et al*., 1986; Lewis *et al*., 1997; Rajan and Ghani, 1997).

1.2.3 Phosphorus (P)

In 1557 Henning Brandt in Germany discovered phosphorus, the name derived from the Greek (phos = light and phorus = bringing) denoting light production (Boyd, 1990), due to its luminescent properties and spontaneous combustion in air. Additionally, outside of agriculture, P is necessary for production of detergents, explosives, matches, and flares (Massey, 2010).

P is not very plentiful in natural ecosystems, but it is a major plant nutrient and plays a fundamental role in agriculture and biogeochemical cycles. P exists in every part of the living organisms. It can form numerous covalent organo-phosphorus compounds and binds to C, N, O, Al, Fe, and Ca. It is involved in the primary transfer processes from energy of radiant electromagnetic energy to chemical (photosynthesis energy) and sustains the growth of the plants (Bahl*et al.*, 1997; Garg and Bahl, 2008).

1.2.3.1 Phosphorus cycle

Phosphorus is one of the lowest biologically available nutrients and yet is abundant in nature. P is similar to the other mineral nutrient cycles in that it exists in minerals, soils, water and living organisms (Schachtman et al. 1998; Ashley *et al.*, 2011). Elemental P is very reactive and when exposed to the air it will violently combine with oxygen (Kirkby & Johnston 2008). P in natural systems (such as water and soil), exists as phosphate ions, forms where each P atom is bounded by four oxygen atoms (Takahashi & Anwar 2007). The simplest phosphate is orthophosphate that exists in water as PO_4^{3-}, in acidic condition as $H_2PO_4^{1-}$ and in alkaline conditions as HPO_4^{2-}(Fardeau & Guiraud 1996).

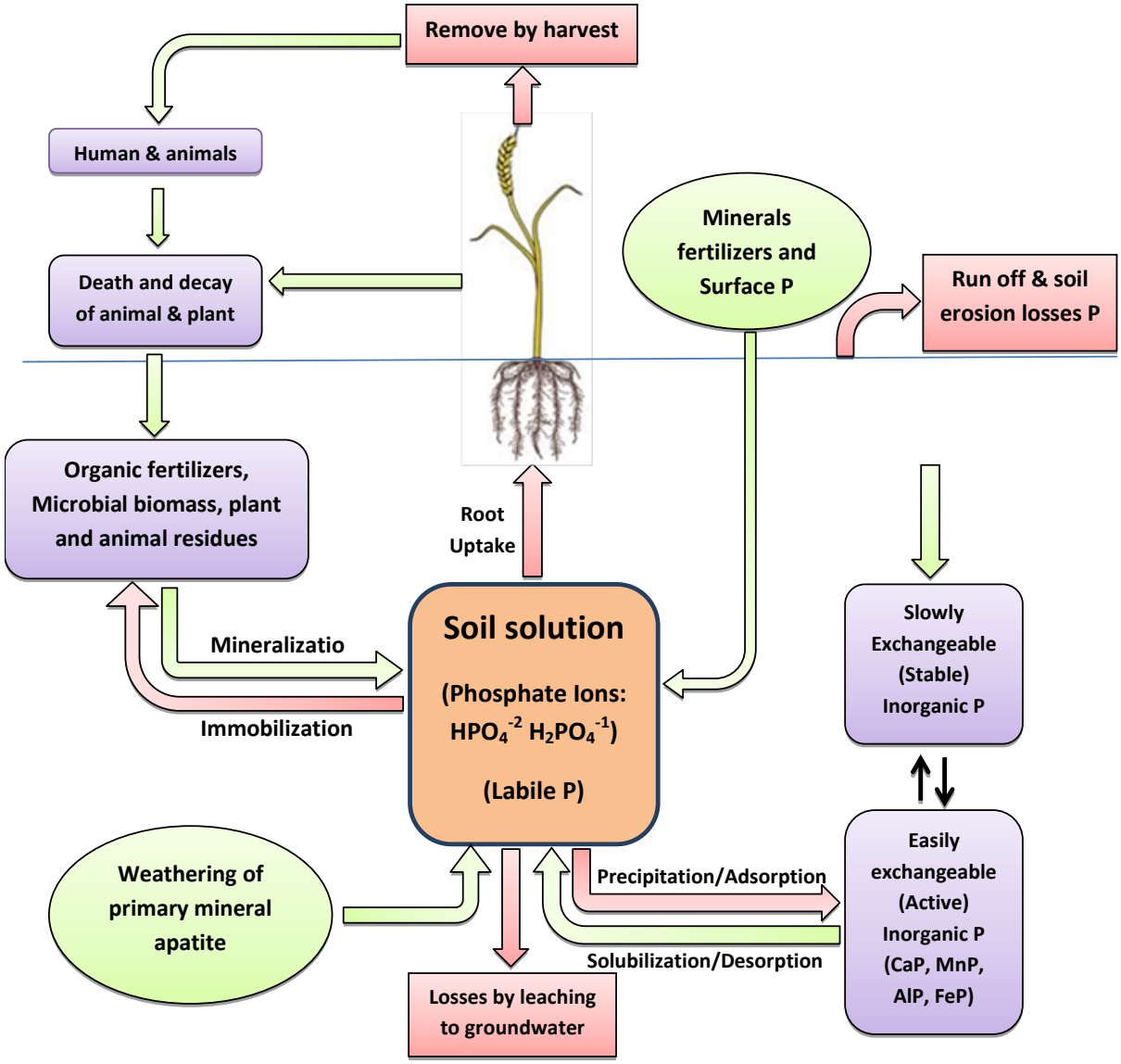

Fig.1.2. Phosphorus transformation and movements in the soil and plant.(Liu & J. Chen 2008;Mackey & Paytan 2009; Adapted from Shen *et al.*, 2011; Suh & Yee 2011).

P is taken up from soil by plants, plants are consumed by human and animals, and returned to soil as organic residues, death and decay in soil (Fig 1.2). The organic phosphate for plant material in soil will be released in the form of inorganic phosphate (mineralization) by soil microorganisms (Rodríguez & Fraga 1999;(Mkhabela & Warman, 2005). P can be lost through run off, soil erosion and leaching to groundwater (Dawson & Hilton, 2011).

Several phosphate salts are not highly soluble in water. Therefore, the majority of P exists in the solid form in natural systems (Vazquez *et al.*, 2000).

1.2.3.2 Soil Phosphorus

Phosphorus is the major essential nutrient element (after nitrogen) most often limiting agricultural production. Compared to other main nutrients, P is the least mobile and least available to plants in the majority of soil conditions (Schachtman et al., 1998; Hinsinger, 2001). However, P is the 11th most abundant element in the soil, which typically has concentrations of 0.1- 3 mg P kg soil (Hedley *et al.*, 1995; Mengel, 1997).

Though the total quantity of P can be high in farmed soils, it is frequently in unavailable forms and the major proportion of available forms may be far from plant roots (outside of the rhizosphere). In the soil more than 80% of P becomes unavailable and immobilized for plant uptake because of transformation to the organic form and adsorption, or precipitation (Holford, 1997). Dissolved inorganic P is important for plant uptake, but a proportion of dissolved organic P may also be utilized (Turner and Haygarth, 2000b).

P is present in soil in different forms of organic and inorganic minerals (Fig. 1.3), (at least 170 different minerals contain phosphate), both organic (P_o) and inorganic (P_i) P are important P sources for microbes and plant uptake (Rao *et al.*, 1999; Richardson, 2003). Inorganic phosphate includes P ions free in the soil solution, unstable P bound with soil particles, mainly clays, salts of insoluble inorganic P_i, for instance aluminium phosphate and iron phosphate in acidic soils or calcium phosphate in alkaline soils, and components of complex organic compounds in the organic material of soil (Rao et al. 1999;Shenoy & Kalagudi 2005). More than 90% of the whole P in the soil-plant-animal cycle is in the soils

and approximately 10% is in the residual biological cycle (Ozanne, 1980; Oberson and Joiner, 2005; Turner, 2007; Kirkby and Johnston, 2008).

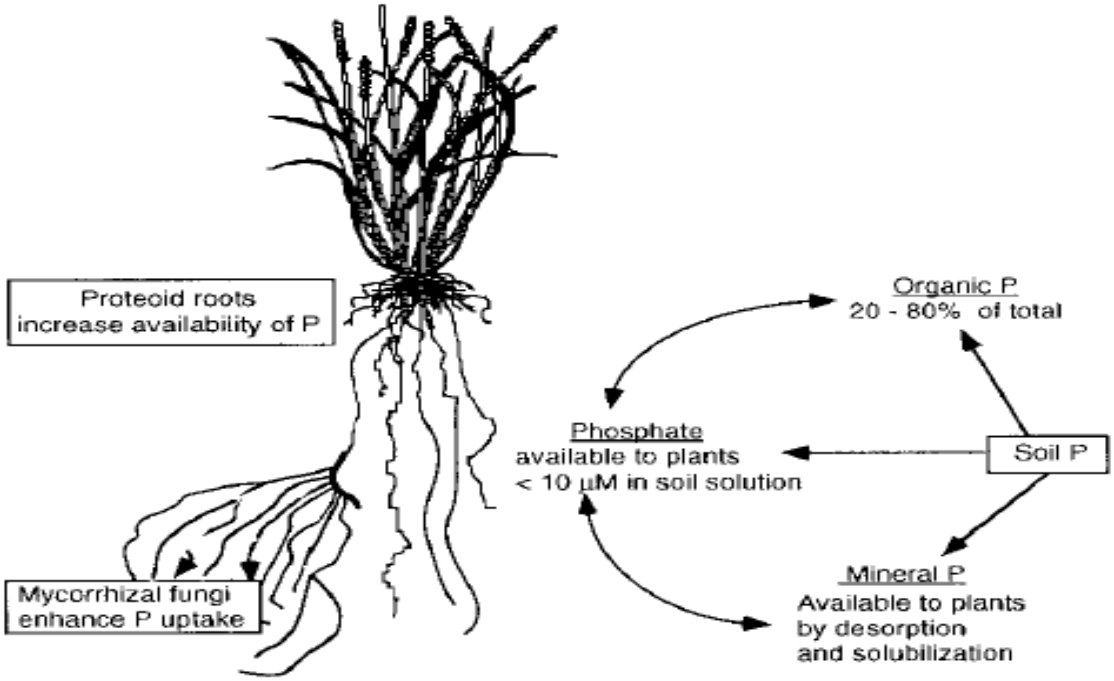

Fig.1.3. Plant acquisition of soil P (Adapted from Schachtman et al., 2001).

1.2.3.3 Phosphorus in plants

Phosphorus is a necessary nutrient for plant growth, development and fertility. It is a component of key molecules like phospholipids in cell membranes, adenosine triphosphate (ATP), adenosine diphosphate (ADP) (ADP and ATP are essential in energy storage and transfer reactions), deoxyribonucleic acids (DNA), and ribonucleic acids (RNA) (RNA and DNA are the two nucleic acid components of genetic information). It helps in the early ripening of plants, decreasing grain moisture, and enhancing crop quality (Sharma, 2002).

As a result, plants cannot grow without a reliable supply of this nutrient (Thedorou and Plaxton, 1993). However in natural and agricultural ecosystems availability of P regularly

limits plant growth (Agren 1988; Vance *et al.,* 2003; Gusewell, 2004). This is because plants can only acquire P via root hairs and via their connected mycorrhizal fungi (MF) taking up P (orthophosphate anions), mostly HPO_4^{-2} and to a lesser amount $H_2PO_4^{-1}$, from the soil solution (Bieleski, 1973; Ullrich-Eberius*et al.,* 1984; Furihata*et al.*, 1992; Schachtman*et al.,* 1998; White, 2003). Orthophosphate ions exist in the soil solution at only very low concentrations (<10 μM) because of the low solubility of P_i salts products (Hedley *et al.*, 1995; Marschner 1995). In addition, during the crop growing season the quantity of P in the soil solution of rhizosphere declines very rapidly, whereas the replenishment of P from soil P sources to the soil solution of rhizosphere is slow (Barber, 1995).

Therefore compensation for P deficits and enhancing the efficiency of P uptake can be helpful to plants' P absorption. This can be achieved by a variety of mechanisms (Fig 1.4) such as change of rhizosphere to enhance P availability by freeing of bio-molecules that are able to release P from organic-P complexes or metallic-P compounds (Marschner, 1995; Johnson et al., 1996), modification of soil exploration and increasing the absorptive area by roots (Lynch,1995), associated with soil microbes like arbuscularmycorrhizal fungi (Schachtman et al., 1998), and enhanced production of phosphates (Bariola*et al.,* 1994). In addition, the application of phosphorus fertilizers can be used to raise soluble P concentrations in the rhizosphere.

The concentration of P in plant tissue ranges between 0.2% and 0.5% of total dry matter under the conditions where P is not limiting (Sanchez, 2007). Plants absorb P only as P_i ions from in the soil solution (Holford, 1997).Therefore for plant nutrition, sources of P should be mineralized or solubilised to release soluble P (Mengel, 1997; Ashley *et al*., 2011). Properties of both the soil and the plant can affect P acquisition by the crop from the soil. Availability of P for plants depends on soil processes such as P solubility or sorption,

mineralization or immobilization, root - soil contact and P transport. Also, release and availability of P in the soil is dependent on soil pH: the highest rates of P uptake occur between pH 5.0 and 6.0 (Ullrich-Eberius*et al.,* 1984; Furtihata*et al.,* 1992).

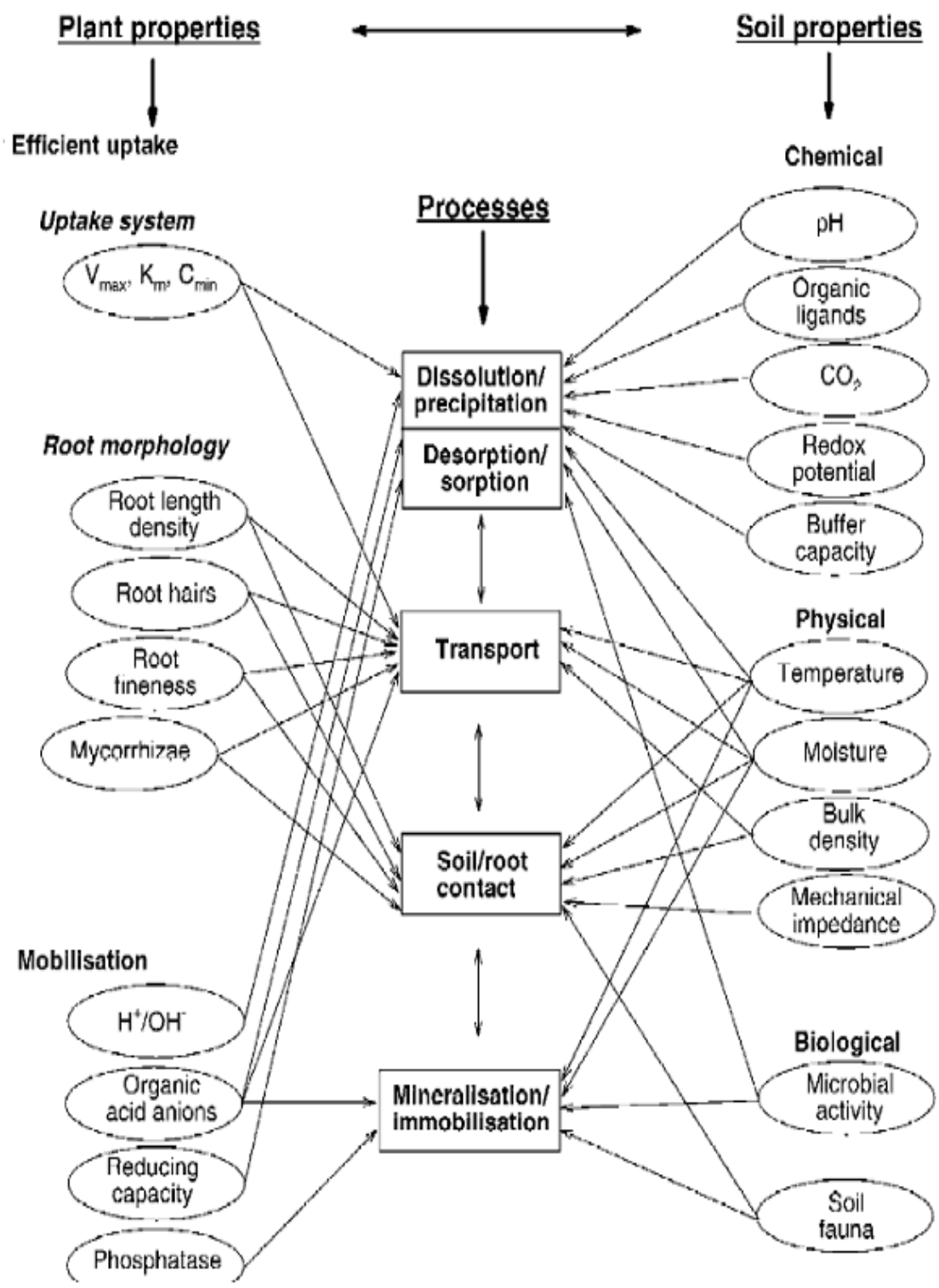

Fig. 1.4. Processes governing acquisition of soil and fertilizer P by crops as affected by plant and soil properties (Adapted from Horst *et al*. 2001).

1.2.4 Bio-inoculants used as phosphorus fertilizers

1.2.4.1 Mycorrhizae

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; Mehrotra, 2005;Pellerin *et al.*, 2007).

Mycorrhizal types

Two main categories of mycorrhizae, depending on mode of penetration of the fungus into the root cells:

1. Ectomycorrhizae are found in trees roots, where the fungus products hyphae between cortex cells of the root together with clear external hyphae encasing roots and also penetrate surrounding soil with hyphae. The majority of ectomycorrhizal fungi producing fruiting bodies (mushrooms) and can be cultured in the laboratory.

2. The most widespread type of mycorrhizal association is the arbuscularmycorrhiza (AM), connecting a wide variety of plant species and fungi from the phylum Glomeromycota (Schubler*et al.*, 2001). Endomycorrhizae, or arbuscular mycorrhizae enters cortex cells of the root

and also establish a diffuse network of external fine hyphae in soil. They are extant in the majority of agronomic plants and vegetable crops and fruits. AM are characterized by the occurrence of arbuscles in the root cortex; vesicles may or may not be present. Also AM can produce spores outside of the root (Fig 1.5).

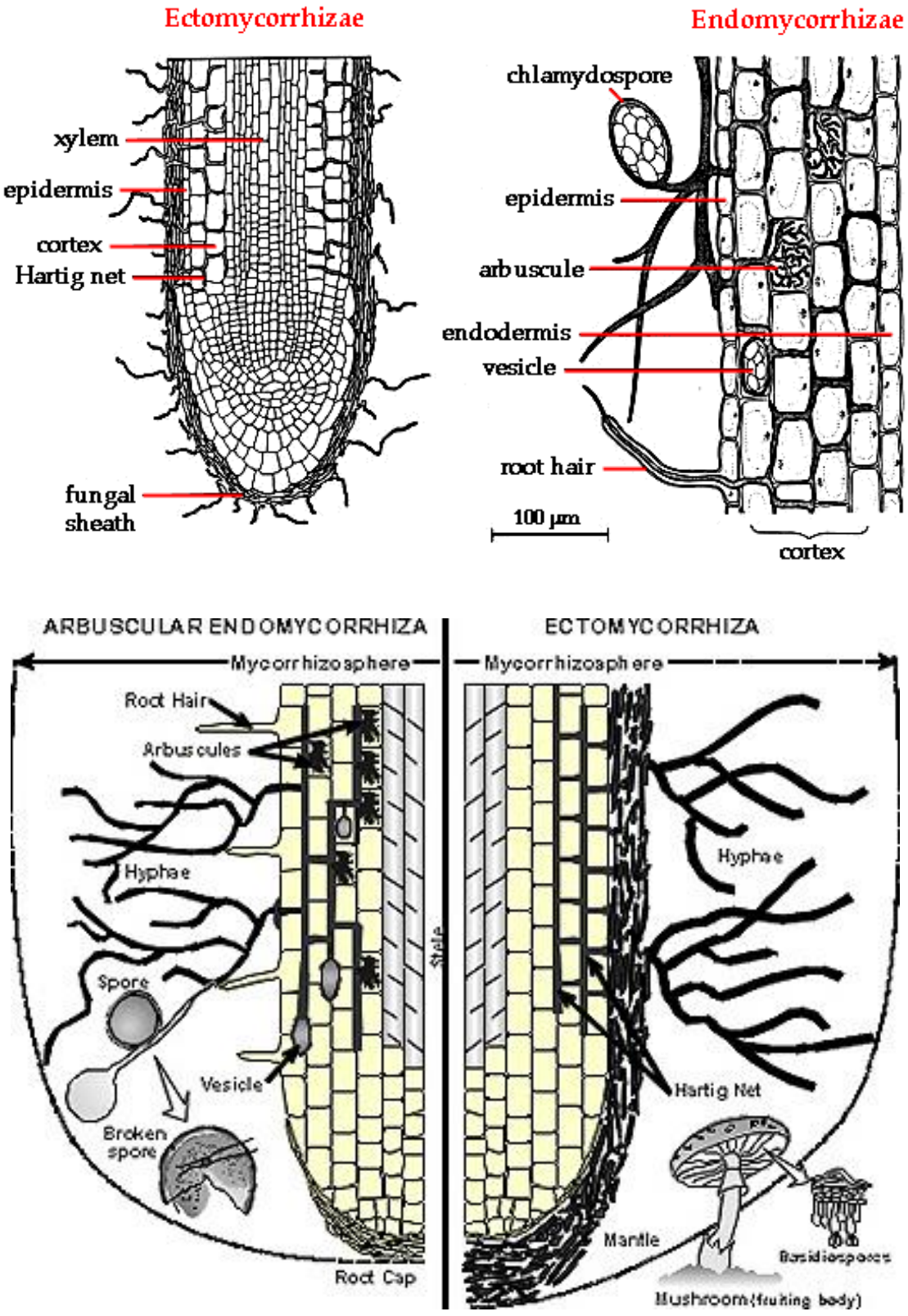

Fig.1.5.Difference in mycorrhizal colonization by ectomycorrhizae and endomycorrhizae (from cals.arizona.edu).

The advantageous effect of mycorrhizal fungi on the P nutrition of crop plants in soils low in P has been reported by many diverse researchers (e.g. Duponnois 2006; Chen *et al*., 2005; Toro *et al*., 1997). Colonization of roots by mycorrhizal fungi connects the root with soil microhabitats through the external mycelium network. Such symbioses increase nutrient cycling and capture in agroecosystems and natural environments by connecting the biotic and geochemical portions of the ecosystem (Bolan, 1991; Jeffries, and Barea, 1994; Joner, and Jakobsen, 1994). AM fungi are known to be ubiquitous in agricultural soils and are believed to enhance P nutrition of plants by scavenging the available P due to the large surface area of their hyphae (Fig. 1.6), and by their high affinity for P uptake mechanisms (Hayman, 1983; Moose, 1980).

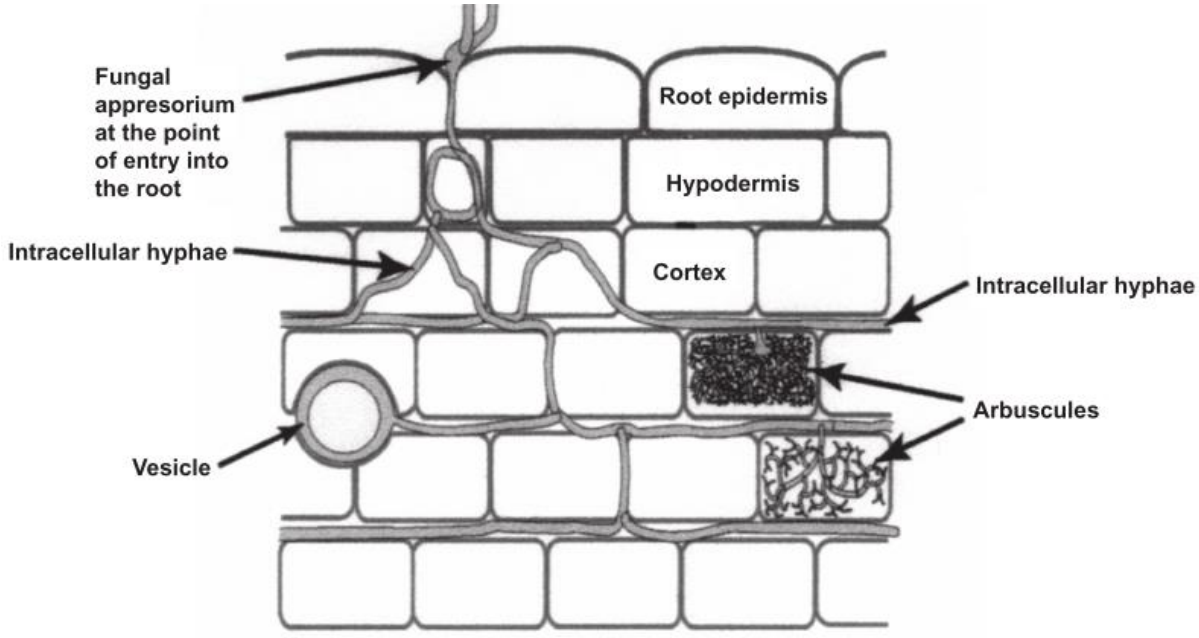

Fig.1.6. Diagram of a longitudinal section of a root showing the characteristic of arbuscularmycorrhizal fungi.http://mycorrhiza.ag.utk.edu/mimag.htmNT Uphoff - 2006 - books.google.com

The role of AM in nutrient absorption from the soil has been long accepted and recognized. Specifically it has been noted in relation to the more immobile plant nutrients in soil, like P, Zn, and Cu (Bolan, 1991; Marschner, and Dell, 1994). For the effect of AM, three main mechanisms have been postulated as being responsible:

i) The larger exploitation of the soil volume by the hyphal system (Haymann, and Mosse, 1972; Georgem*et al*., 1992).

ii) The higher affinity of hyphae for phosphate (Cress *et al*., 1979) and the ability to absorb P at lower solution concentrations than the root themselves (Faquin et al., 1990).

iii) Rhizosphere changes by AM, such as exudation of acids (Rovira, 1969) or chelates (Duff *et al*., 1963). A number of factors control the sorption of P by components of soils and sediments, such as the quantity and characteristics of the components of soil that involves other ions, pH, reaction time, P concentrations, and ionic strength of the soil solution, solid: solution percentage, and organic compounds (Khater*et al*., 2004).

1.2.4.2 Phosphorus solubilizing bacteria (PSB)

Soil and rhizosphere bacteria which are called plant growth promoting bacteria can benefit plant growth by different mechanisms. The ability of the microorganisms to solubilize P is considered to be one of the main factors associated with phosphorus nutrition of plant (Glick, 1995). P mainly moves in soils via diffusion (Lynch, 1995). Soil microbes release immobile forms of P into the soil solution but are also responsible for the immobilization of P (Fig. 1.7). Attempts to improve mineral phosphate solubilisation by utilizing the enhancing capabilities of PSB in the rhizosphere have not been particularly successful because of limitations such as their poor ecological fitness, low metabolite production and variability in

inoculate-delivery systems which have led to inconsistent performance in field applications (Mark *et al*., 2003).

Phosphorus solubilising microorganisms include many types of microorganisms present in soil (bacteria, actinomycetes, and fungi) capable of solubilising the complex insoluble forms of phosphorus into simple ions that can be taken up by plants. There is a large population of PSB in the soil and in the rhizosphere (Sperberg, 1958; Alexander, 1977). These include both aerobic and anaerobic strains, with a prevalence of anaerobic strains in submerged soils (Raghu, 1996). Considerably higher population densities of PSB are commonly found in the rhizosphere in comparison with non-rhizosphere soil (Katznelson*et al*., 1962).

Inorganic forms of P are solubilized by a group of heterotrophic microorganisms excreting organic acids and anions that dissolve phosphatic minerals, substitute for adsorbed P and/or chelate cationic partners of the P ions i.e. PO_4^{3-} directly releasing P into solution (Kucey*et al*., 1989; He *et al*., 2002). PSB fertilizer consists of millions of microorganism per gram that stay near the roots and make the phosphorus available to plants from the soil by releasing various organic acids (Taha*et al*., 1969; El Gibaly*et al*., 1977; Banik and Dey 1981; Kapoor*et al*., 1989;). These acids including acetic, oxalic, malic, citric, butyric, malonic, gluconic, lactic, succinic, glyconic, adipic, fumaric, and 2-ketogluconic acid (Taha*et al.* 1969; Gaur and Ostwal, 1972; Moghimi et al. 1978; Moghimi and Tate, 1978; Banik and Dey, 1982; Kucey, 1987; Leyval and Berthelin 1989).

Illmer and Schinner (1992) have shown that a number of PSB are very effective in solubilizing calcium phosphates without producing organic acids. Several reports have examined the ability of different bacterial species to solubilise insoluble inorganic P compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate (Goldstein, 1986). Bacterial genera which have strains with this capacity include

Pseudomonas, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Microccocus*, *Aerobacter*, *Flavobacterium*and *Erwinia*. Strains of *Pseudomonas putida*and *Pseudomonasfluorescens*have been shown to increase root and shoot elongation in oil seed rape (*Brassica campestris*), lettuce (*Lactucasativa*) and tomato (*Lycopersicumesculentum*) (Hall *et al*., 1996), and also yields in wheat (*Triticumaestivum*) (MirzaeiHeydari et al., 2009) and many other crop species. Wheat yields have been shown to increase by up to 30% with *Bacillus* inoculants (Rodriguez and Fraga, 1999).

Finally, the use of PSB can reduce the negative environmental impacts of chemical fertilizers by making them more available to crops and less prone to leaching. The use of PSB reduces the cost of chemical fertilizer use by improving its efficacy. PSB could, therefore, have a role to play in sustainable agricultural practices.

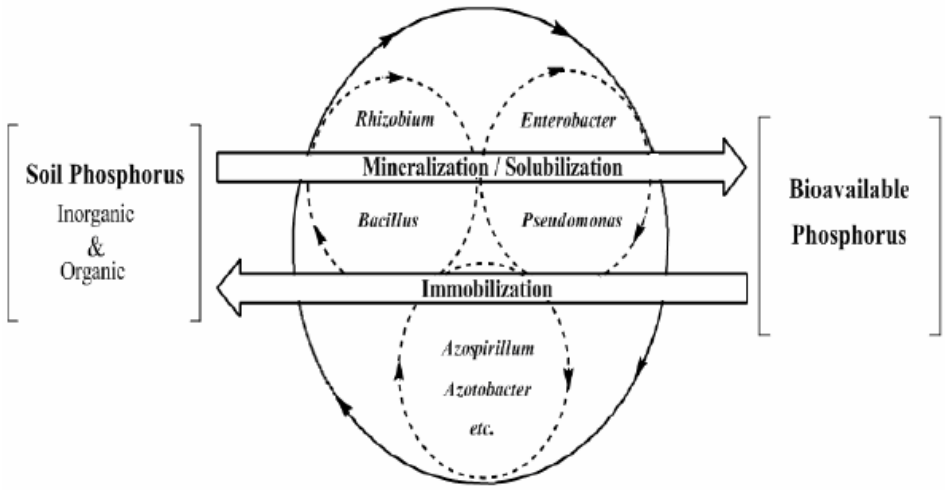

Fig.1.7. Schematic diagram of soil phosphorus mobilization and immobilization by bacteria(Khan *et al*, 2009).

1.2.5 Barley

The test organism in this research is barley, so a short review of this species follows.

Barley (*Hordeum vulgare*L.) is one of the most important cereal crops, ranking fifth in crop production worldwide (over 136 million tonnes of barley are produced annually on about 56 million ha) after maize, wheat, rice, and soybean (area harvested, FAO 2005, Akar*et al*., 2004; USDA, 2007). It is a significant crop that can provide valuable nutrients for animal feed, malt, and human food. It is able to be grown on marginal lands and in challenging environments such as conditions of drought, low temperature, relative salinity and high altitude (Van Oosterom*et al.*, 1993; Maas and Hoffman, 1997; Baum *et al.*, 2004).

Barley, over the last decade has gained increased attention as it has high energy and nutritional value with biologically valuable proteins, high lipids portion, and considerable levels of dietary fibre, It has a favourable saccharine composition (low glycaemic index), and useful amounts of vitamins and mineral substances. Integration of barley in breads can increase their fibre content contributing to the overall aim of an improved dietary fibre intake (Jakubecova, 2004; Demirbas, 2005; Holtekjølen, 2006).

1.2.5.1 Characteristics of barley grain

Physical characteristics of barley grain (covered barley) are that they are brown, through yellow to white, plump, hulled (palea and lemma adhere to the testa), middle hard and consistent in size (Pomeranz, 1974;Taketa, *et al*., 2008). A mutant form, naked barley (where palea and lemma thresh free of the seed) is suitable for use as food (Dickin*et al*, 2012).

Chemical composition of barley grain consists of approximately 65–68% starch (the largest single component in barley grain), 10–17%protein, 4–9% β-glucan, 2–3% free lipids and 1.5–2.5% minerals (Quinde *et al*., 2004). Total dietary fibre ranges from 11 to 34% and soluble dietary fibre 3 - 20% (Fastnaught, 2001). Hulless or de-hulled barley grain contains 11–20% dietary fibre (Fastnaught, 2001; Virkki et al., 2004).

1.2.5.2 Barley plant morphology

Barley is an annual grass belonging to the family Poaceae, the tribe Triticeae, and the genus Hordeum with plant height from 60 to 120 cm (Nilan&Ullrich, 1993). The depth of root (seminal and adventitious roots of barley) is dependent on the environmental conditions, soil texture and structure, soil temperature and soil moisture (Briggs, 1978; Nilan&Ullrich, 1993). The stems of barley (a central stem and 2-5 side stems) aare erect, hollow and cylindrical, sectioned by internodes bearing alternating leaves (Gomez- Macpherson, 2001).

1.2.5.3 Temperature

The lowest temperature for germination of seeds is between 3-4°C, the most favourable temperature being about 20°C, and the maximum temperature range is from 28 to 30°C. Barley can mature even when the season of growing is short, which is important especially in higher latitudes and dry land situations (Boguslawski, 1981; Holtekjolen, *et al.*, 2006).

1.2.5.4 Barley nutrient and P fertilizer

Barley is one of the more responsive crops to fertilizer because of high yield potential and fast growth (Grant, *et al.*, 1991) and also research has shown that barley was more responsive to P fertilizer than either wheat or canola (McKenzie *et al.,* 2003). Spring barley requires adequate P for optimal tillering and plant growth. Phosphorus is usually adequate when the soil test P concentration is greater than 15 to 20 ppm (Robertson & Stark, 1993). Spring barley has a comparatively low phosphorus requirement, but a sufficient quantity of P must be available for optimum plant growth (Fig 1.9). Thus, if the soil level of P is low, the crop will respond to P application(McKenzie *et al.*, 2003;Mahler & Guy, 2007).

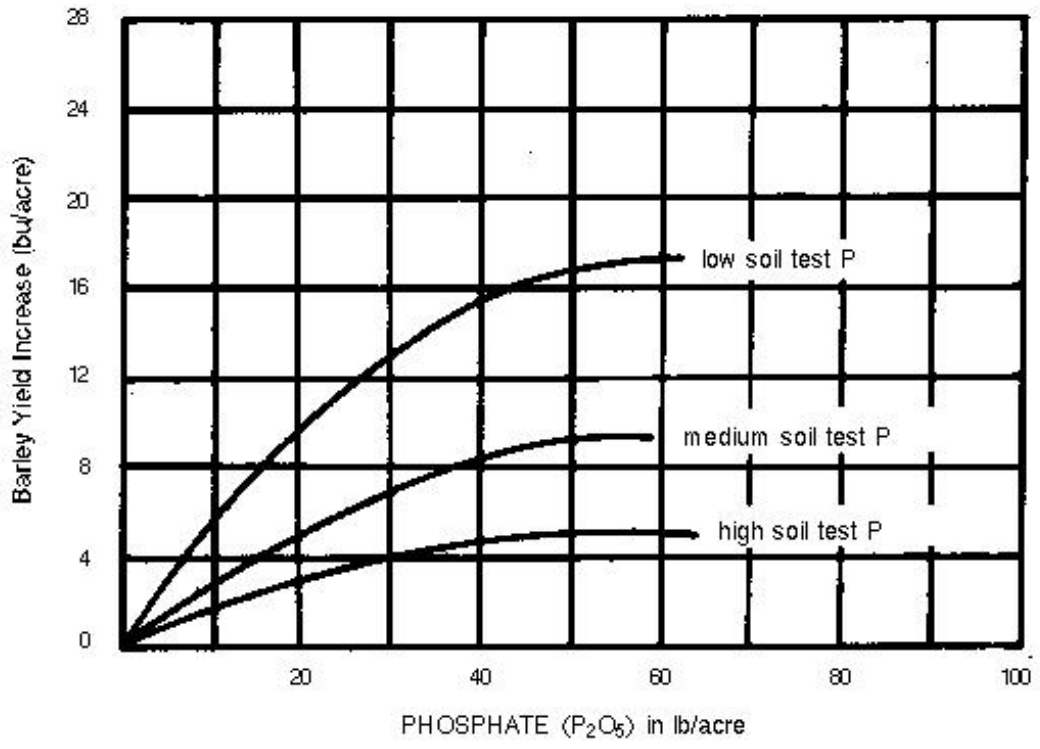

Fig. 1.9. Expected yield increases with phosphorus fertilizer use in Central Alberta. (From, www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/crop1228)

Chapter 2: General materials and methods

2.1. Materials and methods

The objective of this thesis was to further understand the role of mycorrhizae (M) and P solubilizing bacteria (PSB) bio-inoculants and external P sources (super phosphate (SP), struvite (AMP) and rock phosphate (RP) on phosphorus availability in soil and their effects on the growth, yield and P uptake of barley by method of randomized complete block design from 2010 to 2012 in the field and glasshouse (Table 2.1).

2.1.1. Experiments timetable

Table 2.1. The time table of all of the PhD research experiments from start to end in the field and glasshouse.

Experiment	Treatments	Replication	Days	Sowing	Harvesting
Field experiment 1	8	3	119	26/03/2010	23/07/2010
Field experiment 2	8	4	133	12/04/2011	22/08/2011
Pot experiment 1	12	9	126	26/09/2011	30/01/2012
Pot experiment 2	24	6	120	06/01/2012	06/05/2012

2.1.2. Staining and colonization of the roots

For measurement of the mycorrhiza colonization level, root samples were washed in water to remove soil particles then cut into 1-2 cm lengths taken from five different sites around the root and stored in individual vials containing a formalin, acetic acid and alcohol (FAA) solution or fixed in 50% ethanol (C_2H_5OH) for at least 24 h. Root samples were stored in the cold room (5º C) until they had been processed.

The brief procedure of clearing and staining roots using the method of Phillips and Hayman (1970) was as follows:

1. For roots staining, C_2H_5OH removed by washing with deionized water several times and placed in small glass vials.
2. For clearing, 10% (w/v) KOH solution added to cover roots and heated at 90 °C for about 20 minutes and.
3. Washing KOH solution, rinsed with deionized water 3 times.
4. Acidified in 2% HCl for 1-2 min.
5. Stain in 0.05% Trypan blue (made up in a solution of 80% lactic acid: glycerin: distilled water 1:1:1) for 2-3 h at 90 C˚.
6. De-stained in 50% glycerin for approximately 24 h.
7. Mount roots on slides in 50% glycerin.

The degree of infection and colonization was determined by the grid-line intersect method (Fig 2.1) (Giovanetti and Mosse, 1980).

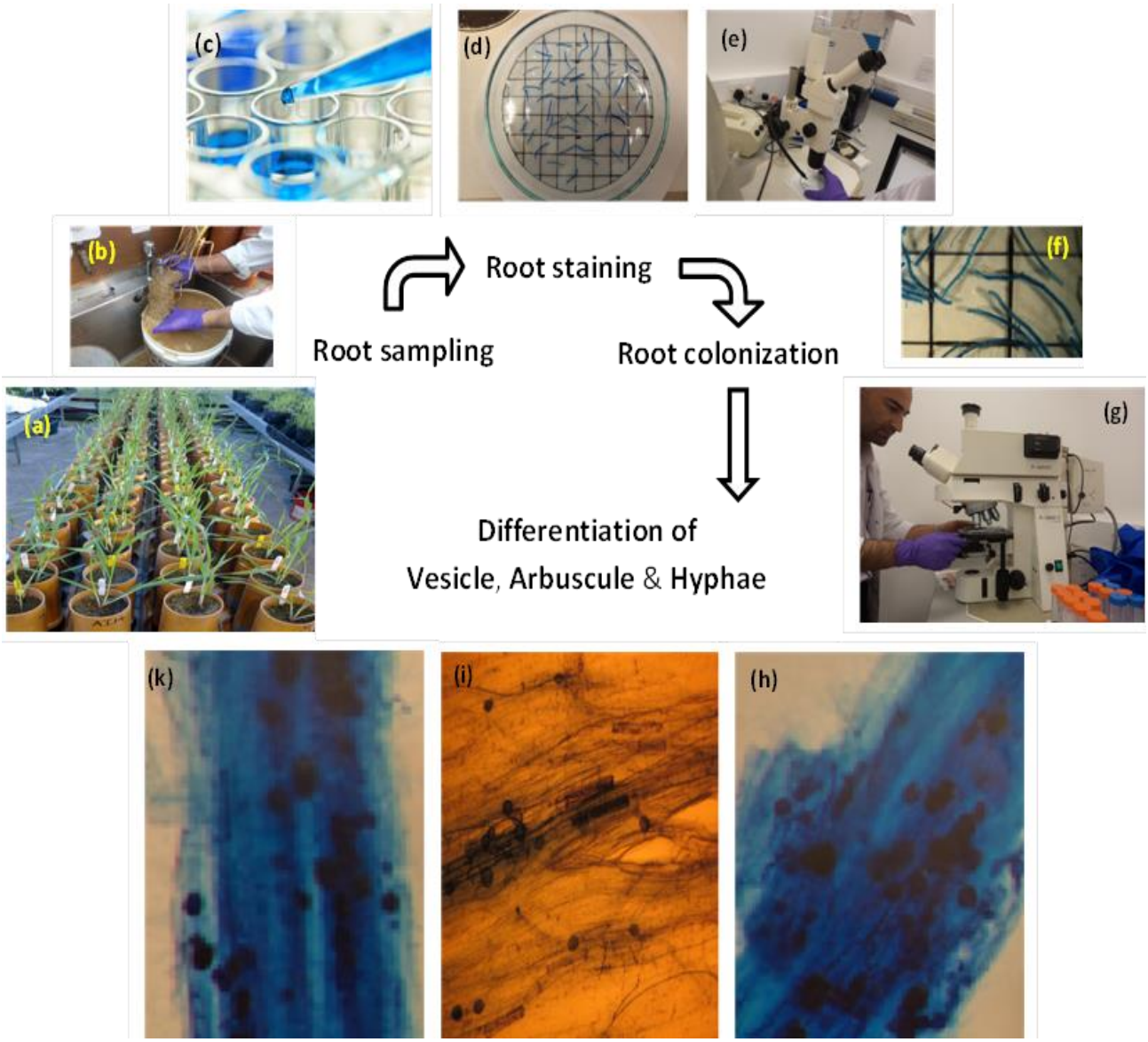

Fig. 2.1. Measurement of VAM infection process. (a) colonized barley plants in the pot experiment; (b) root clearing and sampling; (c) root staining; (d) root parts in grid-line petri dish; (e) binocular microscope; (f) determination of the roots colonization with the grid-line intersect method by binocular; (g) microscopic differentiation of Hyphaes (h), Arbuscules (i) and Vesicles (k).

2.1.3. Phosphorus analyses

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

The brief procedure for phosphate analysis was:

1. Make up 10 mL of 10% (w/v) ascorbic acid.
2. Add 80 µL of sample or standards to all the wells required in a 96 well plate.
3. Add 180 µL of Ames Reagent (kept in fridge) to all the wells required in a 96 well plate.
4. Add 30 µL of ascorbic acid (by multi pipette) to all the wells required in 96 well plate.
5. Measure absorbance at 820 nm in plate reader after 15 minutes (when standards are blue enough). Keep reading at 30 min intervals until all strips are read.
6. The readings were converted to mg P kg^{-1}dwt from standard curves (Fig 2.2).

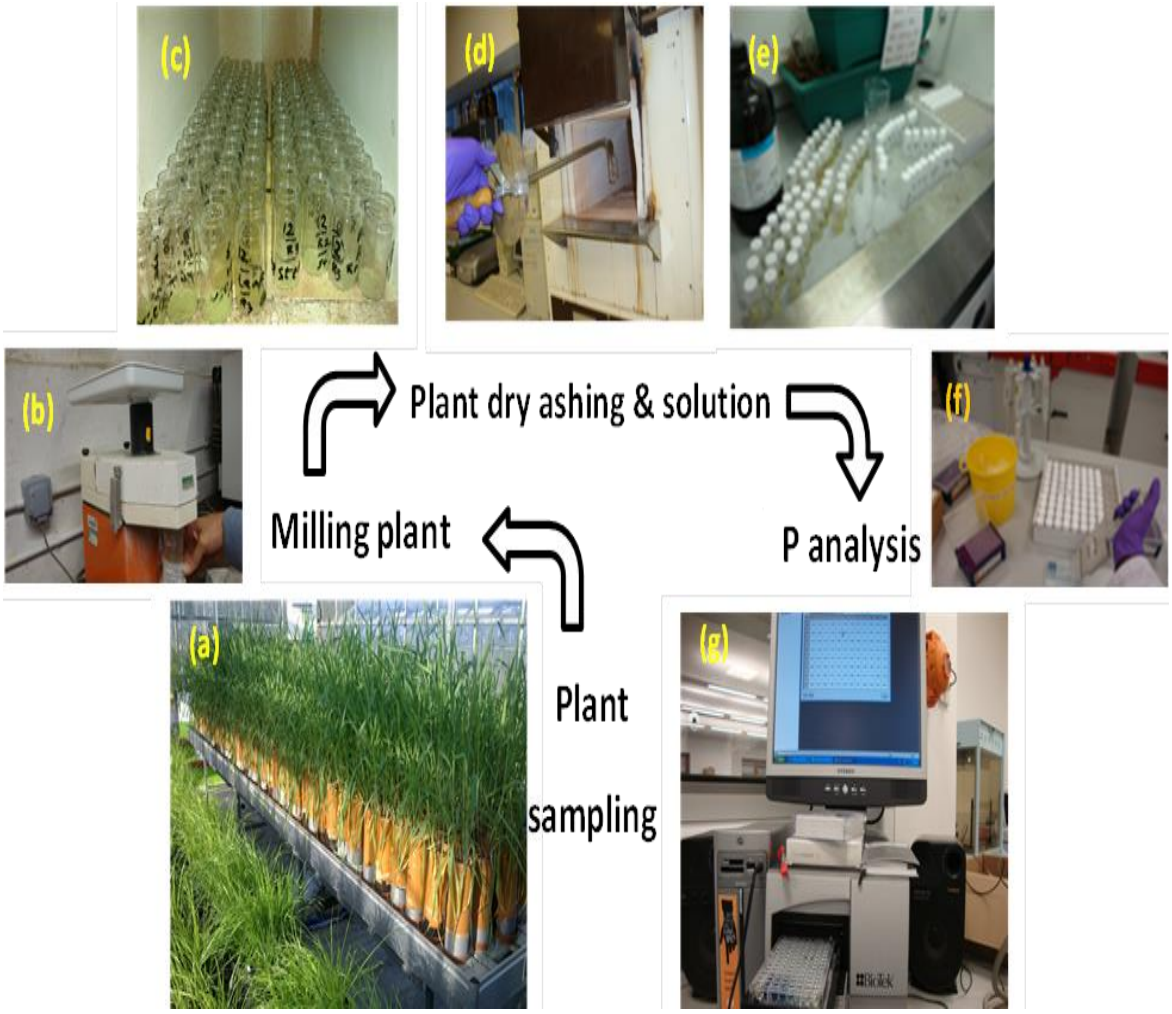

Fig. 2.2. Phosphorus analysis process. (a) Plants sampling; (b) Plant milling; (c) Sampling and weighting; (d) Plants dry ashing; (e) Preparation of samples solution; (f) Preparation of the reagent and standard solutions; (g) Spectrophometeric analysis and determination of P concentration.

2.1.4. Statistical analyses

Data were analysed byone-way and tow-way analysis of variance (ANOVA) to determine main treatment effects. Mean values were compared by Tukey test (GenStat 14th Edition, SPSS version 19, and Sigma Plot version 12). Tukey multiple range tests were used to compare significant differences between means at $P = 0.05$.

Chapter 3: Field experiments

3.1. Introduction

Phosphorus (P) is one of the primary macronutrients involved in various essential biochemical reactions in plant growth and has immense importance in root shoot and grain formation. P in soil exists in two forms i.e. organic and inorganic (Ehrlich, 1990; Paul and Clark 1989). When fertilizer-P is applied to soil, some portion of the soluble P (fertilizer-P) is used by the plant and the remaining P is immobilized in to soil organic matter, incorporated in to the microbial biomass or converted/precipitated into inorganic forms of varying solubility. Application of P fertilizers with low nutrient use efficiency (range from 10% to 30% uptake in the year of application) is of grave concern among environmentalists (water pollution due to P eutrophication) and economists (high cost of production) (Ghost and Bhat, 1998; McLaughlin, *et al*., 1991; Miller *et al*. 2001;Xie, *et al*., 2011). The use of P fertilizer can be minimized by using certain biological means such as integrated use of mycorrhiza and phosphorus solubilizing bacteria (PSB), capable of extracting phosphorus from raw material (rock phosphate) used in the production of fertilizer (Sudhakarapeddy*et al*., 2002; Harrier, *et al*., 2003;Chen *et al*., 2006).

The PSB induced chemical changes in the rhizosphere enable the plant to enhance P uptake and crop yield by solubilisation of P fertilizers and mineralization of RP (Taha*et al*., 1969; Banik and Dey, 1981; Kim *et al*., 1998; Chen *et al*., 2006). The PSB produce a number of organic acids to dissolve phosphates by means of anion exchange or chelating Ca, Fe or Al ions linked with the phosphates. These organic acids include citric, butyric, succinic, malic, acetic, glyconic, adipic, oxalic, malonic, lactic, gluconic, fumaric, and 2-ketogluconic acid. (Louw and Webley 1959; Taha*et al*., 1969; Moghimi*et al*., 1978; Moghimi and Tate 1978;

Illmerans Schinner, 1995; Rodriguez and Fraga, 1999; Gyaneshwar*et al*., 2002; Chen *et al*., 2006;Hameeda*et al*., 2008).

The vesicular-arbuscular mycorrhizal (VAM) develop a beneficial symbiotic relationship between soil and plant roots (Vierheilig, *et al*., 1998; Sieverding, 1991; Schreiner *et al*. 2003). The symbiosis enables the host plant to obtain mineral nutrients from outside the normal root area (rhizosphere) by the extraradical fungal mycelium and mycorrhizal hyphae, whereas in return the fungi takes photosynthetically manufactured carbon compounds from the host (Smith, and Read, 1997). Mycorrhiza are of two types: ectomycorrhiza (extracellular fungal growth in the root cortex) and endomycorrhizas (intracellular fungal growth in root cortex). Vesicular arbuscular mycorrhiza (endomycorrhiza) is a widely distributed in the world (Smith and Read, 1997). Colonization of plant roots by AMF enhance P uptake from soil solution through the hyphal network thus increasing surface area of roots and improve resistance against drought stress (Janson, 1980a; Janos, 1980b; Dela Cruz, 1987; Sieverding, 1991; George *et al*. 1995; Chen *et al*. 2005; Ruiz-Lozano, 2006).

Application of rock phosphate (RP) directly to P-deficient acidic soils is recommended as an alternative to P fertilization and has received significant interest in recent years; however solubility of RP is low in non-acidic soils. RP is a cheaper raw material generally used in the manufacture of P fertilizer (Owumu-Bennoah*et al*., 2002). The use of PSB and VAM can increase the availability of P from RP as well as soil and resultantly enhance P uptake of plants (Gyaneshwar*et al*., 2002).

This chapter reports the effects of biological P (M and PSB) inoculation and natural sources of phosphorus (RP) on growth and production of spring barley (*Hordeum vulgare* L. var. Static) in laboratory and field studies in comparison to P fertilizers. After analysis of the results of the first field experiment where significant effects of M and PSB, on plant growth and production were, a found second field experiment was amended to use M with chemical

P fertilizers (AMP, SP) and test the ability of M for efficiency and increase P uptake and decreasing of chemical P fertilizers.

3.2. Materials and methods

3.2.1. 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, UK (53.14^0 N, 4.01^0 W) with a temperature hyperoceanic climate and a seasonal temperature varying between -3to 10 ^{0}C in winter and 12 to 25 ^{0}C in summer and the annual rainfall is about 1000 mm) (Figure 3.2.1). During the first season, 2009-2010, the experiment investigated the effects of biological P (M and PSB) and natural sources of phosphorus (RP) on growth and production of barley (*Hordeum vulgare* L. var. Static). There were seven P-source treatments plus a control (zero P) of P fertilizers treatments of rock phosphorus (RP), phosphorus solubilising bacteria: *Pantoeaagglomerans 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. The treatments were arranged as a 2*4 factorial mycorrhiza and P source being the main effect.

External P sources were rock phosphate (RP), triple super-phosphate (TSP) and ammonium magnesium phosphate (AMP, Struvite, a waste product from the water industry). There were two control treatments, one with no mycorrhiza or external P (C), and one with just mycorrhiza (M).

3.2.2. Experimental soil

The soil used for the field study was a sandy loam, which had received no P fertilizer. Soil samples were taken at depths of 0-30 cm after removing 3 cm of the soil surface, for soil analysis. Initial analysis showed that the 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 mgL-1 and available Mg at 42 mg L-1and pH was 6.4. The experimental area was used for sheep grazing and had not been cultivated for many years previously. The annual rainfall and temperature of the experimental site and soil are given in Figure 3.2.1.

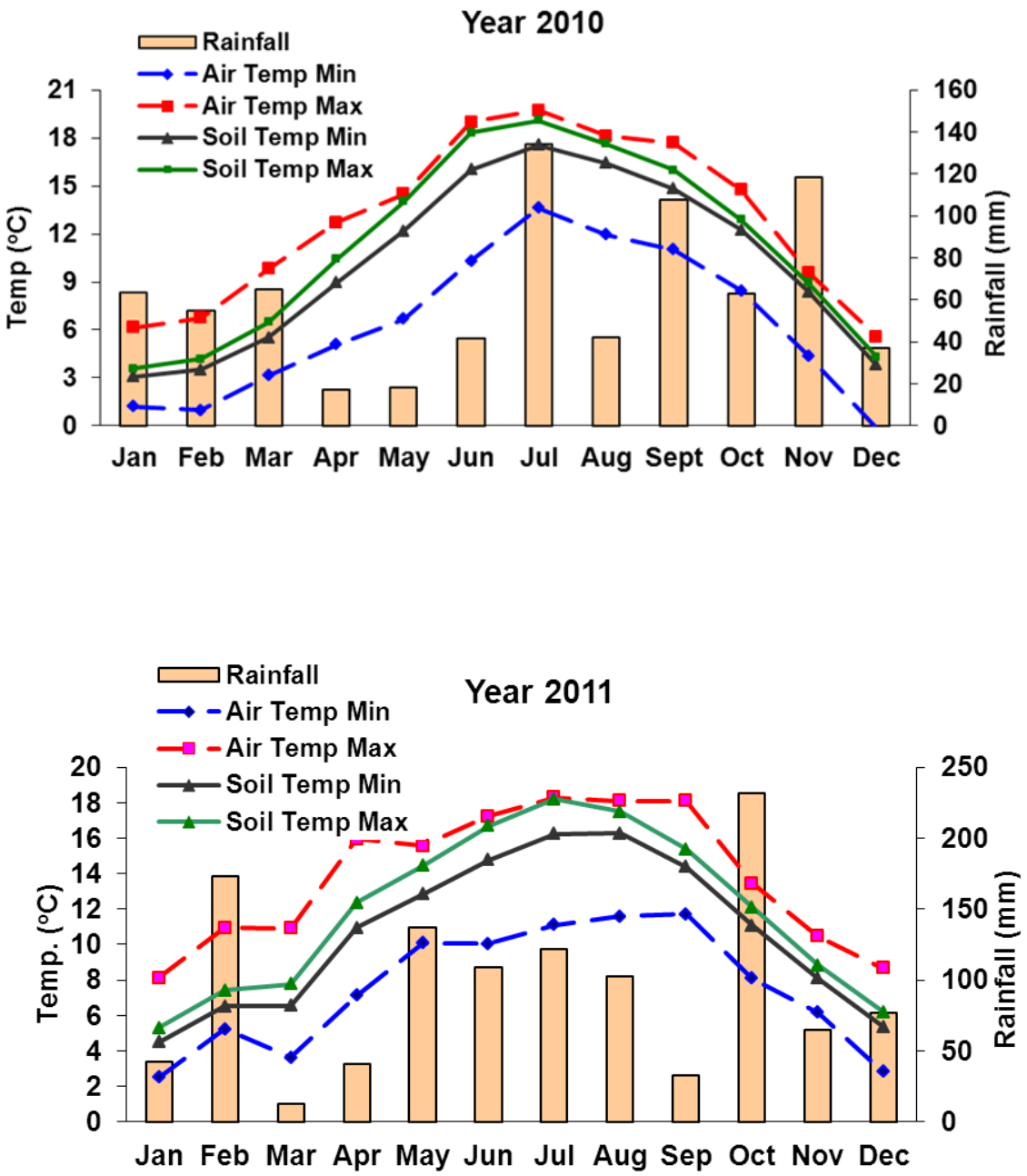

Fig. 3.2.1. Annual temperature (Soil and atmosphere) and rain fall in the experimental site(years of 2010 and 2011). Data from a Campbell Automatic Weather Station (Campbell Scintific Ltd, Shepshed, UK) Henfaes Meteorological Station.

3.3.3. Experimental design

The first experimental design was a randomized complete block design with three replications (Fig 3.2.2). 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. Barley seeds were sown on 26th March, 2010 and harvested on 22th July. The seeds were inoculated with M and PSB in the appropriate treatments before sowing and RP was applied with the seeds.

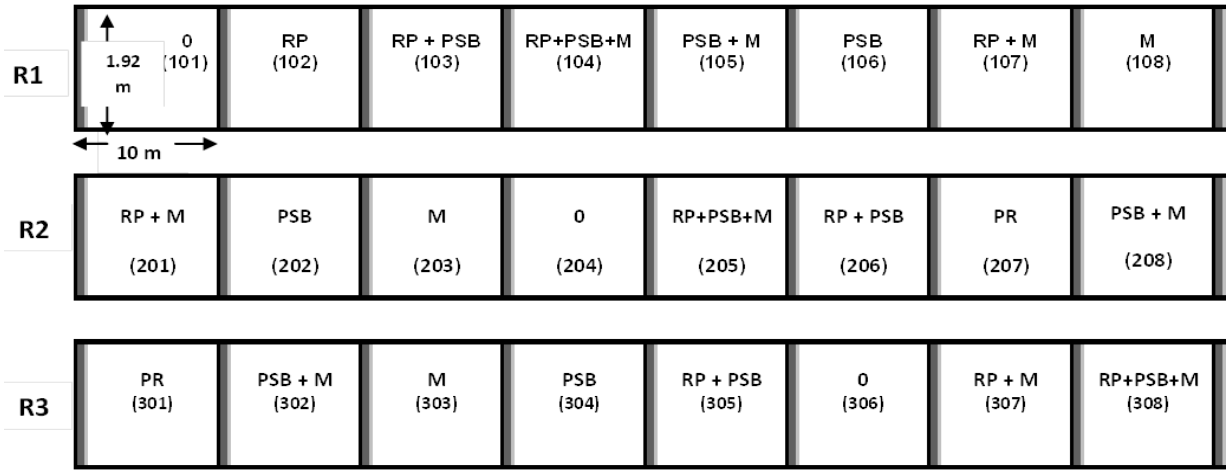

Fig. 3.2.2. First field experimental design (3 replicates) with barley under eight nutrient treatments (Year 2010).

The second experimental design was a 2*4 factorial arranged as a randomized complete block design with four replications (Fig 3.2.3). 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 (80 kg P ha^{-1}) 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. The crop was harvested at physiological maturity.

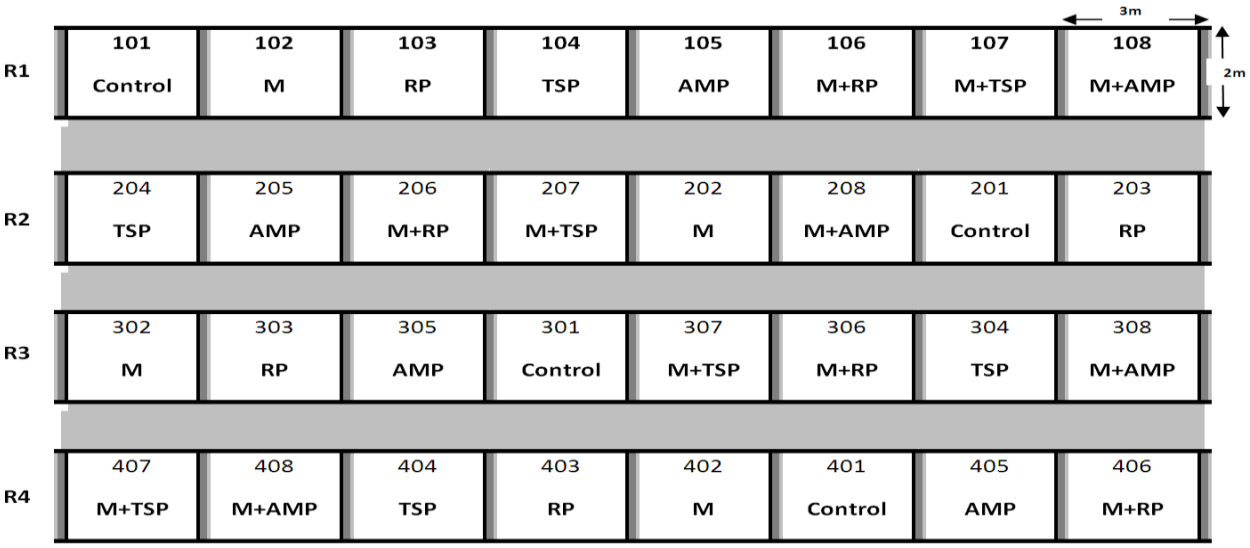

Fig. 3.2.3. Second field experimental design (4 replicates) with barley under eight nutrient treatments (Year 2011).

3.2.4. Measurements and data recorded

In the first experiment samples were taken at 43, 53, 64, 73, 83, 94 and 104 days after sowing 50 cm row lengths of barley plants were randomly taken from each plot to determine leaf area index, stem dry matter, leaf dry matter and straw dry matter.For the final sampling and harvesting (8^{th}Sample), at Growth Stage 87, Hard dough (HGCA, 2006) 119 days after sowing plants were harvested at maximum grain dry matter but before ripening to avoid bird

damage, which is a major problem in this area. 2m row lengths of barley plant were randomly taken from each plot to determine straw dry matter, ear dry matter, and root dry matter(root systems were harvested with the whole plant after washing and dry the roots), grain yield, 1000 seeds weight, plant height, and number of ears.

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. The crop was harvested at grain maturity and to avoid damage birds nets were used (Fig 3.2.4).

Fig. 3.2.4. Some problems and controlling trough the field experiments.

3.2.5. Staining and colonization of the roots

Refer to chapter 2

3.2.6. Phosphorus analyses

Refer to chapter 2

3.2.7. Statistical analyses

Refer to chapter 2

3.3. Results

3.3.1. First Field Experiment (year 2010) results

3.3.1.1. Dry matter accumulates

The significant effect of treatments on dry weight of plant parts were seen in the RP+PSB+M treatment on root, stem, leaf, and ear in the all sample stages (Figs 3.3.1.1, 3.3.1.2, 3.3.1.3, 3.3.1.4 and Table 3.3.1.1, 3.3.1.2, 3.3.1.3, 3.3.1.4) and there was also a significant effect of RP+PSB+M treatment on dry root, stem, leaf, and ear weight in the final sample (Fig 3.3.1.5 and Table 3.3.1.5).

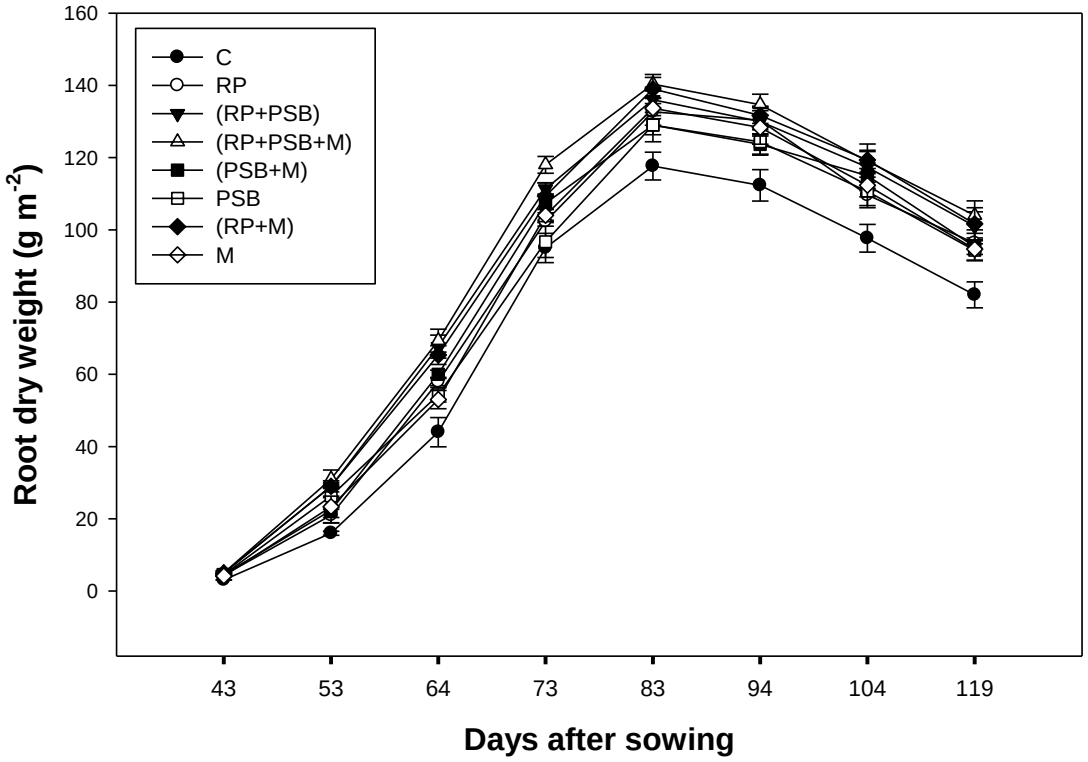

Fig. 3.3.1.1. Mean values of root dry weight by P sources at eight samples(43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.1.Table of mean values, standard errors of differences and P values for data presented in Figure 3.3.1.1. Significant levels of P are in bold script.

Treatments	Root dry matter (g m^{-2})							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
C	3.1	16.0	44.0	95.0	117.7	112.3	97.7	82.0
RP	4.4	21.0	58.0	102.3	132.7	130.3	109.7	96.3
RP+PSB	5.0	29.0	67.7	111.3	136.0	130.0	117.3	101.0
RP+PSB+M	5.0	31.0	69.3	118.0	140.3	134.7	119.0	104.0
PSB+M	4.6	22.3	60.0	107.3	129.0	123.7	115.0	95.3
PSB	4.5	26.3	54.3	96.7	129.0	124.3	110.7	94.3
RP+M	5.0	29.0	65.3	109.3	139.0	131.7	119.3	101.7
M	4.2	23.3	53.0	104.0	133.7	128.3	112.3	94.7
SED	0.801	5.972	8.990	8.413	8.509	8.443	8.791	8.122
P	**0.025**	**0.009**	**<.001**	**0.001**	**0.008**	**0.015**	**0.023**	**0.01**

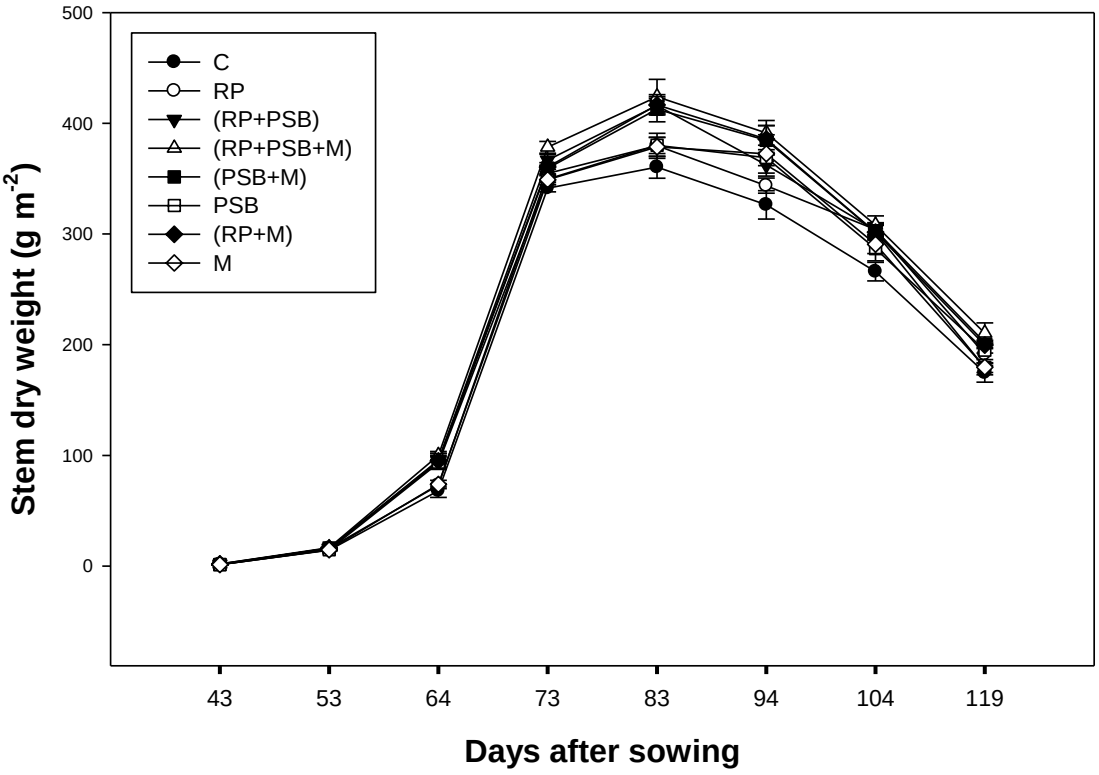

Fig. 3.3.1.2. Mean values of Stem dry weight by P sources at eight samples(43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.2.Table of mean values, standard errors of differences and P values for data presented in Figure 3.3.1.2. Significant levels of P are in bold script.

Treatments	Stem dry matter (g m^{-2})							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
C	1.3	14.3	68.0	341.3	360.3	326.3	266.0	174.7
RP	1.3	16.0	73.0	354.7	379.7	343.7	304.0	191.3
RP+PSB	1.7	16.0	95.7	366.7	416.0	362.3	303.3	202.0
RP+PSB+M	2.0	16.7	100.0	378.3	424.0	391.3	308.0	210.3
PSB+M	1.3	16.3	94.0	359.3	412.7	384.7	301.3	179.3
PSB	1.3	14.7	93.0	349.7	380.0	369.0	286.7	195.0
RP+M	2.0	16.0	95.0	360.3	416.7	385.7	301.7	199.7
M	1.3	14.7	73.7	349.0	378.7	372.3	290.7	179.7
SED	0.509	1.100	14.206	12.830	27.267	27.332	17.446	15.376
P	0.398	**0.022**	**0.001**	**0.001**	**0.003**	**0.016**	**0.031**	**0.015**

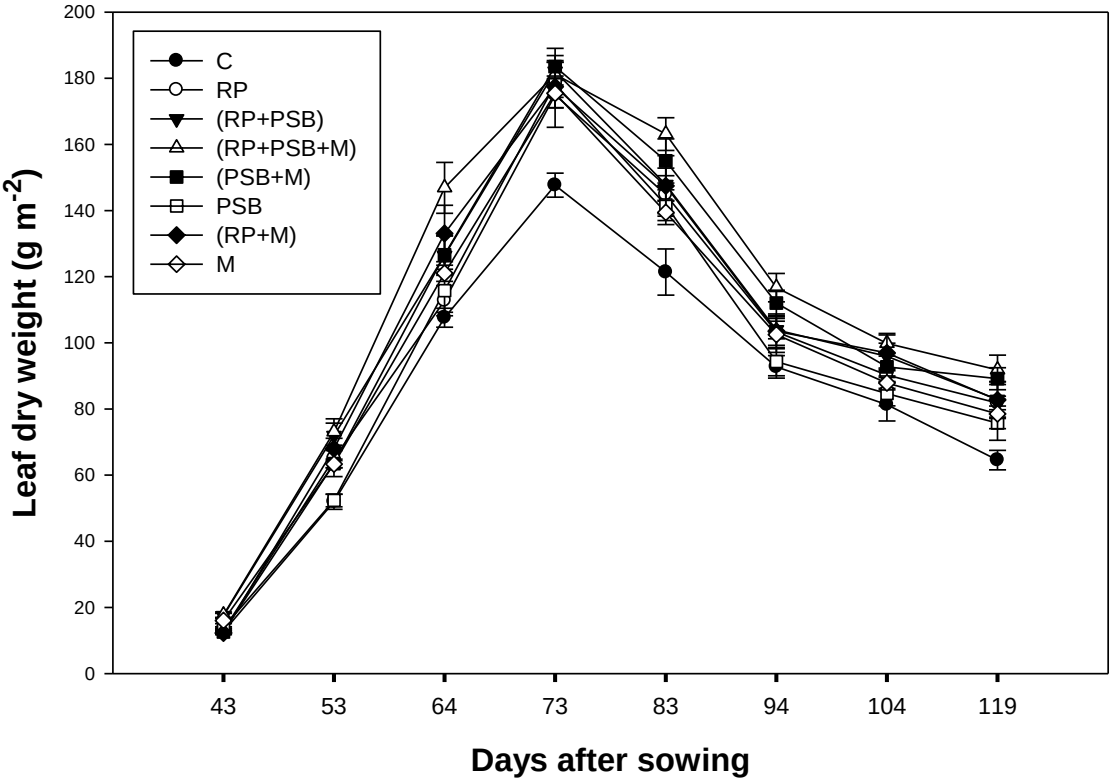

Fig. 3.3.1.3. Mean values of leaf dry weight by P source at eight samples(43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.3. Table of mean values, standard errors of differences and P values for data presented in Figure 3.3.1.3. Significant levels of P are in bold script.

Treatments	Leaf dry matter (g m^{-2})							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
C	12.7	51.9	107.6	147.7	121.4	92.7	81.3	64.5
RP	13.1	65.0	112.8	175.0	144.8	103.3	90.3	81.8
RP+PSB	17.5	71.9	126.4	182.1	147.8	103.9	95.9	82.9
RP+PSB+M	17.7	73.1	146.9	181.2	163.1	116.7	99.8	91.8
PSB+M	12.6	63.5	126.6	183.5	155.0	112.1	92.8	89.2
PSB	13.9	52.3	115.8	178.2	141.6	94.3	84.6	75.7
RP+M	12.3	68.0	133.1	177.4	147.4	103.5	97.0	82.7
M	16.0	63.3	121.0	175.4	139.4	102.5	87.9	78.5
SED	2.36	8.77	14.65	13.74	14.59	9.80	8.14	10.19
P	**<.001**	**0.001**	**0.006**	**0.010**	**0.010**	**0.016**	**0.035**	**0.013**

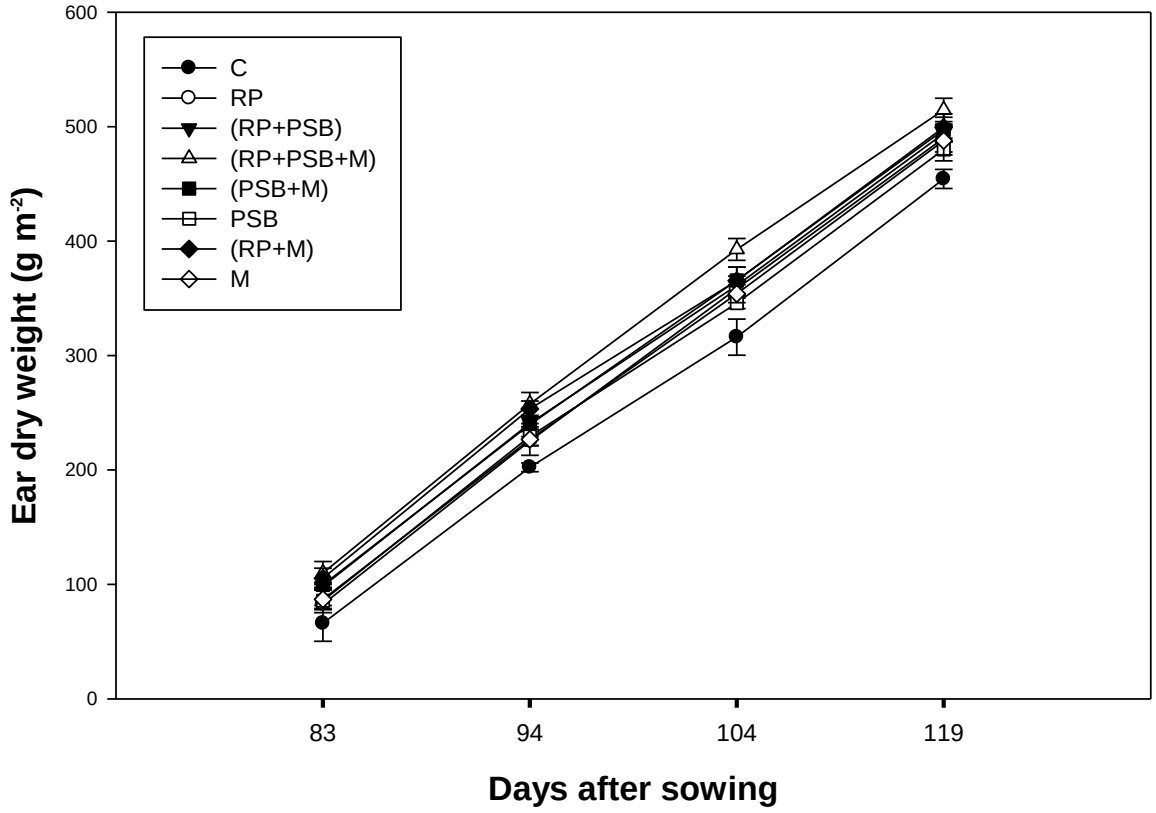

Fig. 3.3.1.4. Mean values of ear dry weight by P source at four samples(83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.4.Table of mean values, standard errors of differences and P values for data presented in Figure 3.3.1.4. Significant levels of P are in bold script.

Treatments	Ear dry matter (g m^{-2})			
	Sample 5	Sample 6	Sample 7	Sample 8
C	66.0	202.3	316.1	454.3
RP	82.7	225.3	358.3	490.0
RP+PSB	98.3	240.7	361.3	494.0
RP+PSB+M	110.0	257.7	392.7	514.6
PSB+M	99.7	239.7	365.7	497.3
PSB	85.7	229.3	345.7	480.0
RP+M	105.3	253.3	365.4	499.4
M	87.0	226.7	354.0	487.8
SED	18.7	20.3	25.2	22.6
P	0.054	**0.004**	**0.005**	**0.043**

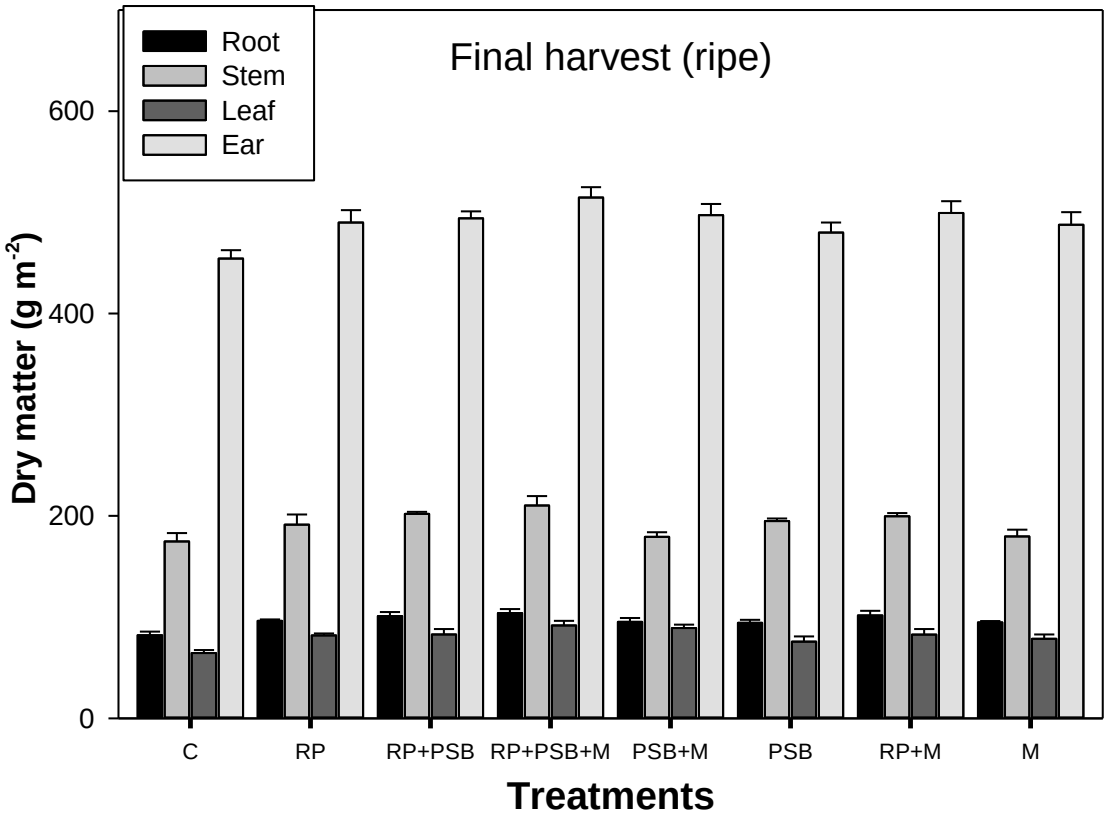

Fig. 3.3.1.5. Mean values of root, stem, leaf and ear dry weight by P source at the final sample.Error bars show standard error of means (n=3).

Table 3.3.1.5. Table of mean values, standard errors of differences and P values for data presented in Figure 3.3.1.5. Significant levels of P are in bold script.

Treatments	Final dry matter (g m^{-2})			
	Root	Stem	Leaf	Ear
C	82.0	174.7	64.5	454.3
RP	96.3	191.3	81.8	490.0
RP+PSB	101.0	202.0	82.9	494.0
RP+PSB+M	104.0	210.3	91.8	514.6
PSB+M	95.3	179.3	89.2	497.3
PSB	94.3	195.0	75.7	480.0
RP+M	101.7	199.7	82.7	499.4
M	94.7	179.7	78.5	487.8
SED	8.12	15.38	10.19	22.56
P	**0.01**	**0.015**	**0.013**	**0.043**

3.3.1.2. Yield and yield components of barley

In this study application of RP+PSB+M treatment significantly increased number of ears per plant, 1000 seed weight and grain yield compared to the control treatment by RP+PSB+M at the final sample stage (Table 3.3.1.6 and Fig 3.3.1.6).

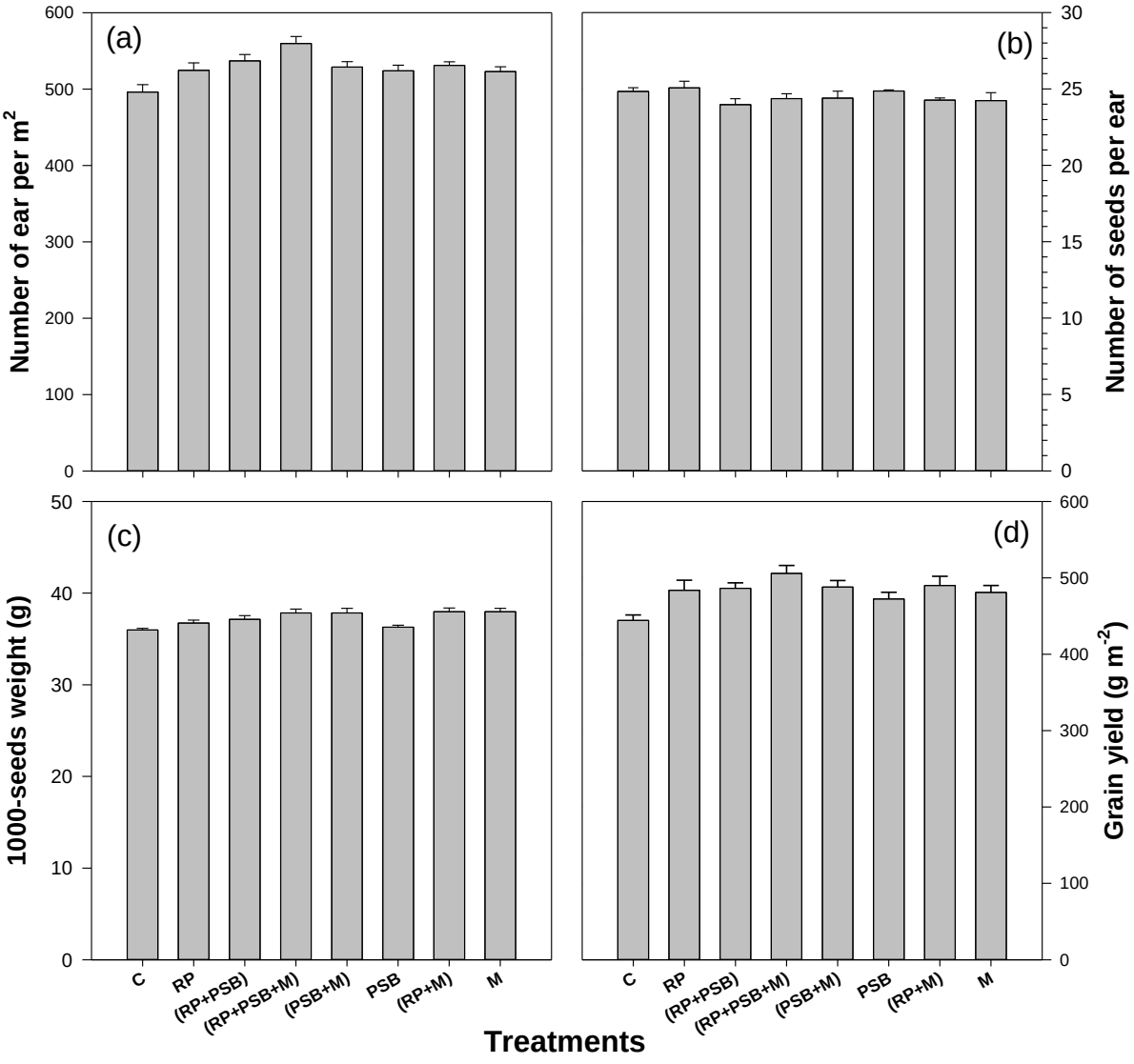

Fig. 3.3.1.6. Effect of P source on number of ear per plant(a), number of grains per plant(b), 1000-seed weight(c) and grain yield(d) at final sample stage. Error bars show standard error of means (n=3).

Table 3.3.1.6. Table of mean values, standard errors of differences and P values for data presented in Figure 3.3.1.7. Significant levels of P are in bold script.

Treatments	Yield and yield components of barley			
	Nu. Of ears per m^{-2}	Nu. Of seeds per ear	1000-seeds weight (g)	Grain yield (g m^{-2})
C	496.2	24.8	36.0	444.3
RP	524.5	25.1	36.7	483.6
RP+PSB	537.0	24.0	37.1	486.3
RP+PSB+M	559.6	24.4	37.8	505.8
PSB+M	528.8	24.4	37.8	487.9
PSB	523.9	24.9	36.3	472.3
RP+M	531.0	24.3	38.0	490.0
M	522.8	24.2	38.0	480.8
SED	20.359	0.628	0.937	22.021
P	**0.004**	**0.390**	**0.004**	**0.022**

3.3.1.3. P concentration

Analysis showed that the P concentration in root, stem and leaf were significantly by treatments affected by the treatments in the all sample stages (Tables 3.3.1.7, 3.3.1.8 and 3.3.1.9). The highest P concentrations in root, stem and leaf were in the first sample (43 days after sowing) (Figs 3.3.1.7, 3.3.1.8 and 3.3.1.9).

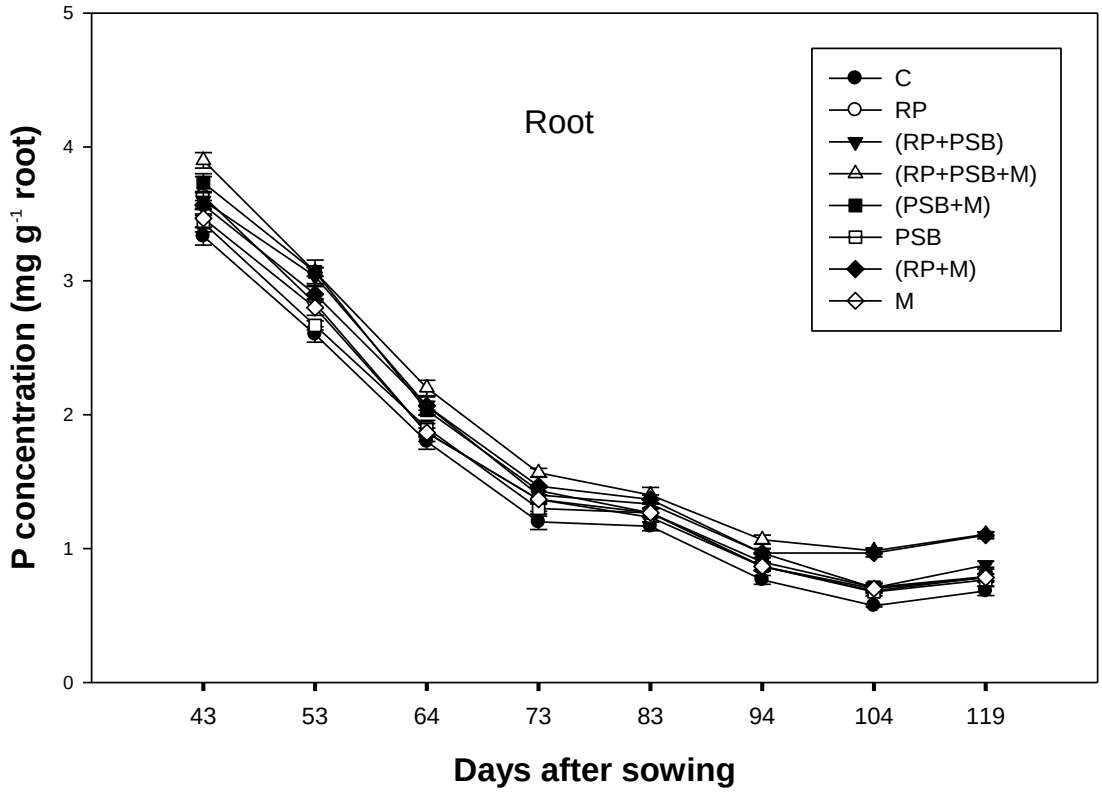

Fig. 3.3.1.7. Mean values of root P concentration by P source at eight samples(43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.7. Table of mean values, standard errors of differences and P values for data presented in Figure 3.3.1.7. Significant levels of P are in bold script.

Treatments	P concentration (mg g^{-1} root)							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
C	3.33	2.60	1.80	1.20	1.17	0.77	0.57	0.68
RP	3.63	2.83	1.87	1.37	1.23	0.87	0.68	0.79
RP+PSB	3.60	3.03	2.07	1.40	1.33	0.97	0.71	0.88
RP+PSB+M	3.90	3.07	2.20	1.57	1.40	1.07	0.98	1.11
PSB+M	3.73	3.07	2.03	1.43	1.27	0.90	0.71	0.79
PSB	3.43	2.67	1.90	1.30	1.27	0.87	0.68	0.77
RP+M	3.57	2.90	2.07	1.47	1.37	0.97	0.97	1.10
M	3.47	2.80	1.87	1.37	1.27	0.87	0.70	0.78
SED	0.204	0.190	0.151	0.133	0.090	0.102	0.144	0.159
P	**0.002**	**<.001**	**0.001**	**0.013**	**0.009**	**0.002**	**<.001**	**<.001**

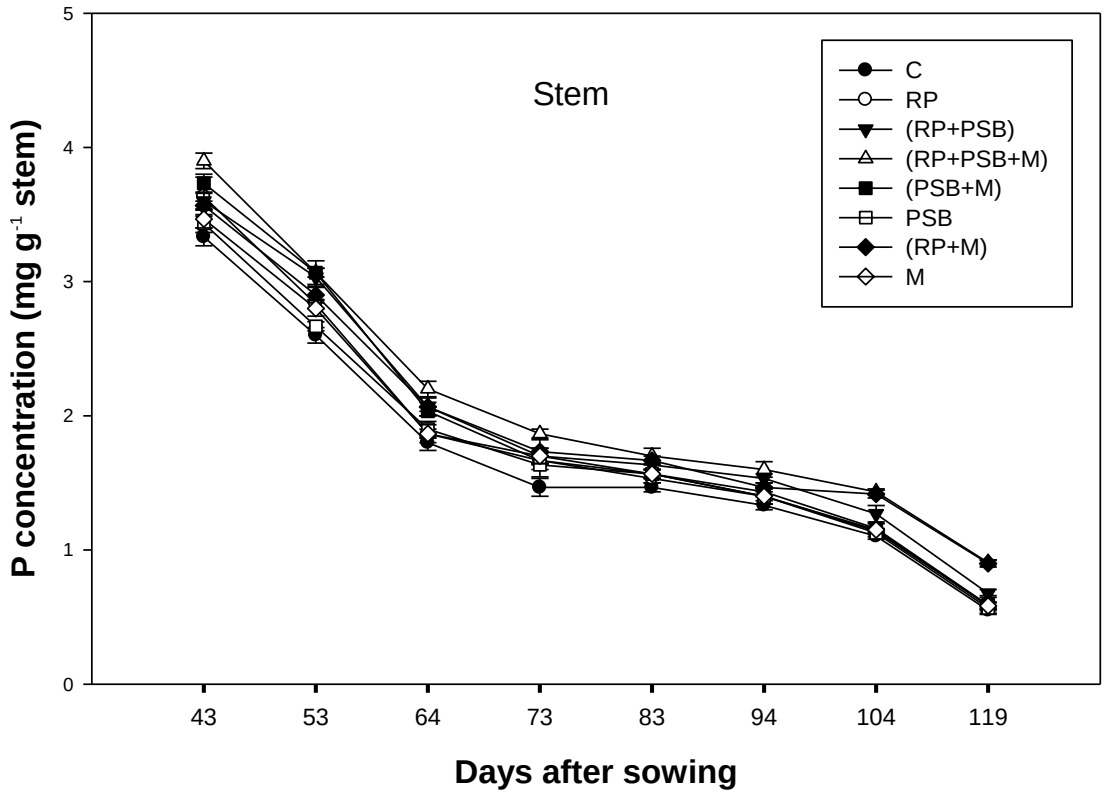

Fig. 3.3.1.8. Mean values of stem P concentration by P source at eight samples(43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.8. Table of mean values, standard errors of differences and P values for data presented in Figure 3.3.1.8. Significant levels of P are in bold script.

Treatments	P concentration (mg g^{-1} stem)							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
C	3.33	2.60	1.80	1.47	1.47	1.33	1.10	0.55
RP	3.63	2.83	1.87	1.67	1.53	1.40	1.14	0.59
RP+PSB	3.60	3.03	2.07	1.70	1.63	1.53	1.27	0.68
RP+PSB+M	3.90	3.07	2.20	1.87	1.70	1.60	1.43	0.91
PSB+M	3.73	3.07	2.03	1.67	1.57	1.43	1.16	0.59
PSB	3.43	2.67	1.90	1.63	1.57	1.40	1.13	0.57
RP+M	3.57	2.90	2.07	1.73	1.67	1.47	1.42	0.90
M	3.47	2.80	1.87	1.70	1.57	1.40	1.15	0.58
SED	0.204	0.190	0.151	0.141	0.090	0.106	0.135	0.150
P	**0.002**	**<.001**	**0.001**	**0.034**	**0.009**	**0.023**	**<.001**	**<.001**

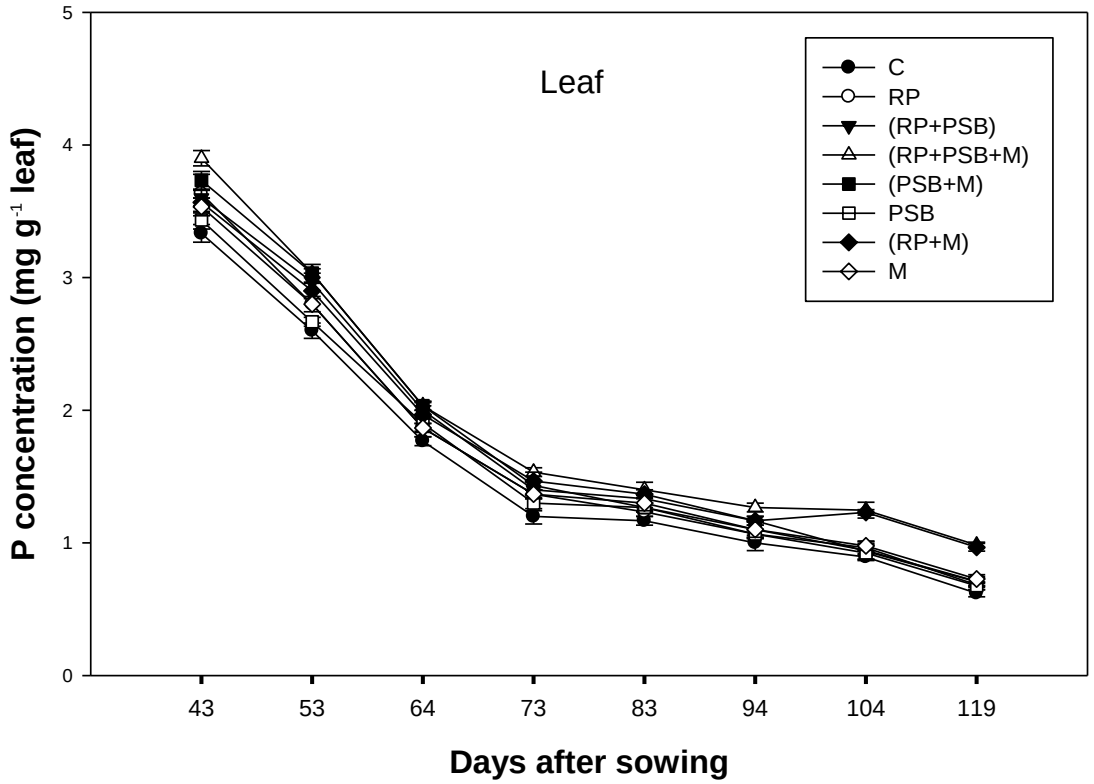

Fig. 3.3.1.9. Mean values of leaf P concentration by P source at eight samples(43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.9.Table of mean values, standard errors of differences and P values for data presented in Figure 3.3.1.9. Significant levels of P are in bold script.

Treatments	P concentration (mg g^{-1} leaf)							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
C	3.33	2.60	1.77	1.20	1.17	1.00	0.89	0.62
RP	3.63	2.80	1.87	1.37	1.23	1.07	0.96	0.68
RP+PSB	3.60	2.97	2.00	1.40	1.33	1.17	0.94	0.71
RP+PSB+M	3.90	3.03	2.03	1.53	1.40	1.27	1.25	0.98
PSB+M	3.73	3.03	2.03	1.43	1.27	1.10	0.94	0.71
PSB	3.43	2.67	1.90	1.30	1.27	1.07	0.92	0.68
RP+M	3.57	2.90	1.97	1.47	1.37	1.17	1.23	0.97
M	3.53	2.80	1.87	1.37	1.30	1.10	0.98	0.73
SED	0.200	0.172	0.116	0.120	0.093	0.101	0.148	0.137
P	**0.003**	**<.001**	**0.016**	**0.006**	**0.021**	**0.020**	**<.001**	**<.001**

3.3.1.4. 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 3.3.1.10, 3.3.1.11, 3.3.1.12, 3.3.1.13 and 3.3.1.14). 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 3.3.1.10, 3.3.1.11, 3.3.1.12, 3.3.1.13 and 3.3.1.14).

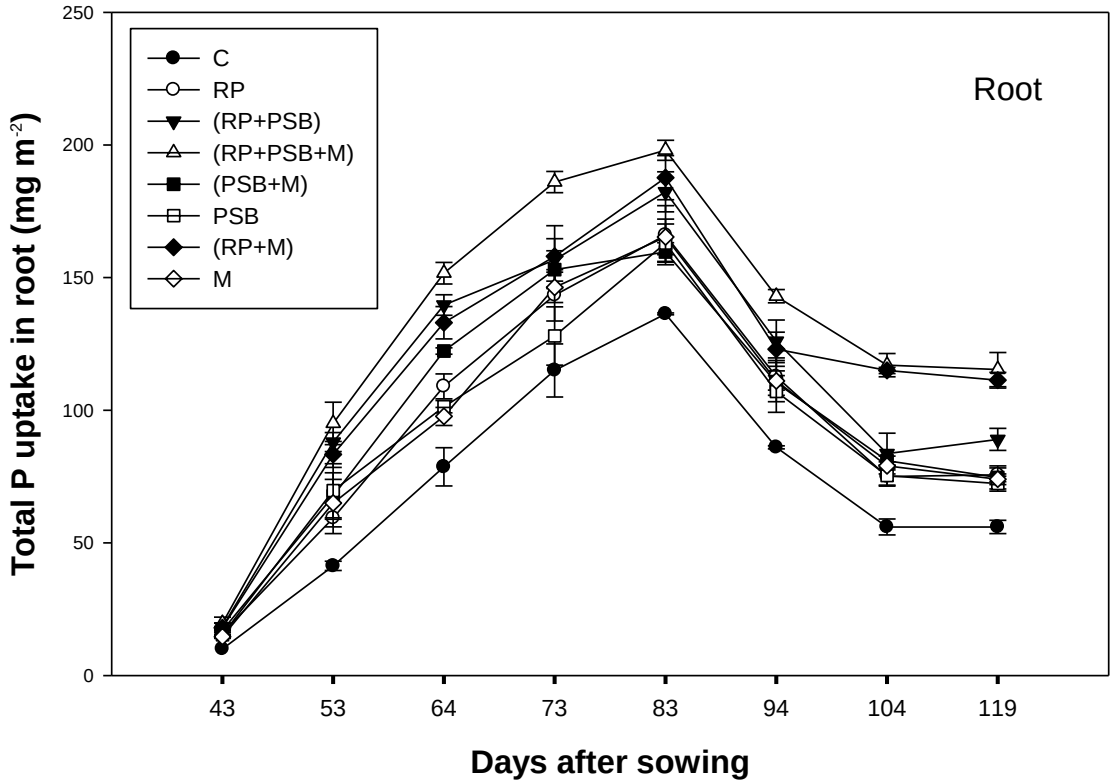

Fig. 3.3.1.10. Mean values of P uptake in root by P source at eight samples(43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.10. Table mean values, standard errors of differences and P values for data presented in Figure 3.3.1.10. 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**

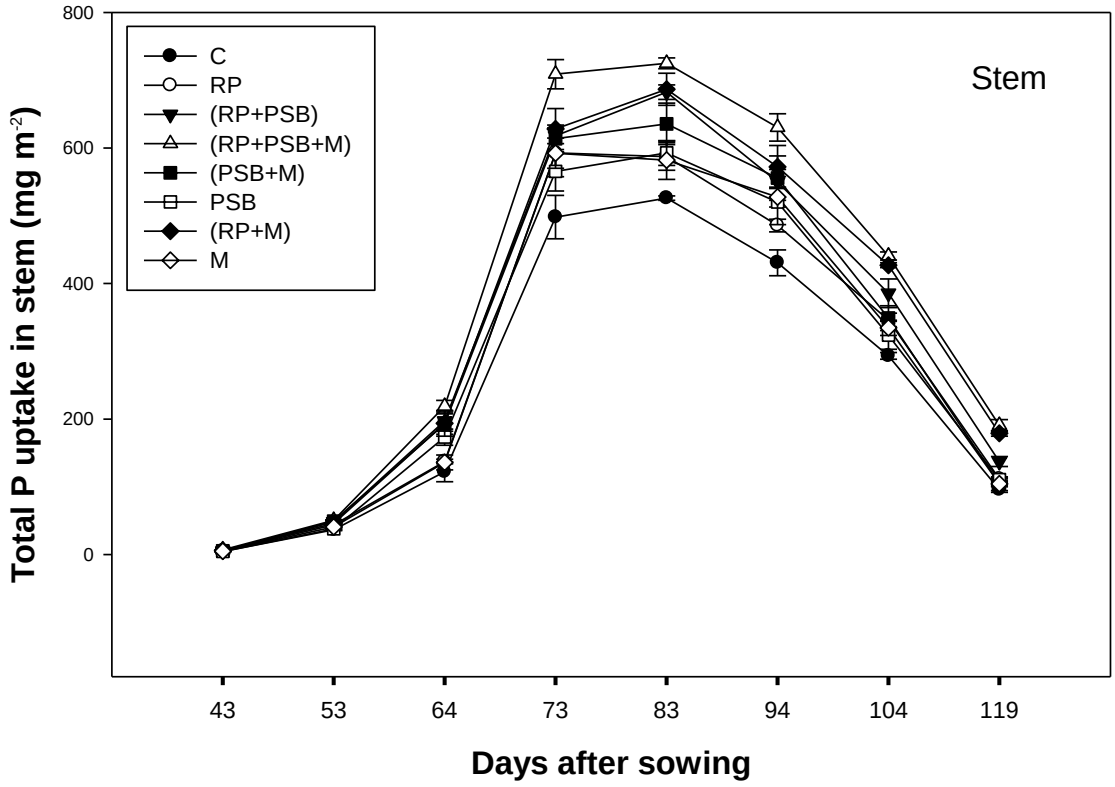

Fig. 3.3.1.11. Mean values of P uptake in stem by P source at eight samples (43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.11. Table mean values, standard errors of differences and P values for data presented in Figure 3.3.1.11. 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**

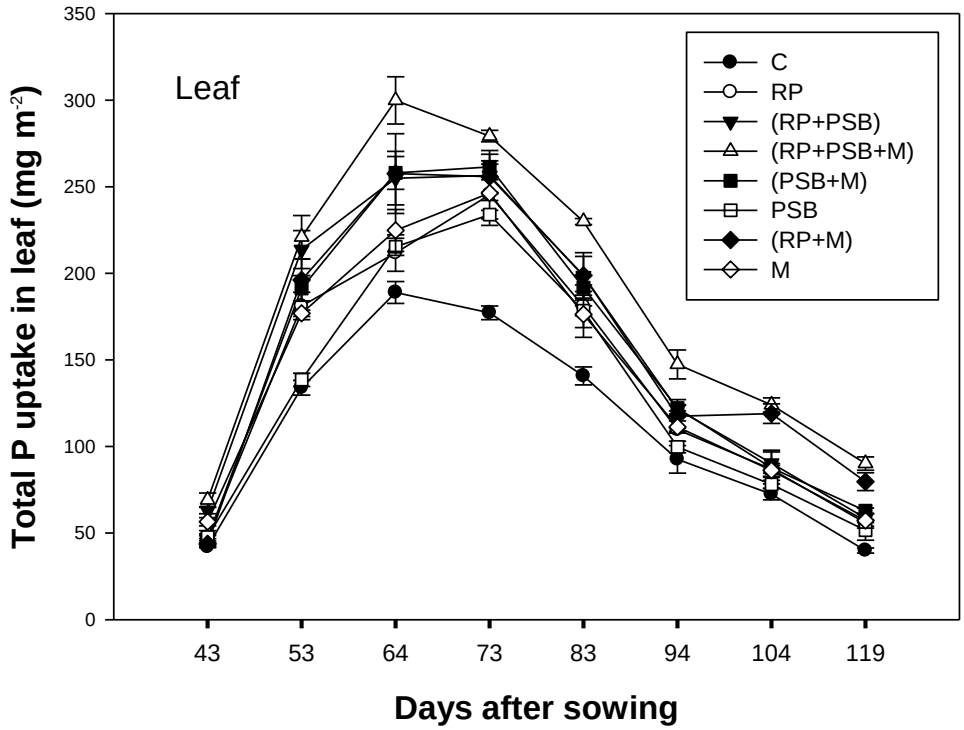

Fig. 3.3.1.12. Mean values of P uptake in leaf by P source at eight samples (43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.12. Table mean values, standard errors of differences and P values for data presented in Figure 3.3.1.12. 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**

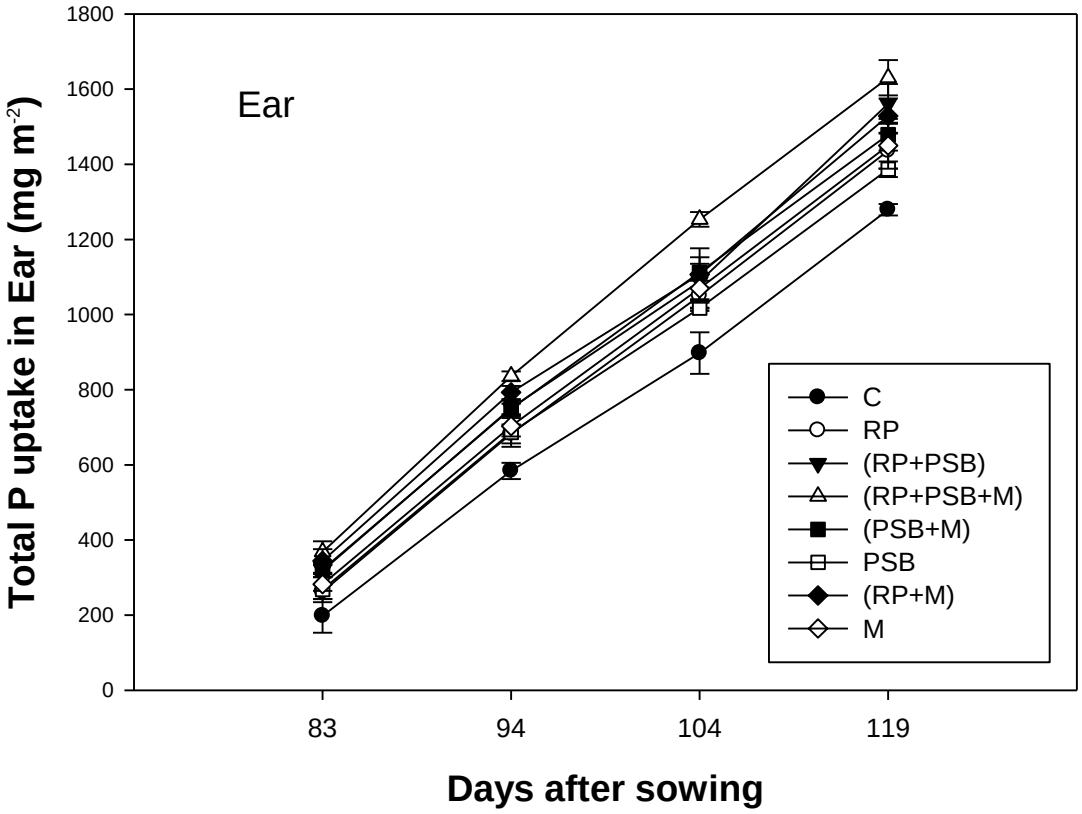

Fig. 3.3.1.13. Mean values of P uptake in ear by P source at four samples(83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.13. Table mean values, standard errors of differences and P values for data presented in Figure 3.3.1.13. 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**

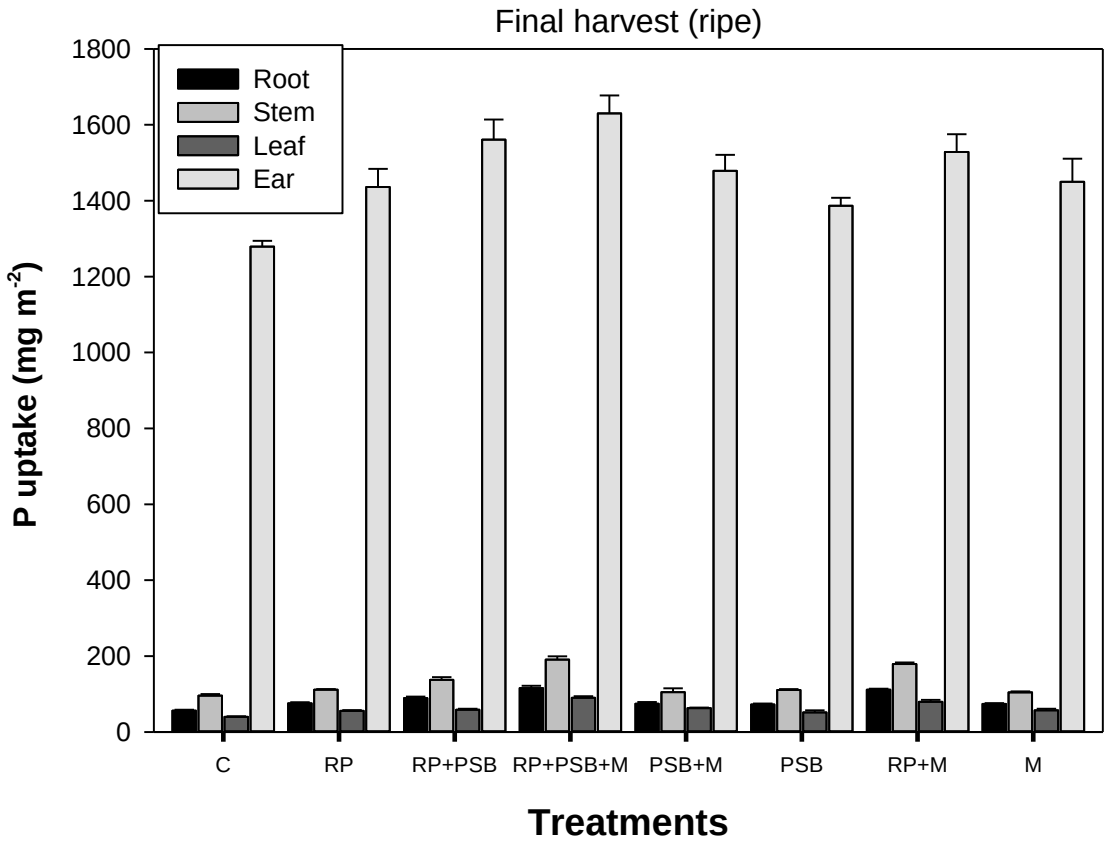

Fig. 3.3.1.14. Mean values of total P uptake in root, stem, leaf and ear by P source at the final sample.Error bars show standard error of means (n=3).

Table 3.3.1.14. Table mean values, standard errors of differences and P values for data presented in Figure 3.3.1.14. 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**

3.3.1.5. Root colonization

The root colonization was significantly affected by P sources in the all of eight samples (Table 3.3.1.15). 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 3.3.1.15).

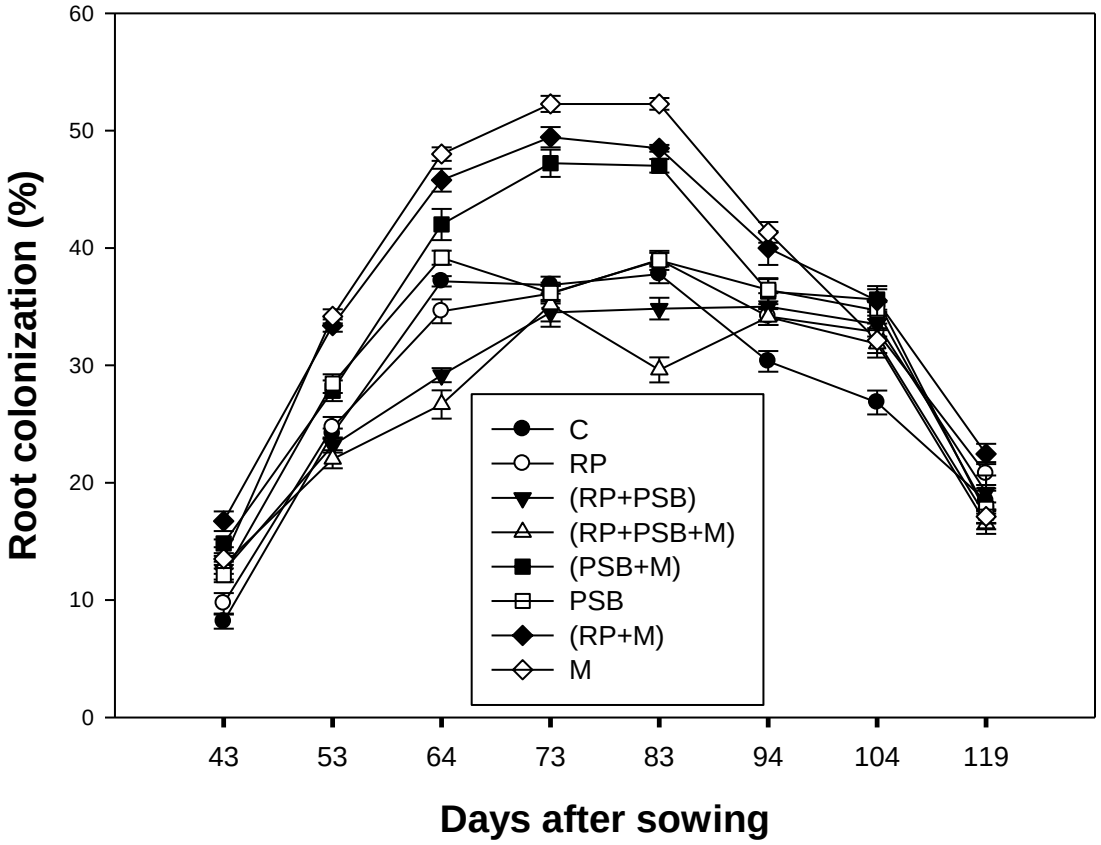

Fig. 3.3.1.15. Mean values of root colonization by P source at eight samples (43, 53, 64, 73, 83, 94, 104 and 119 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.15. Table mean values, standard errors of differences and P values for data presented in Figure 3.3.1.15. 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**

3.3.1.6. Leaf area index (LAI)

Leaves had senesced by 104 days. Analysis showed that the LAI was significantly affected by P source in the all of eight samples (Table 3.3.1.16). The highest LAI of 4th sample (73 das) was observed in the RP+PSB+M and PSB+M treatments (Fig 3.3.1.16).

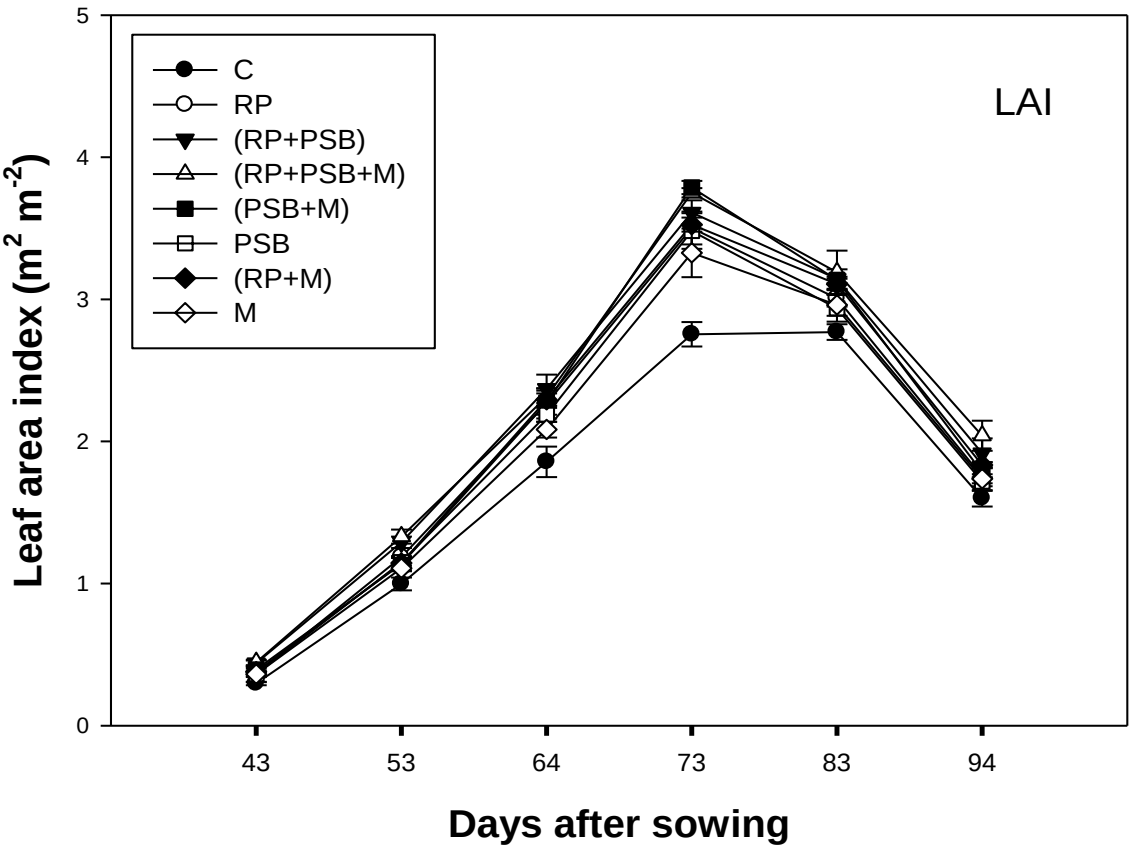

Fig. 3.3.1.16. Mean values of LAI by P source at six samples(43, 53, 64, 73, 83 and 94 days after sowing).Error bars show standard error of means (n=3).

Table 3.3.1.16. Table mean values, standard errors of differences and P values for data presented in Figure 3.3.1.16. Significant levels of P are in bold script.

Treatments	LAI (m^{-2} m^{-2})					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
C	0.297	0.997	1.857	2.754	2.769	1.599
RP	0.364	1.187	2.267	3.502	3.003	1.750
RP+PSB	0.440	1.290	2.370	3.610	3.146	1.915
RP+PSB+M	0.445	1.330	2.313	3.750	3.188	2.036
PSB+M	0.394	1.137	2.273	3.788	3.134	1.783
PSB	0.362	1.150	2.187	3.481	2.938	1.718
RP+M	0.379	1.143	2.293	3.526	3.108	1.833
M	0.361	1.107	2.083	3.327	2.962	1.735
SED	0.059	0.114	0.196	0.341	0.177	0.163
P	**0.021**	**<.001**	**0.007**	**<.001**	**0.029**	**0.017**

3.3.2. Second field experiment results

3.3.2.1. Dry matter

Analysis showed that the root, straw (stem+leaf) and ear dry weight were significantly affected by non-biological P (NBP) and biological P (BP) in the all of five samples, except straw dry weight that was affected only by NBP in the fifth sample and root dry weight that was affected only by BP in the third sample (Table 3.3.2.1, 3.3.2.2, 3.3.2.3 and 3.3.2.4). The highest root, straw and ear dry weight of the majority of the samples and also final sample were most affected by M+AMP and M+TSP treatments (Fig 3.3.2.1, 3.3.2.2, 3.3.2.3 and 3.3.2.4). In the results, biological P refers to the + or – mycorrhiza main effect, and non-biological P sources (RP, TSP, AMP).

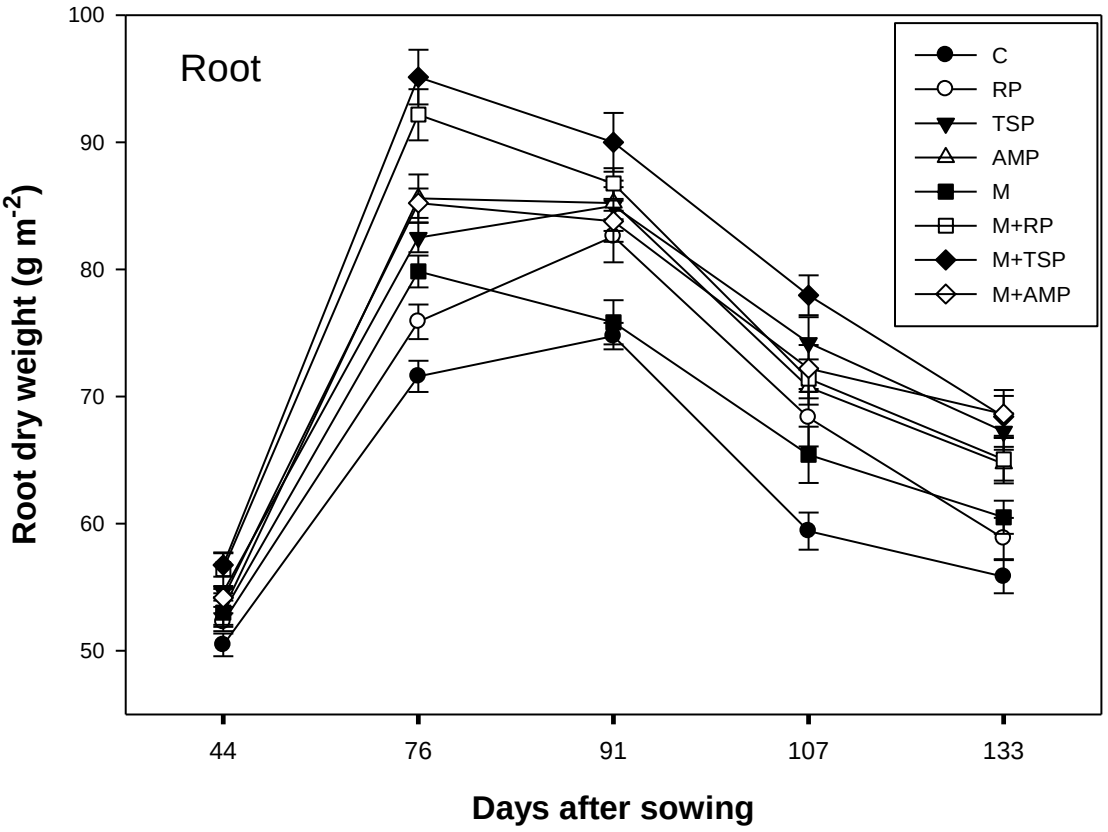

Fig. 3.3.3.1. Mean values of root dry weight by P source at five samples(44, 76, 91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.3.1. Table of standard errors of difference and P values for data presented in Figure 3.3.3.1. Significant levels of P are in bold script.

Treatments	Root dry matter (g 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	0.973	**0.005**	1.532	**<.001**	1.622	**<.001**	1.782	**<.001**	1.212	**<.001**
Biological P	0.688	**0.002**	1.083	**<.001**	1.147	0.068	1.260	**0.010**	0.857	**<.001**
Non-Biological P* Biological P	1.377	0.444	2.167	**<.001**	2.294	0.208	2.520	0.651	1.714	0.240

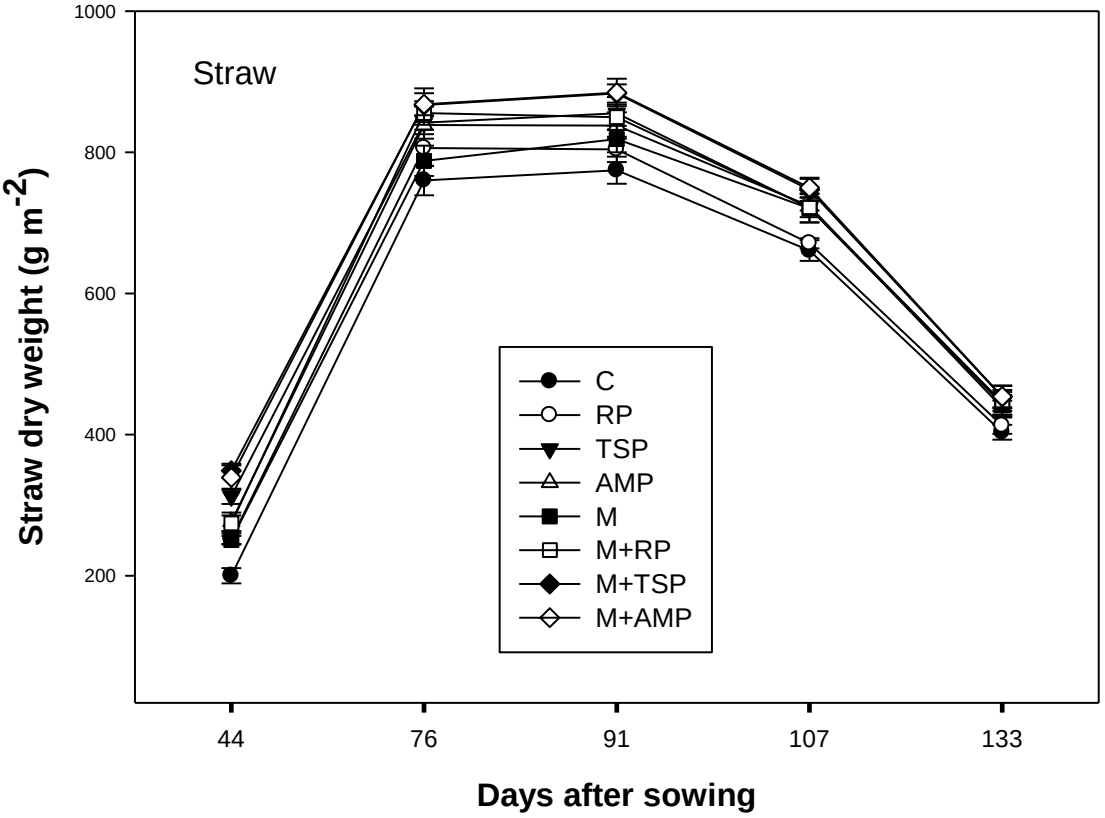

Fig. 3.3.3.2. Mean values of straw dry weight by P source at five samples (44, 76, 91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.3.2. Table of standard errors of difference and P values for data presented in Figure 3.3.3.2. Significant levels of P are in bold script.

Treatments	Straw dry matter (g 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	11.2	**<.001**	17.0	**<.001**	17.9	**0.002**	13.7	**0.003**	12.9	**0.157**
Biological P	8.0	**<.001**	12.0	**0.012**	12.7	**0.004**	9.7	**<.001**	9.1	**0.015**
Non-Biological P* Biological P	15.9	0.322	24.0	0.882	25.3	0.947	19.3	0.492	18.3	0.605

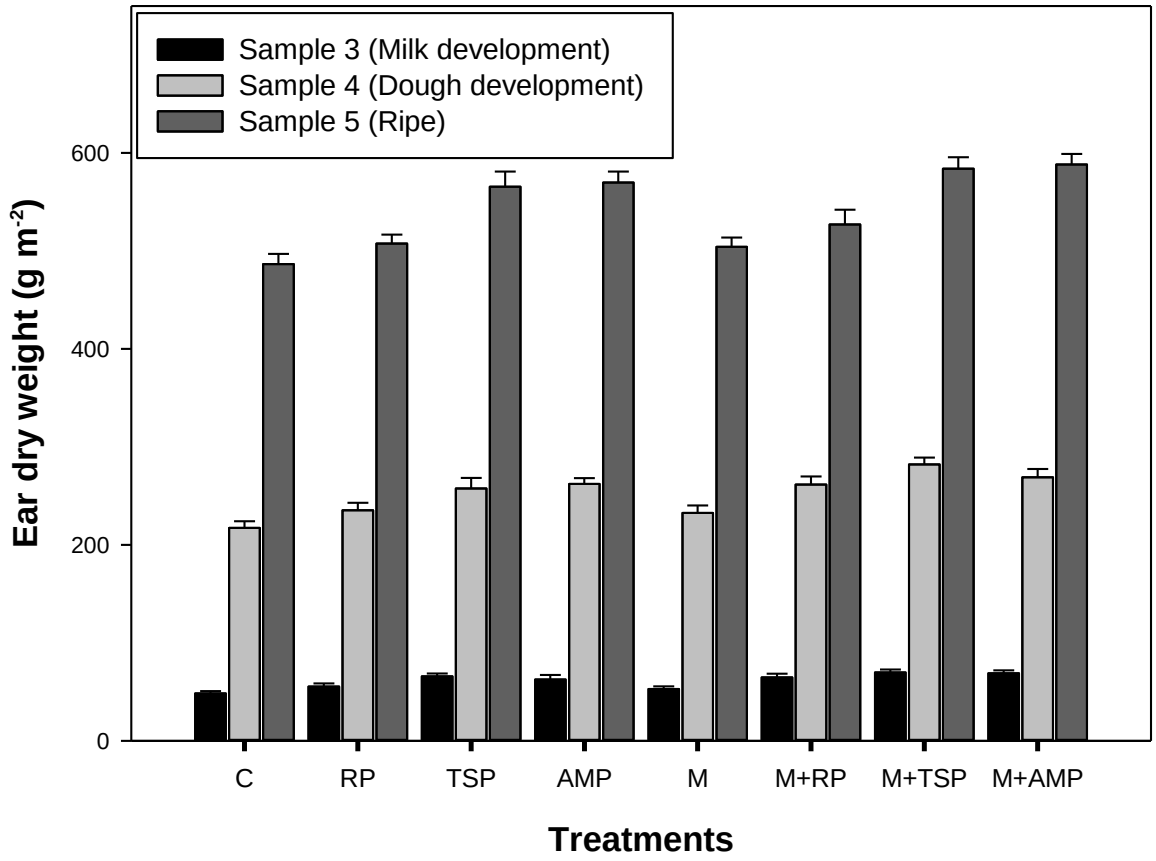

Fig. 3.3.3.3. Mean values of ear dry weight by P source at 3 samples(91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.3.3. Table of standard errors of difference and P values for data presented in Figure 3.3.3.3. Significant levels of P are in bold script.

Treatments	Ear dry matter (g m^{-2})					
	Sample 3		Sample 4		Sample 5	
	SED	P	SED	P	SED	P
Non-Biological P	3.180	**<.001**	4.740	**<.001**	10.730	**<.001**
Biological P	2.250	**0.014**	3.350	**<.001**	7.590	**0.024**
Non-Biological P* Biological P	4.500	0.828	6.710	0.172	15.180	1.000

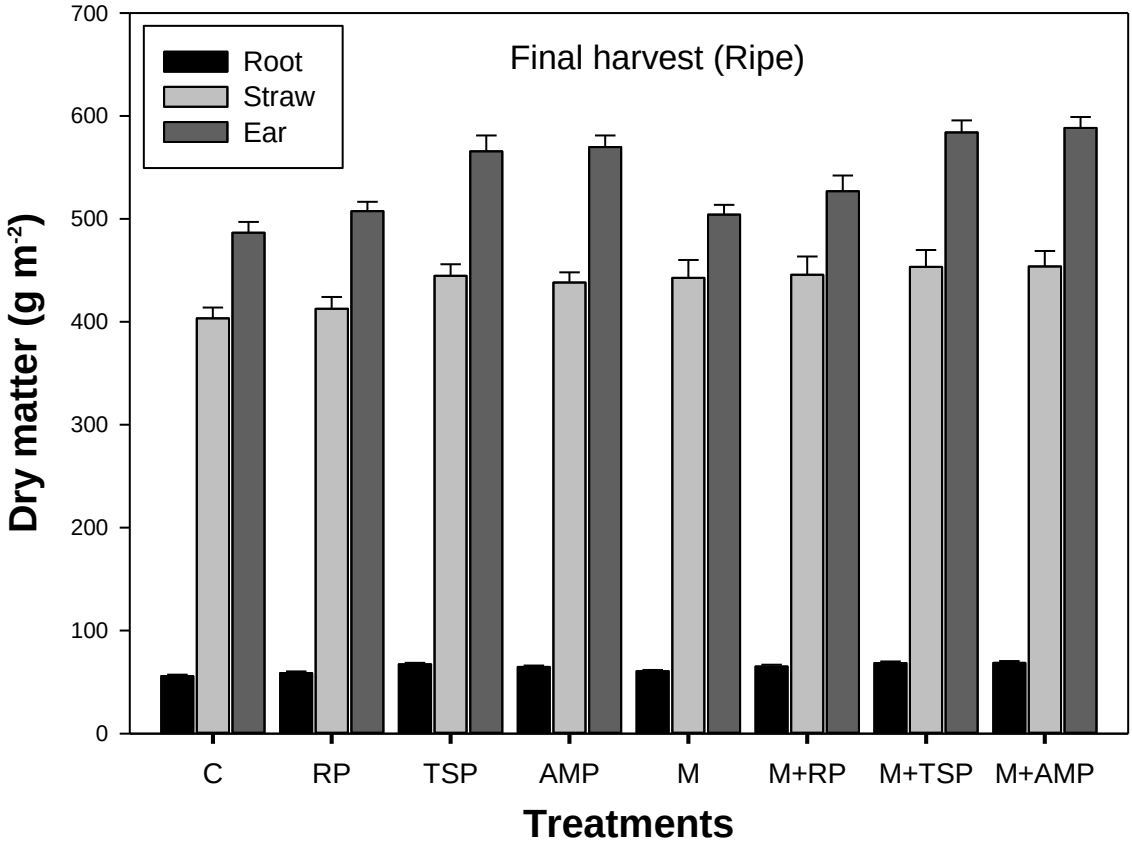

Fig. 3.3.2.4. Mean values of root, straw and ear dry weight by P source at final sample (133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.2.4. Table of standard errors of difference and P values for data presented in Figure 3.3.2.4. Significant levels of P are in bold script.

Treatments	Final dry matter (g m^{-2})					
	Root		Straw		Ear	
	SED	P	SED	P	SED	P
Non-Biological P	1.212	**<.001**	12.920	0.157	10.730	**<.001**
Biological P	0.857	**<.001**	9.140	**0.015**	7.590	**0.024**
Non-Biological P* Biological P	1.714	0.240	18.270	0.605	15.180	1.000

3.3.2.2. P concentration

Analysis showed that the root, straw and ear P concentration were significantly affected by NBP and BP in the all of five samples, except straw P concentration that was affected by only BP in the fifth sample and ear P concentration that was affected by only BP in the fourth sample (Tables 3.3.2.5, 3.3.2.6, 3.3.2.7 and 3.3.2.8). The highest root, straw and ear P concentration were greatest in the M+TSP and M+AMP treatments (Fig 3.3.2.5, 3.3.2.6, 3.3.2.7 and 3.3.2.8). There were no significant interactions.

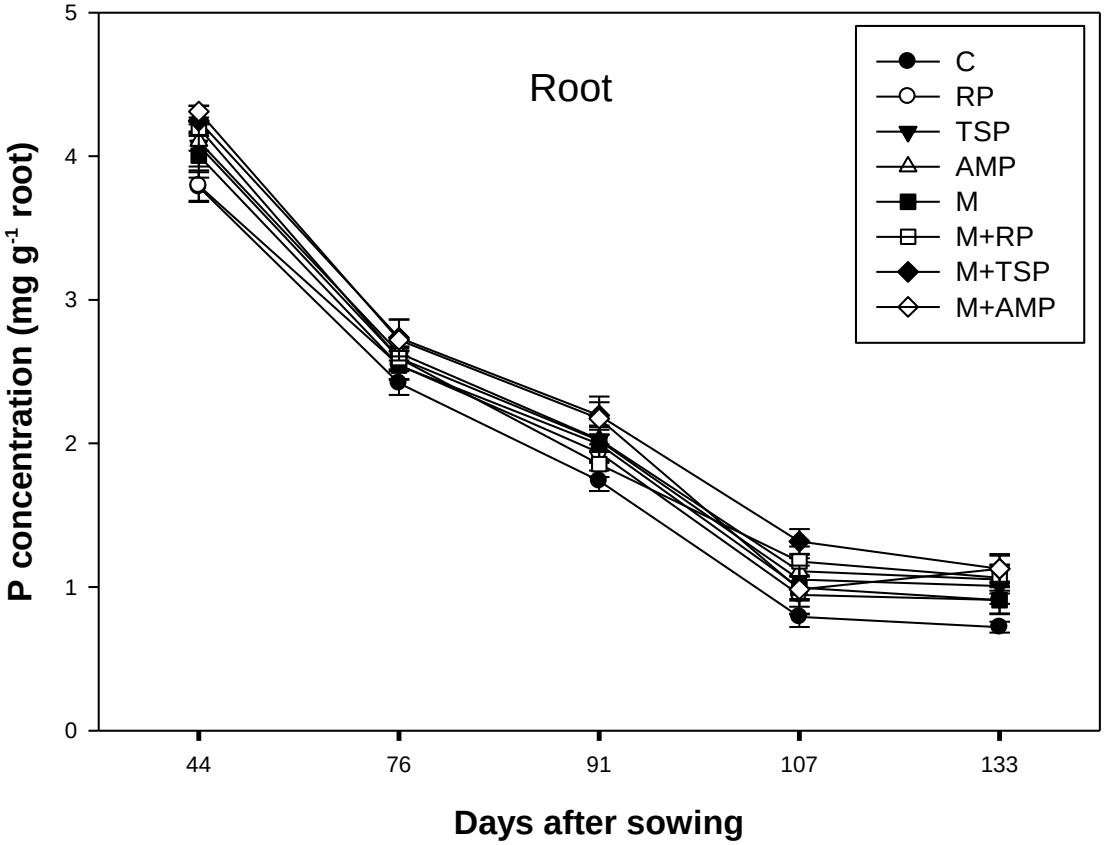

Fig. 3.3.2.5. Mean values of root P concentration by P source at five samples(44, 76, 91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.2.5. Table of standard errors of difference and P values for data presented in Figure 3.3.2.5. Significant levels of P are in bold script.

Treatments	P concentration (mg g^{-1} root)									
	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
	SED	P	SED	P	SED	P	SED	P	SED	P
Non-Biological P	0.098	**0.016**	0.058	**0.010**	0.074	**0.003**	0.093	**0.040**	0.043	**<.001**
Biological P	0.070	**0.002**	0.041	**0.027**	0.052	**0.026**	0.066	**0.040**	0.030	**<.001**
Non-Biological P* Biological P	0.139	0.642	0.083	0.831	0.104	0.152	0.132	0.158	0.060	0.611

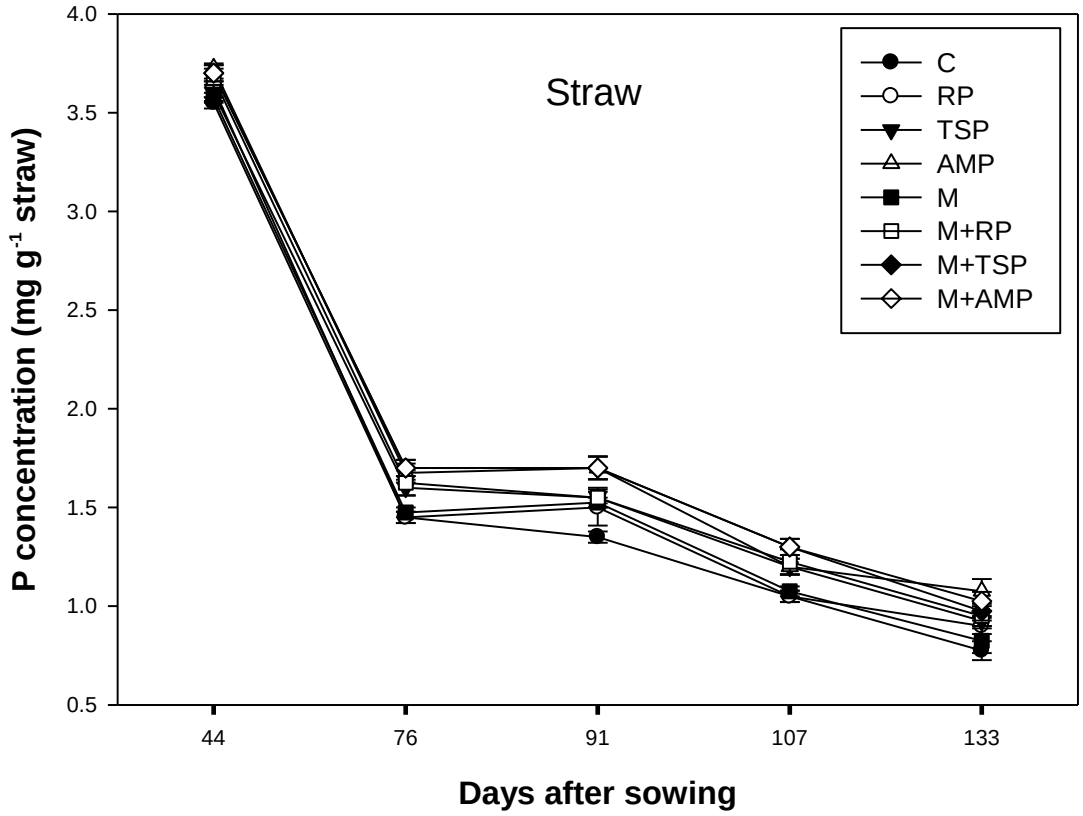

Fig. 3.3.2.6. Mean values of straw P concentration by P source at five samples (44, 76, 91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.2.6. Table of standard errors of difference and P values for data presented in Figure 3.3.2.6. Significant levels of P are in bold script.

Treatments	P concentration (mg g^{-1} straw)									
	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
	SED	P	SED	P	SED	P	SED	P	SED	P
Non-Biological P	0.031	**0.001**	0.025	**<.001**	0.048	**<.001**	0.025	**<.001**	0.046	**<.001**
Biological P	0.022	**0.047**	0.018	**<.001**	0.034	**0.008**	0.018	**<.001**	0.033	0.281
Non-Biological P* Biological P	0.043	0.261	0.036	0.095	0.068	0.213	0.035	0.175	0.065	0.576

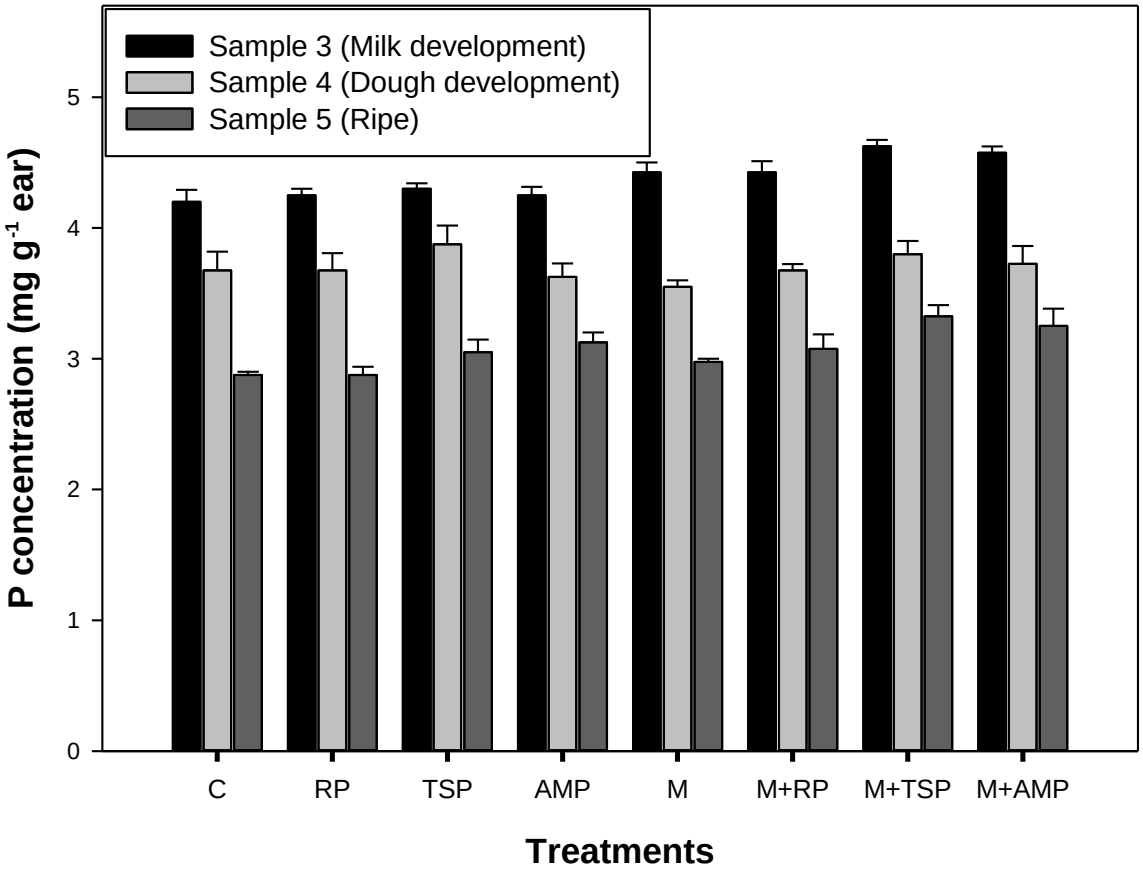

Fig. 3.3.2.7. Mean values of ear P concentration by P source at 3 samples(91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.2.7. Table of standard errors of difference and P values for data presented in Figure 3.3.3.3. Significant levels of P are in bold script.

Treatments	P concentration (mg g^{-1} ear)					
	Sample 3		Sample 4		Sample 5	
	SED	P	SED	P	SED	P
Non-Biological P	0.060	0.040	0.099	0.215	0.089	**0.011**
Biological P	**0.042**	<.001	0.070	0.662	0.063	**0.018**
Non-Biological P* Biological P	0.085	0.522	0.140	0.755	0.126	0.678

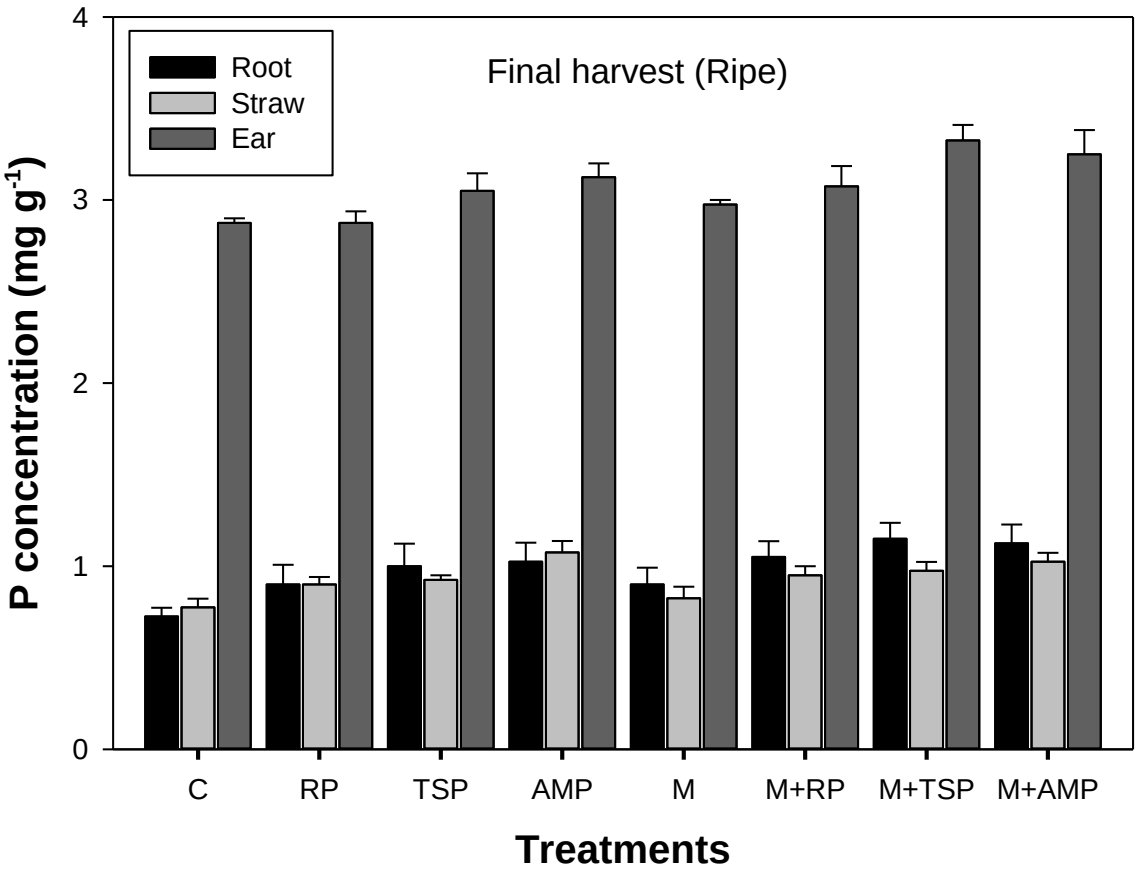

Fig. 3.3.2.8. Mean values of root, straw and ear P concentration by P source at final sample (133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.2.8. Table of standard errors of difference and P values for data presented in Figure 3.3.2.8. Significant levels of P are in bold script.

Treatments	Final P concentration (mg g^{-1})					
	Root		Straw		Ear	
	SED	P	SED	P	SED	P
Non-Biological P	0.043	**<.001**	0.046	**<.001**	24.230	**<.001**
Biological P	0.030	**<.001**	0.033	0.281	17.130	**0.046**
Non-Biological P* Biological P	0.060	0.611	0.065	0.576	34.260	0.493

3.3.2.3. Total P uptake

Analysis showed that the total P uptake in root, straw and ear were significantly affected by NBP and BP in the all of the five samples (Tables 3.3.2.10, 3.3.2.10, 3.3.2.11 and 3.3.2.12). The highest root, straw and ear P concentration of the samples and final sample were in the M+TSP and M+AMP treatments (Figs 3.3.2.10, 3.3.2.10, 3.3.2.11 and 3.3.2.12).

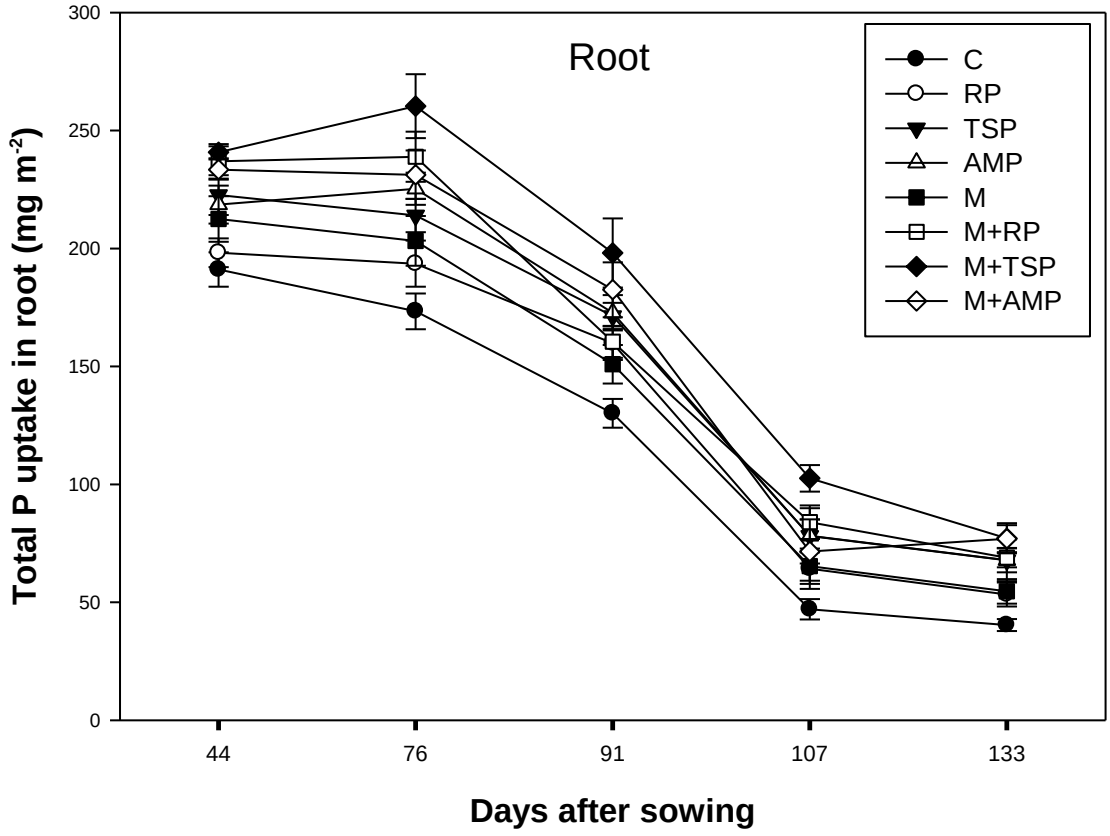

Fig. 3.3.2.9. Mean values of P uptake in root by P source at five samples(44, 76, 91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.2.9. Table of standard errors of difference and P values for data presented in Figure 3.3.2.9. 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

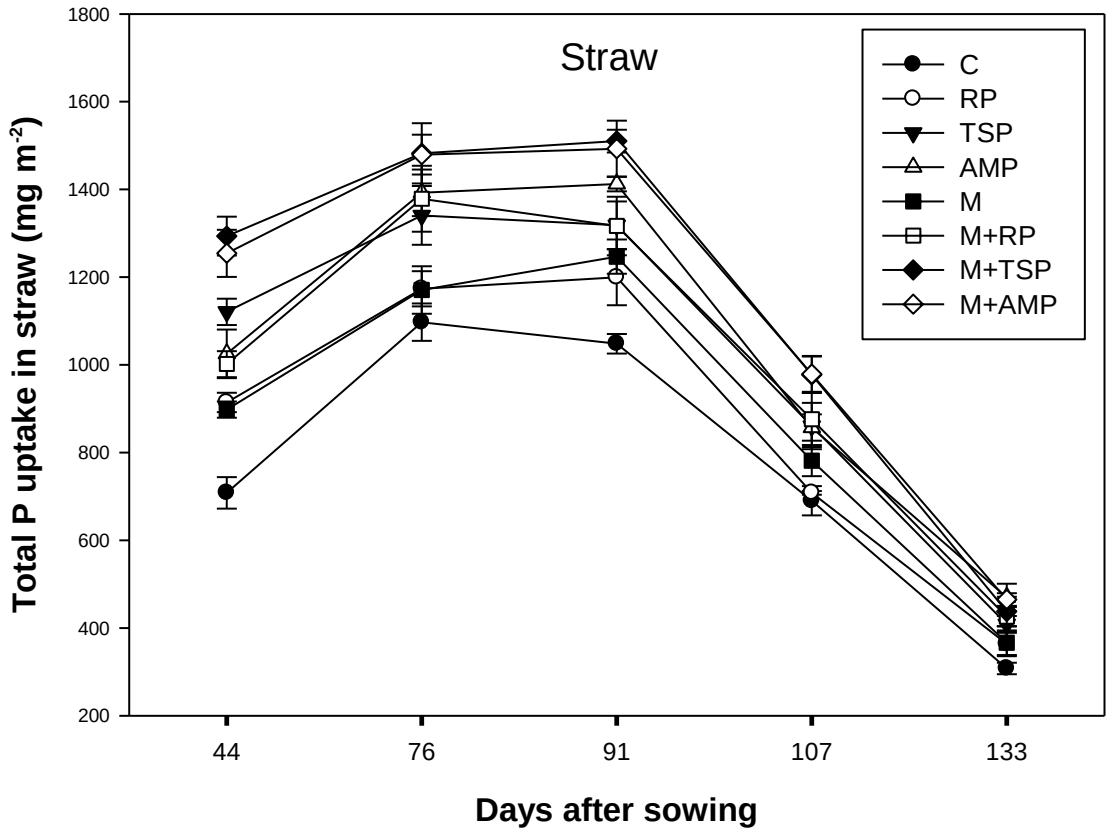

Fig. 3.3.2.10. Mean values of P uptake in straw by P source at five samples(44, 76, 91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.2.10. Table of standard errors of difference and P values for data presented in Figure 3.3.2.10. 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

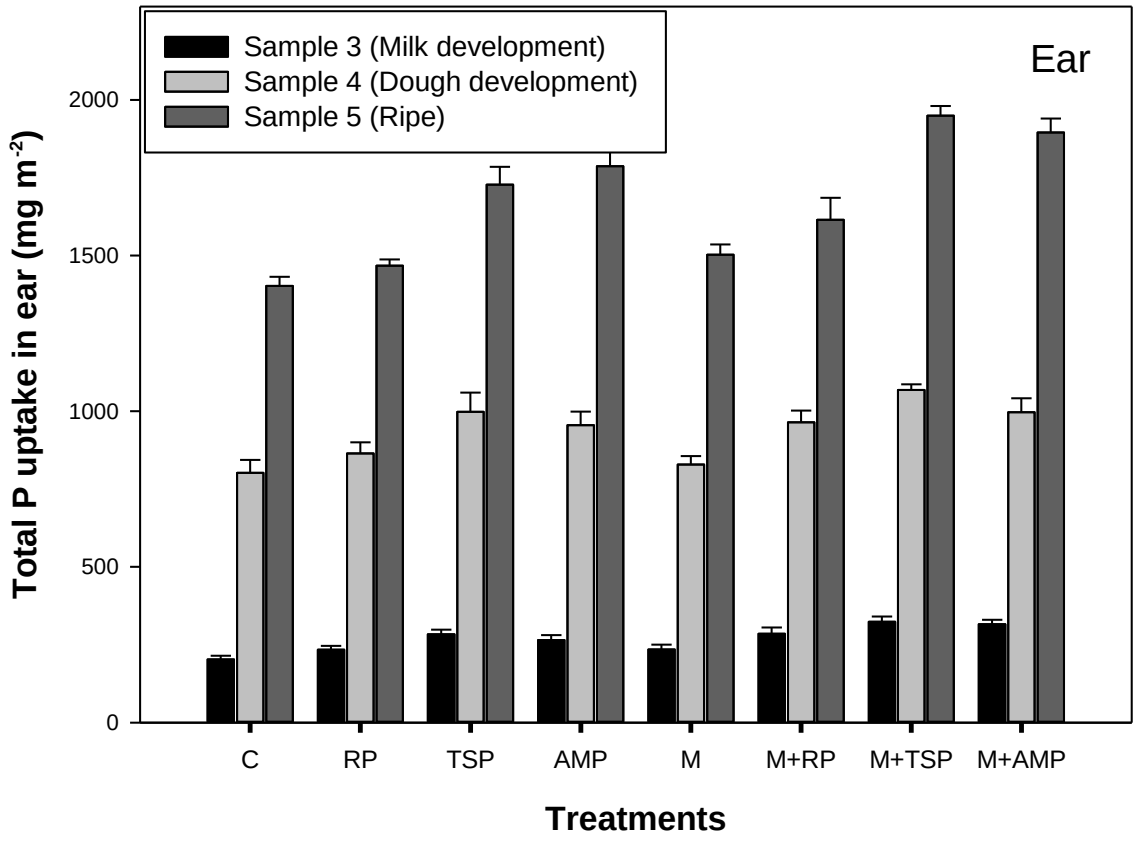

Fig. 3.3.2.11. Mean values of P uptake in ear by P source at 3 samples(91, 107 and 133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.2.11. Table of standard errors of difference and P values for data presented in Figure 3.3.2.11. 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

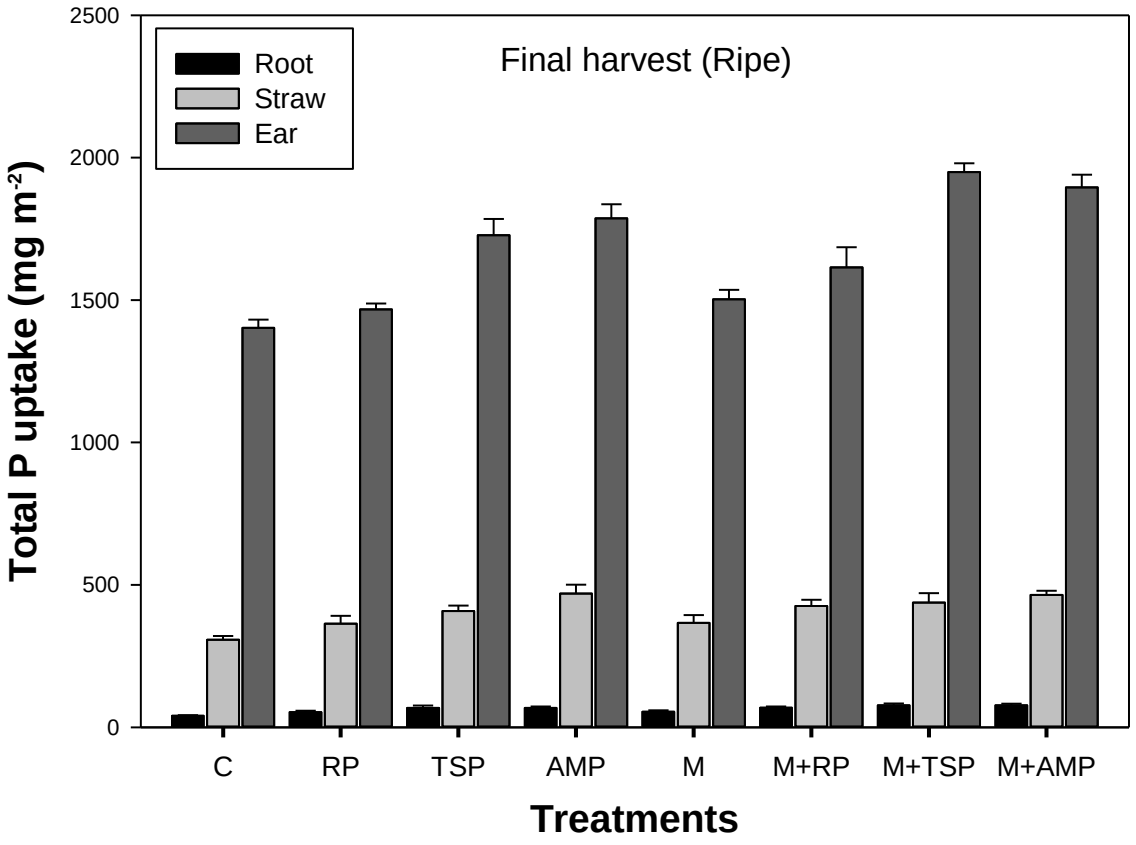

Fig. 3.3.2.12. Mean values of P uptake in root, straw and ear by P source at final sample (133 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.2.12. Table of standard errors of difference and P values for data presented in Figure 3.3.2.12. 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

3.3.2.4. Root colonization

Analysis showed that the root colonization by mycorrhiza was significantly affected by NBP and BP and their interaction in the all of five samples (Table 3.3.2.13). The highest root colonization of the all fifth samples was found in M+RP (Fig 3.3.2.13).

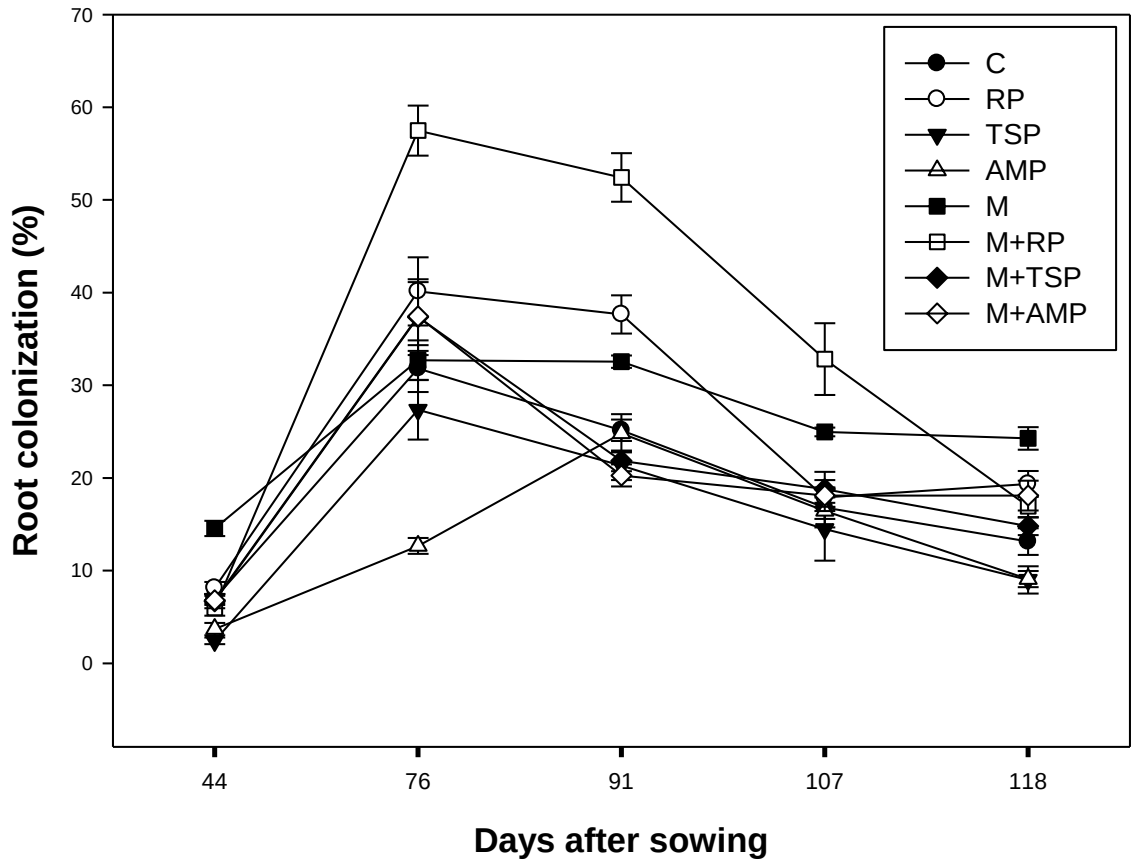

Fig. 3.3.2.13. Mean values of root colonization by P source at five samples(44, 76, 91, 107 and 118 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.2.13. Table of standard errors of difference and P values for data presented in Figure 3.3.2.13. 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**

3.3.2.5. Leaf area index (LAI)

Leaves had senesced by 133 days. Analysis showed that the LAI was significantly affected by NBP and BP in the all samples except LAI that was affected by BP in the second sample (Table 3.3.2.14). The highest LAI of the samples were observed in M+AMP and M+TSP treatments (Fig 3.3.2.14).

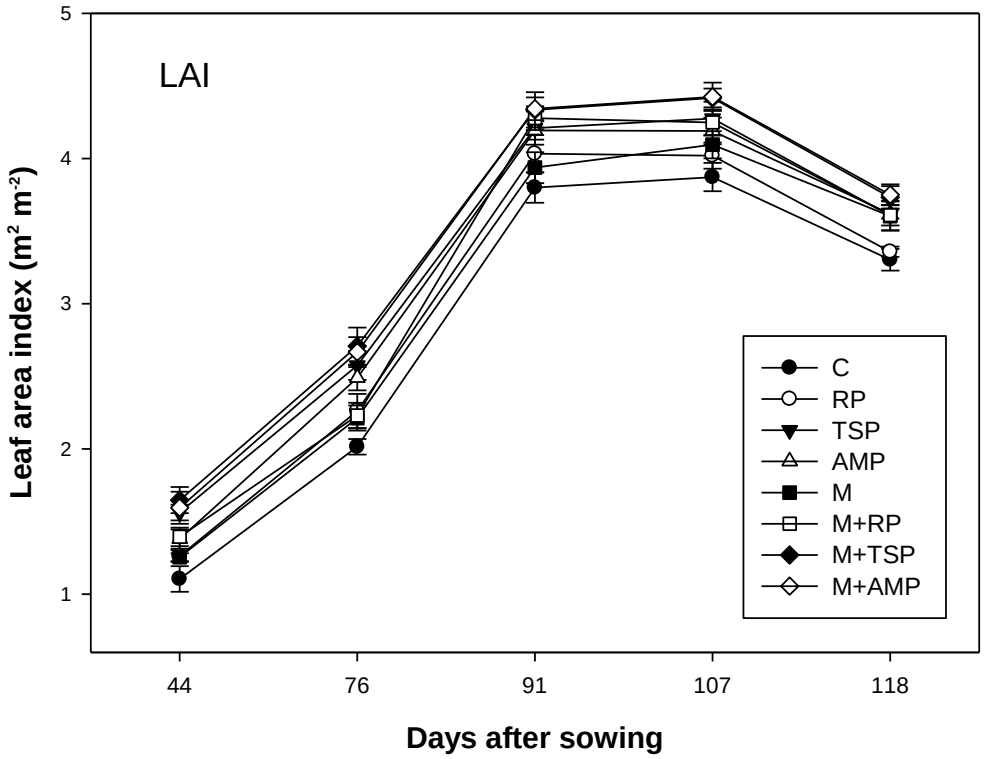

Fig. 3.3.2.14. Mean values of LAI by P source at five samples(44, 76, 91, 107 and 118 days after sowing).Error bars show standard error of means (n=4).

Table 3.3.2.14. Table of standard errors of difference and P values for data presented in Figure 3.3.2.14. Significant levels of P are in bold script.

Treatments	LAI (m^2 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	0.073	<.001	0.099	<.001	0.085	<.001	0.089	0.002	0.068	0.003
Biological P	0.051	0.010	0.070	0.105	0.060	0.012	0.063	0.004	0.048	<.001
Non-Biological P* Biological P	0.103	0.835	0.140	0.641	0.120	0.882	0.127	0.947	0.097	0.492

3.3.2.6. Yield and yield components of barley

In this study application of NBP significantly increased grain yield, number of ears per plant and number of seeds per ear, and grain yield and number of seeds per ear were significantly affected by BP in the final harvest (Table 3.3.2.15 and Fig 3.3.2.15).

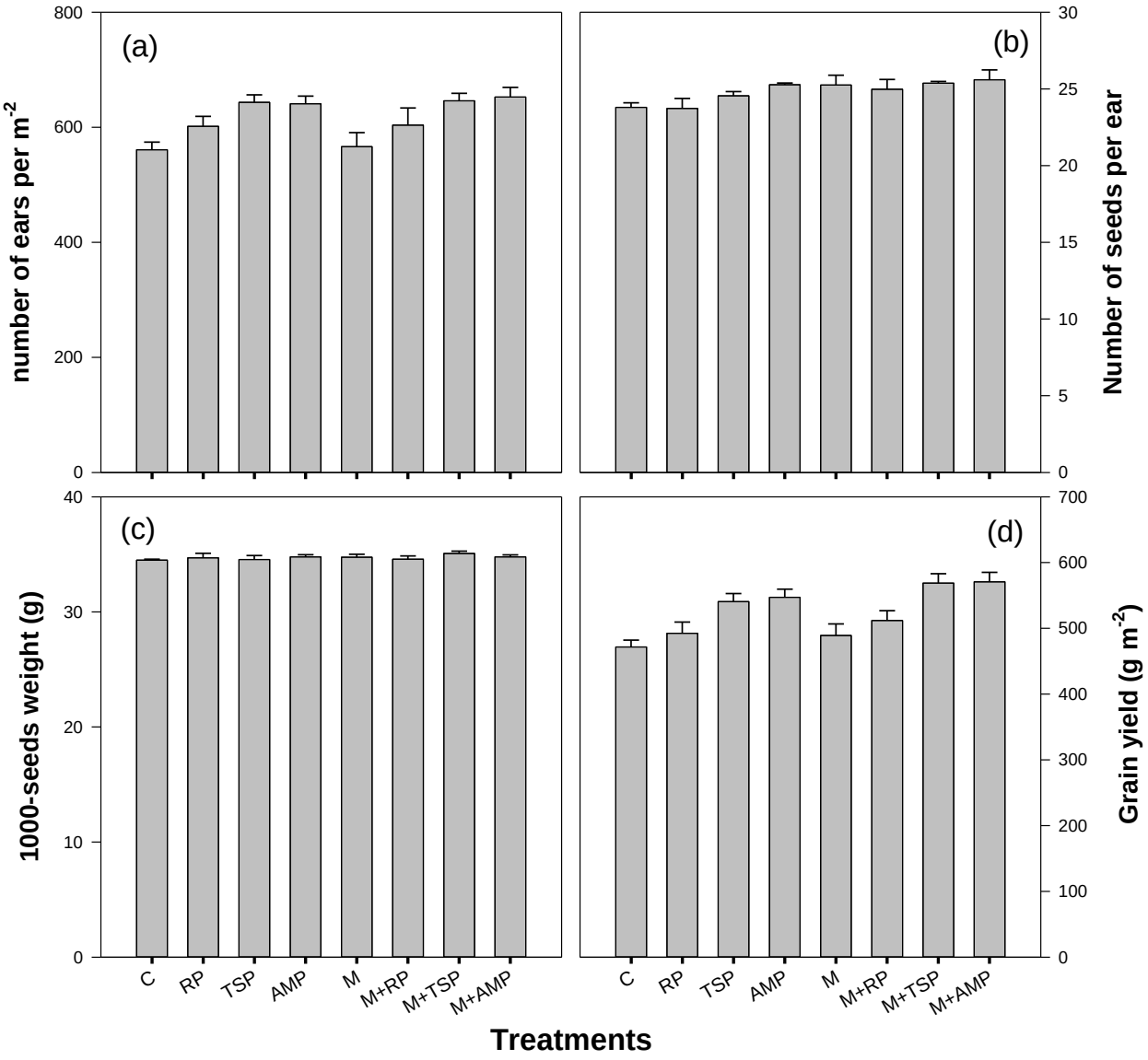

Fig. 3.3.2.15. Effect of P sources on grain yield (a), number of grain per plant (b), number of ear per plant (c) and 1000-seed weight (d) at final sample stage. Error bars show standard error of means (n=4).

Table 3.3.2.15. Table ofstandard errors ofdifferences and P values for data presented in Figure 3.3.2.15. Significant levels of P are in bold script.

Treatments	Yield and yield components							
	Nu of seeds per ear		Nu of ears per m^{-2}		1000-seeds weight (g)		Grain yield (g m^{-2})	
	SED	P	SED	P	SED	P	SED	P
Non-Biological P	0.43	0.087	13.7	**<.001**	0.23	0.811	0.43	**<.001**
Biological P	0.31	**0.005**	9.7	0.576	0.16	0.32	0.31	**0.018**
Non-Biological P* Biological P	0.61	0.594	19.4	0.984	0.33	0.532	0.61	0.972

3.3.1.7. P concentration and P content in grain

The final P concentration in grain was significantly affected by NBP and BP and also the final total P uptake of grain were significantly affected by NBP and BP. (Table 3.3.2.16). The highest and lowest P concentrations and P uptake in grain were in the M+TSP and C (control) treatments respectively (Fig 3.3.2.16).

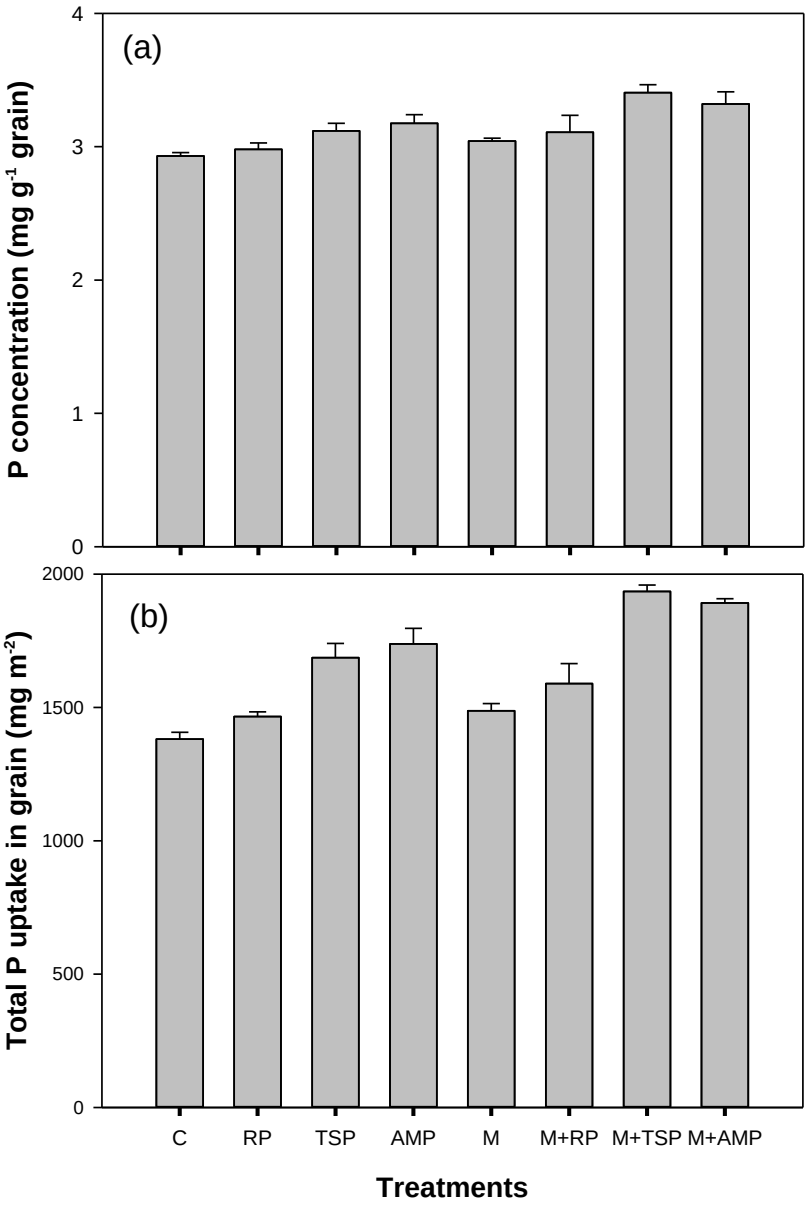

Fig. 3.3.2.16. Effect of P source on P concentration of final grain(a) and P content of final grain(b).Error bars show standard error of means (n=4).

Table 3.3.2.16. Table ofstandard errors ofdifferences and P values for data presented in Figure 3.3.2.16. Significant levels of P are in bold script.

Treatments	Final harvesting			
	P concentration (mg g^{-1} grain)		P uptake in grain (mg m^{-2})	
	SED	P	SED	P
Non-Biological P	0.0744	**0.002**	37.9	**<.001**
Biological P	0.0526	**0.004**	26.8	**<.001**
Non-Biological P* Biological P	0.1053	0.635	53.6	0.272

3.4. Discussions

The results of two field experiments (Fig 2.5.1) showed that M applied in combination and PSB applied in combination with P sources significantly increased root and shoot growth, P concentration, P uptake in the plant parts and finally increased the grain yield, rather than singly M or PSB by treatments.

Application of PSB (microbial inoculation) and/or M (mycorrhiza colonization) are playing significant roles in the optimization of P solubilization (solubilisation of inorganic phosphate), promoting of growth and dry matter production, 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 dry matter (grain yield in particular), P concentration, 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 uptake of P. 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 biofertilizers (M and PSB) for insoluble soil P to be released for uptake by the roots.

In the first experiment the results may have been affected by the weather conditions (drought stress at anthesis of barley which is known to reduce yield and yield components) (Table 3.2.1), and low soil fertility, so increasing the P concentration in grains did not lead to higher grain yields. Rehman and Nautiyal (2002) reported that in drought conditions P solubilizing and N fixing bacteria can not demonstrate their potential ability in P and N utilization in the soil. Because of the drought stress and the availability of minerals and on the other hand, because of the unsuitable conditions for vegetative growth, the concentration of minerals, especially P, will increase in the plant tissue. This result is supported by results reported by Horst *et al.,* (2001).Water deficit during seed development increased grain protein, P, N and micronutrient concentrations except for Fe (Haberle*et al.*, 2008). It can be concluded that water deficit is able to enhance nutritional value in drought stressed conditions.

The results of field experiment showed that LAI of barley increased with P treatments as the control treatment was least compared to all treatments. The combination of M and PSB produced the maximum LAI (Fig 3.3.1.16). This may have been due to the important role of phosphorous (such as PSB with RP) and M for helping the development of a more extensive root system and thus enabling barley to extract water and nutrients from deeper depth and producing higher LAI to compared to the control.

The results of second field experiment showed application of non-biological P fertilizers (RP, TSP and AMP) combined with M significantly increased both the yield and yield components, P concentration and total P uptake, plant dry matter, LAI and concentration of P

in the grain. 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.

Several researchers (Mehrotra, 2005;Pellerin et al., 2007; Li et al., 2011; Bongard, 2012) have concluded that mycorrhizae are 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 biologic phosphorus fertilizers (PSB and M) could allow the achievement of satisfactory crop growth and yield with reduced amounts of non-biological phosphorus fertilizers, and so decrease of fertilization costs and environmental pollution. For these reasons the application of M and/or PSB with non-biological phosphorus fertilizers (organic and inorganic p fertilizers) could be encouraged in barley growing.

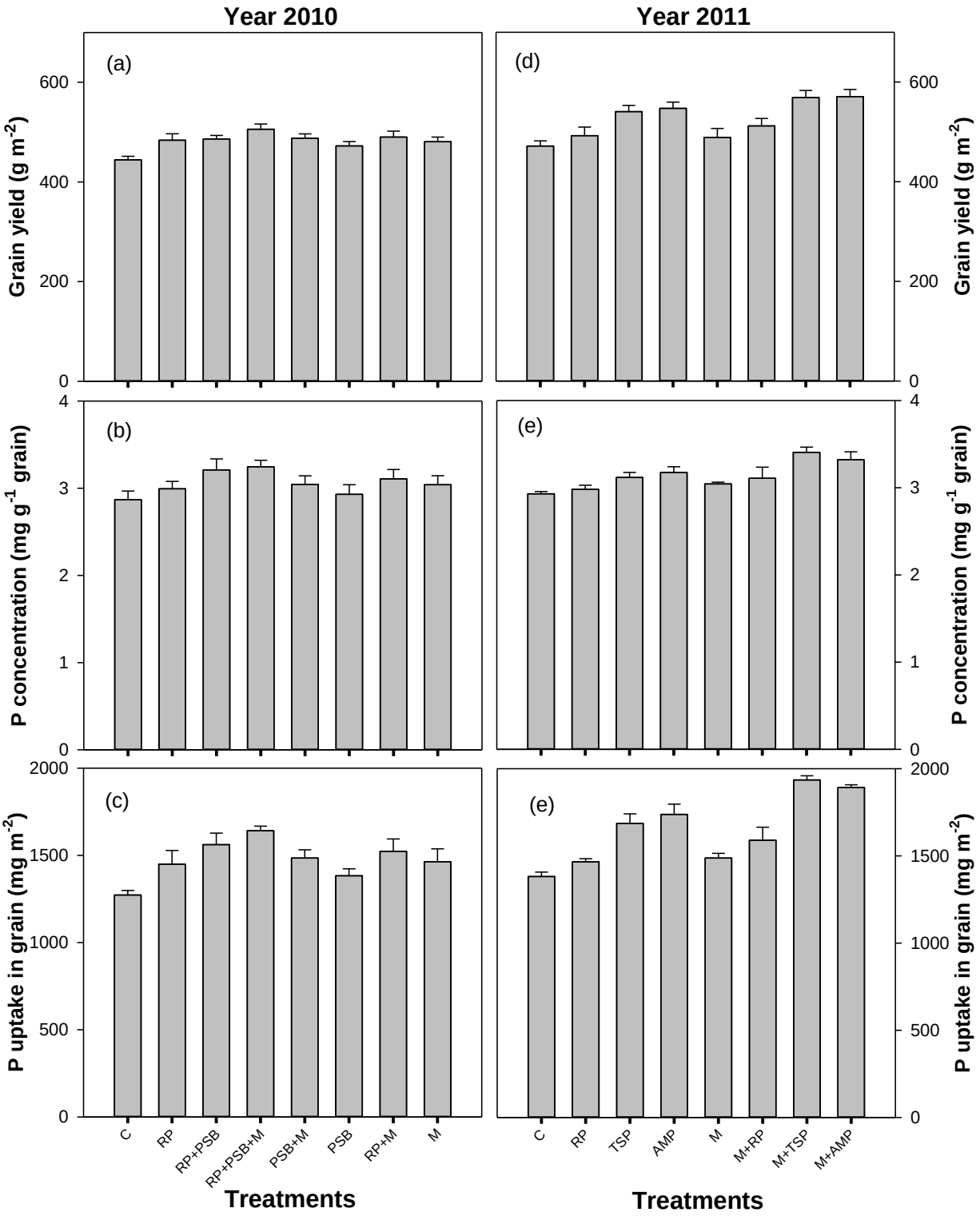

Fig. 3.4.1. Mean values of effect of P sources on grain yield, P concentration and total P uptake in grain of plant.Year 2010: Grain yield (a), P concentration (b), total P uptake in grain (c) and year 2011: Grain yield (d), P concentration (e), total P uptake in grain (f).Error bars show standard error of means (for year 2010 n=3 and for year 2011 n=4).

Chapter 4: Pots Experiments

Effect of P bio-inoculants (VAM and PSB), Non-biological P fertilizers (SP, AMP and RP) and soil sterilization on barley (Hordeum vulgare L.) growth and nutrients uptake

4.1. Introduction

Phosphorus (P) is one of the most limiting and important elements in plant growth and crop yield, andis often immobilized in the soil (Hinsinger, 2001; Wang *et al*., 2010;Ryan et al., 2012). P exists in soil in two forms: organic and inorganic. In the organic form, P is found in organic matter, plant and animal residues and manures and in microbes. A large quantity of organic P occurs in the upper soil layer. On the other hand, inorganic P in the soil occurs in the form of apatite, aluminium and iron phosphates (Rodrı et al., 2006;Rose & Wissuwa, 2012).

Large proportions of soil P may not be available for plant uptake and nutrition (Goldstein & Krishnaraj, 2007; Rose & Wissuwa 2012;Yang et al., 2012). The amount of available P in soils is on average approximately 0.05% by weight, (Schachtman et al., 1998). By contrast the concentration of P uptake in plant tissues is approximately 0.2% of plants, dry weight basis although this varies greatly (Theodorou & Plaxton, 1993). Therefore P reserves of soil becoming rapidly depleted and there is no alternative to P sources, so it is important to help preserve these reserves for future generation.

Some plant species are capable of taking up immobilized P through: modification of the rhizosphere pH, increased root hair density and root length, encouraging mycorrhiza colonization, and the activity of P solubilizing microorganisms (Johansen, 1996;Ekin, 2010; Bünemann et al., 2012).

4.1.1. Mycorrhizae

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 (Fig 4.1.1) (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).

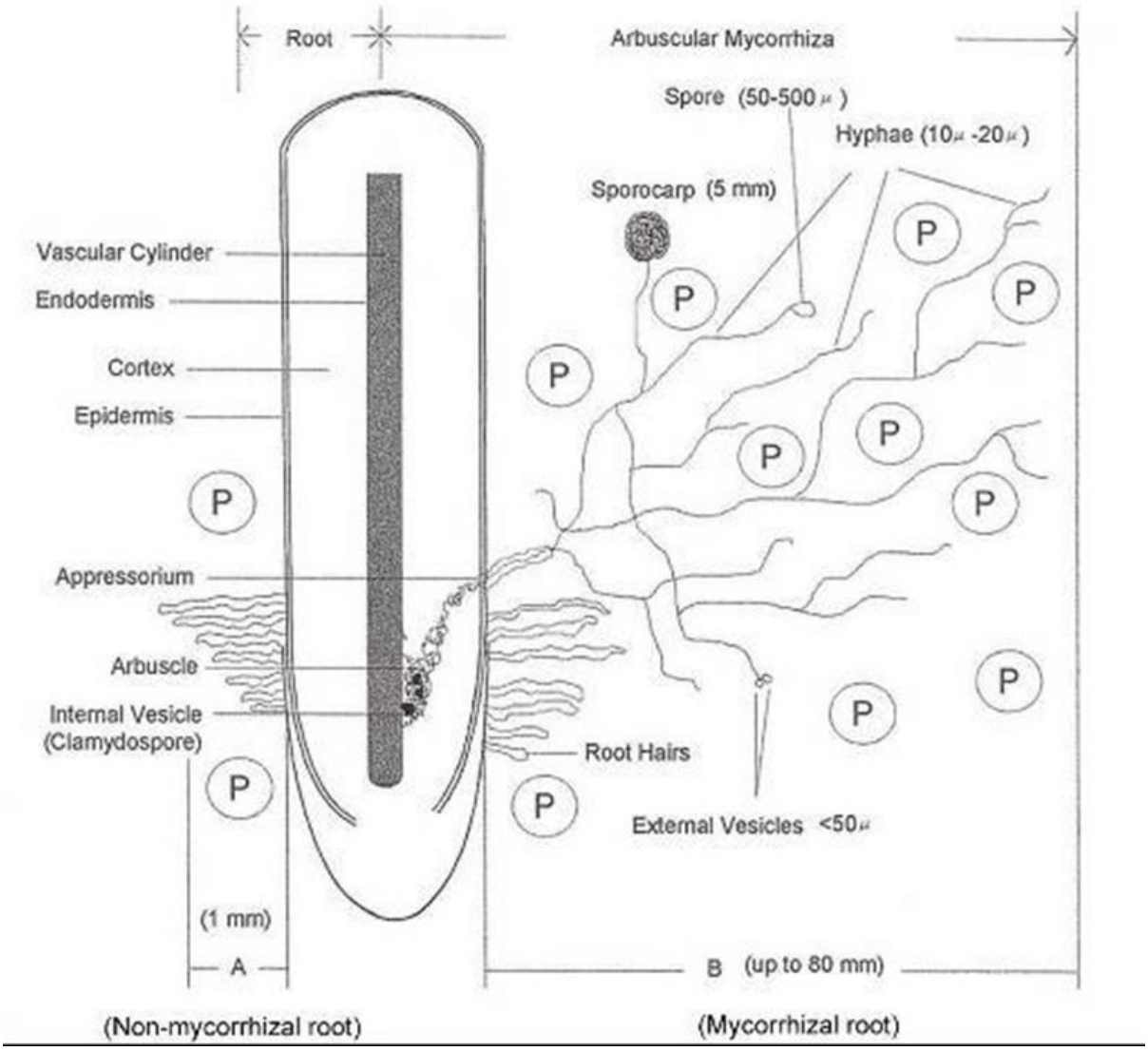

Fig. 4.1.1. Diagram of root P uptake by mycorrhiza (B) and non-mycorrhia (A).*P=phosphate ion.* http://mycorrhiza.ag.utk.edu/mimag.htm(accessed September 2012).

4.1.2. Phosphorus solubilizing bacteria

Phosphorus solubilizing microorganisms (PSM) include many types of microorganism (such as bacteria, actinomycetes and fungi)which are present in soil. These PSM are capable of solubilizing the complex insoluble forms of Pinto simple ions that can be taken up by plants(Hadley, 1978; Khan et al. 2009). There is a large population of PSB in the soil and in the rhizosphere(Gibaly et al., 1977;Hadley, 1978). Containing aerobic and

anaerobic types, with the anaerobic type dominating in waterlogged soil(Raghu & MacRae, 1966). Generally, a greater density of PSB occur in the rhizosphere in comparison to non-rhizosphere(Khan et al. 2009; Singh et al. 2011).

PSB comprise a group of unrelated bacteria (*Pseudomonas*, *Bacillus*, *Aspergillus*, *Penicillium*, *Mycobacterium*, *Micrococus*, *Flavobacterium*, *Selerotium* and *Fusarium*) which are able to decrease the pH of the surroundings, solubilize inorganic phosphate, mineralize organic phosphate and increase the availability of P for plants in the rhizosphere by manufacturing organic and inorganic acids(Kim et al., 1998; Stevenson et al., 2005; Abou-el-Seoud & Abdel-Megeed, 2012), alkaline and acid phosphatases (Rao et al. 1999;Rodríguez & Fraga, 1999) as well as through the production of H^+(Illmer & Schinner, 1992;Smith, 2002). PSB are capable of being used as biofertilizer inoculants to increase crop growth and yield (Fig 4.1.2)(Rodríguez & Fraga, 1999; Vessey 2003; Khan et al. 2009; Yadav et al. 2011).

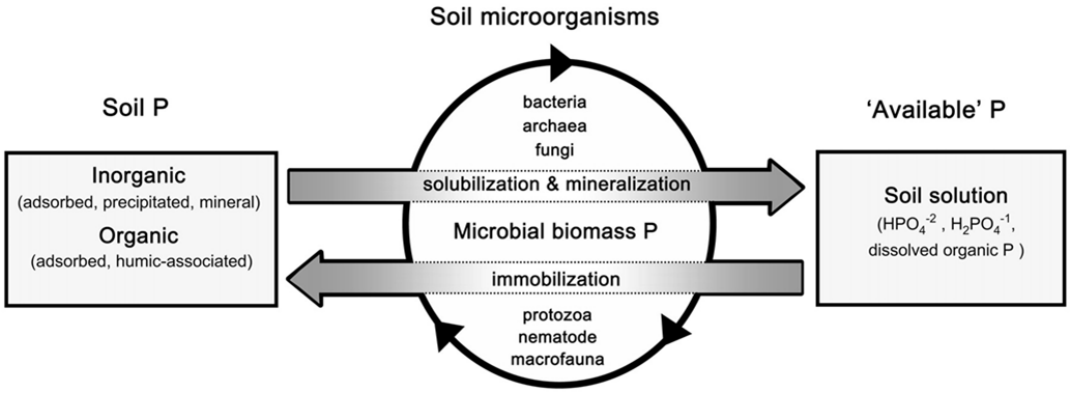

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Fig. 4.1.2. Schematic diagram of soil phosphorus mobilization and immobilization by bacteria(from Khan et al. 2009).

4.1.3. Interactions of soil microorganisms and growth of plants

The rhizosphere is the main zone of interaction between plant roots and soil microorganisms. Several studies have shown that there are a large number of soil microorganisms with beneficial, neutral or deleterious effects on plant growth and crop production(Burr et al. 1984;Gaskins et al. 1985;Hetrick et al. 1988;Nehl et al. 1997;Berggren et al. 2001;Stark & Kytöviita 2006). The soil can have an effect on plant growth and production via chemical, physical and/or biological factors. Interactions between plant roots and soil microorganisms and also interactions between deleterious and beneficial microorganisms within the rhizosphere are important in plant growth and nutrition and crop production(Osmond, 1972; Cakmakçi et al., 2006). Plants and microorganisms mobilize and absorb nutrients by the same mechanisms. Therefore there is intense competition between plants and microorganisms for nutrient uptake. It seems that microorganisms are more competitive than plant roots(Turkington & Klein, 1991;Fernández Bidondo et al., 2012), microorganisms are able to take up nutrients bound to plant-derived compounds, and also to decompose plant-derived material and nutrients immobilized in biomass before they spread to the root surface (Fig 4.1.3) (Marschner et al. 2011).

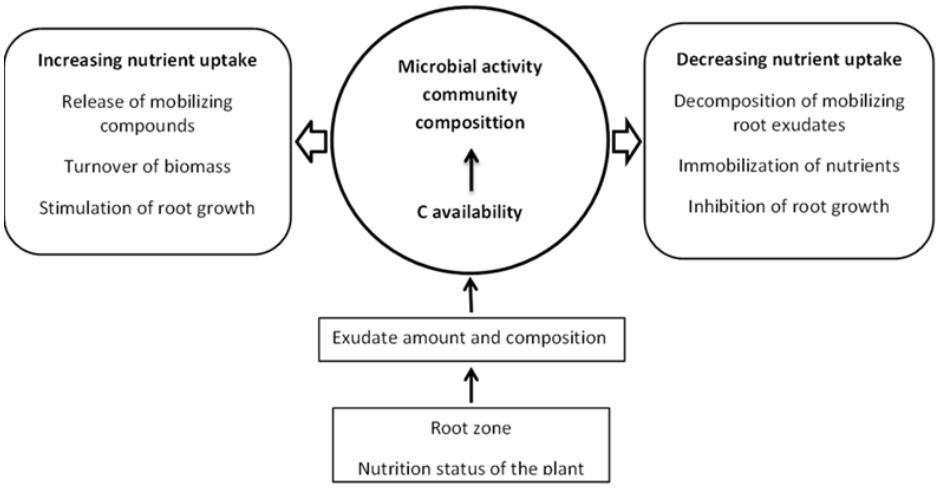


Fig. 4.1.3. Mechanism by which microorganisms in the rhizosphere increase or decrease nutrient availability to plants and some of the factors influencing the relative magnitude of mechanisms(Redrawn from Marschner et al., 2011).

4.1.3.1. Positive interaction

In plant growth and development, plant roots release quantities of organic combinations that include, carbohydrates, amino acids and carboxylic acids, which are energy and nutrient sources available for microorganisms in the rhizosphere. Microorganisms in the rhizosphere are able to positively influence plants, for example by promoting plant growth (by obtaining and absorbing mineral nutrients, providing the materials for plant growth) and also by decreasing susceptibility to diseases that are caused by pathogens of plants such as bacteria, fungi, viruses and nematodes,(Fig 4.1.5) (Johansson et al. 2004;Avis et al. 2008; Adesemoye & Kloepper 2009;Marschner et al. 2011; Rose & Wissuwa 2012).

4.1.3.2. Negative interaction

Microorganisms in the rhizosphere may also have negative effects on plants, for example via raising the risk of contamination with parasites or plant pathogens. Roots can exude toxic compounds, such as phytotoxins, antimicrobial, nematicide and insecticide combinations which might also prevent the growth of beneficial organisms in the rhizosphere (Suslow & Schroth 1982;Nehl et al., 1996;Berggren et al., 2001).

Deleterious microorganisms can have negative effects on plant growth, such as altering the provision of water and ions, as well as changing the morphology and structure of

plant roots with consequent alterations of root functions (Nehl et al. 1997;Berggren et al., 2001). Alterations in soil nutrient status, soil temperature, cropping system and plant genotype can further have a deleterious effect on microorganisms (Fig 4.1.4) (Fredrickson & Elliott 1985; Adesemoye & Kloepper 2009).

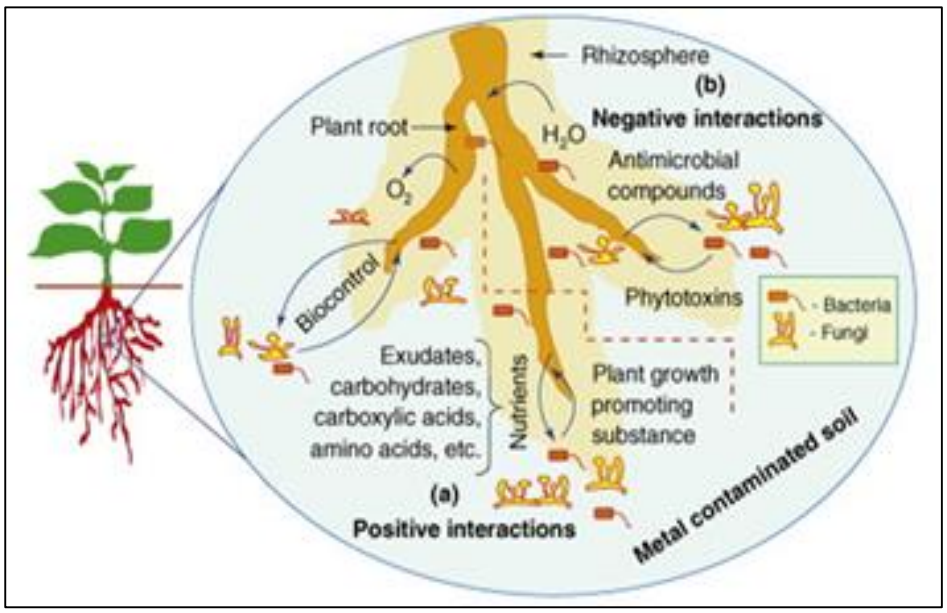


Fig. 4.1.4. Intraction between plants and rhizosphere microbes(from Rajkumar et al. 2010).

4.1.4. Total root length of whole plant

Roots are the main plant organs that supply nutrients, water, hormones and physical support for the plant. In addition roots are able to enhance soil organic matter by contributing to the soil resources of nitrogen, organic carbon and microbial biomass. 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 thefine root system is necessary for good plant growth and development and consequently for satisfactory production (Mollier & Pellerin, 1999;Gao et al. 2010;Tara Singh Gahoonia et al., 1997;Fageria & Moreira, 2011;Marschner et al., 2011).

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 tothe phosphorus status of the plant (Romer et al., 1986;Römer & Schenk, 1998; Zhu et al., 2003;Fageria & Moreira, 2011). 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, 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.

4.2. Materials and methods

4.2.1. Experiment 1: Horticultural sand experiment

4.2.1.1. Experimental design and culture practices

The pot experiments were conducted in a horticultural fine,silver sand(easy extracting whole root systems from the horticultural sand) (Table 4.2.1.1) with nutrient supplied by Long Ashton nutrient solution (Hoagland – see Appendix 1). 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.

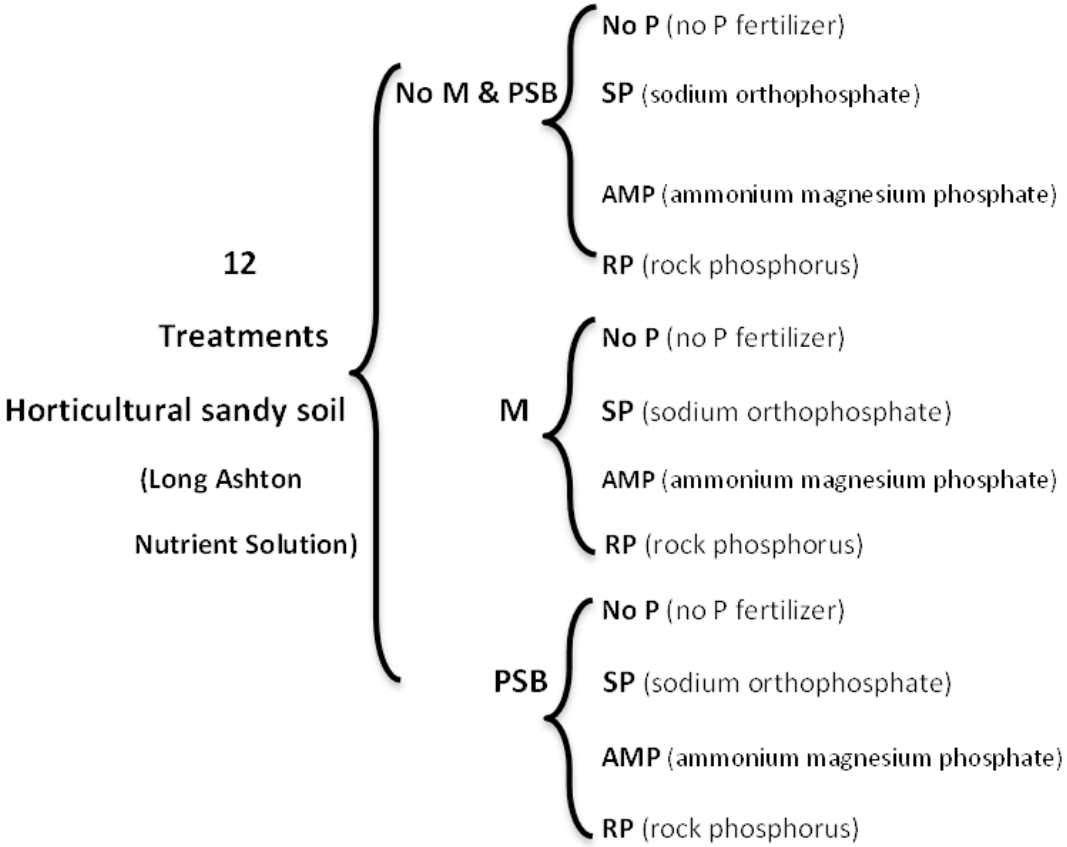
The experiment was a completely randomized 3×4 factorial design with 9 replicates per treatment (Table 4.2.1.2) with 3 replications each being harvested at three sampling date. There were three biological P treatments (vesicular arbuscular mycorrhizal inoculum (M), phosphorus solubilising bacteria inoculums (PSB) 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 rock phosphorus (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). Barley seeds (cv. Static) 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).

Table 4.2.1.1. Properties of the growing used in medium experiment 1.

Sand (%)	100
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 4.2.1.2. Twelve treatments in the horticultural sand experiment.



4.2.1.2. Measurements and data recorded

At22 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).

4.2.1.3. Staining and colonization of the roots

Refer to chapter 2

4.2.1.4. Phosphorus analyses

Refer to chapter 2

4.2.1.5. Determination of root length

Roots were scanned and analyzed using the WinRHIZO software (WinRhizo 5.0a, Regent Instruments Inc., Canada).

The procedure for determination of root length used was (Fig 4.2.1.1):

1. Before removing soil and roots from the pots, watering and soaking the pots was useful.
2. Slowly wash away soil with a gentle spray while supporting roots with hands and a mesh.
3. Remove dead root remnants and other unwanted particles.
4. Preserve roots in 50% ethanol and water in the cold room when there were many samples and could not be stained and scanned immediately.
5. Put some water in a white tray and spread the roots out so that if possible they are not touching. The root system may need to be divided (for spreading of the roots two combs were used).
6. Make sure the root parts are clean. WinRhizo will classify small piece of grit as root length and this can make a difference to total length.
7. Click “preview”. This will do a quick scan and bring up a preview of roots. Check that they are all in the picture and not overlapping, and adjust the marquee to the required size.
8. Click Scan. After scanning WinRhizo, the image was scanned.

9. Analysis of images.

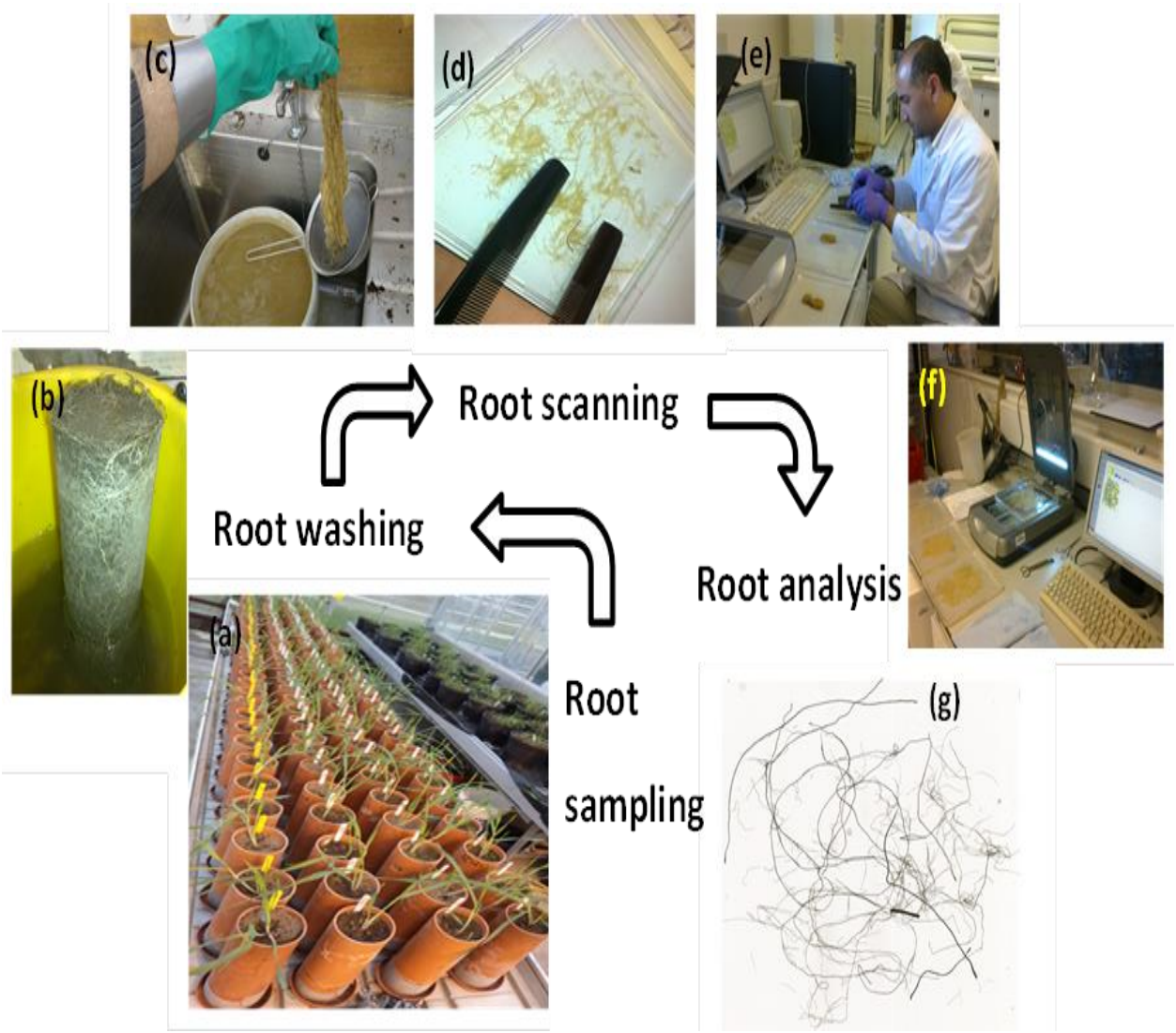


Fig. 4.2.1.1. Measurement of root length process.(a) Plants sampling; (b) root clearing and sampling; (c) root washing; (d) *Put root with some water in the tray*; (e) *Spread the roots in the tray*; (f) Scanning of the roots; (g) Analysis of the root images.

4.2.1.6. Statistical analyses

Refer to chapter 2

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

4.2.2.1. Soil characteristics and experimental site

The soil used for the second pot experiment in the glasshouse was sandy loam (Table 4.2.3) 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 4.2.1.3. 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

4.2.2.2. 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

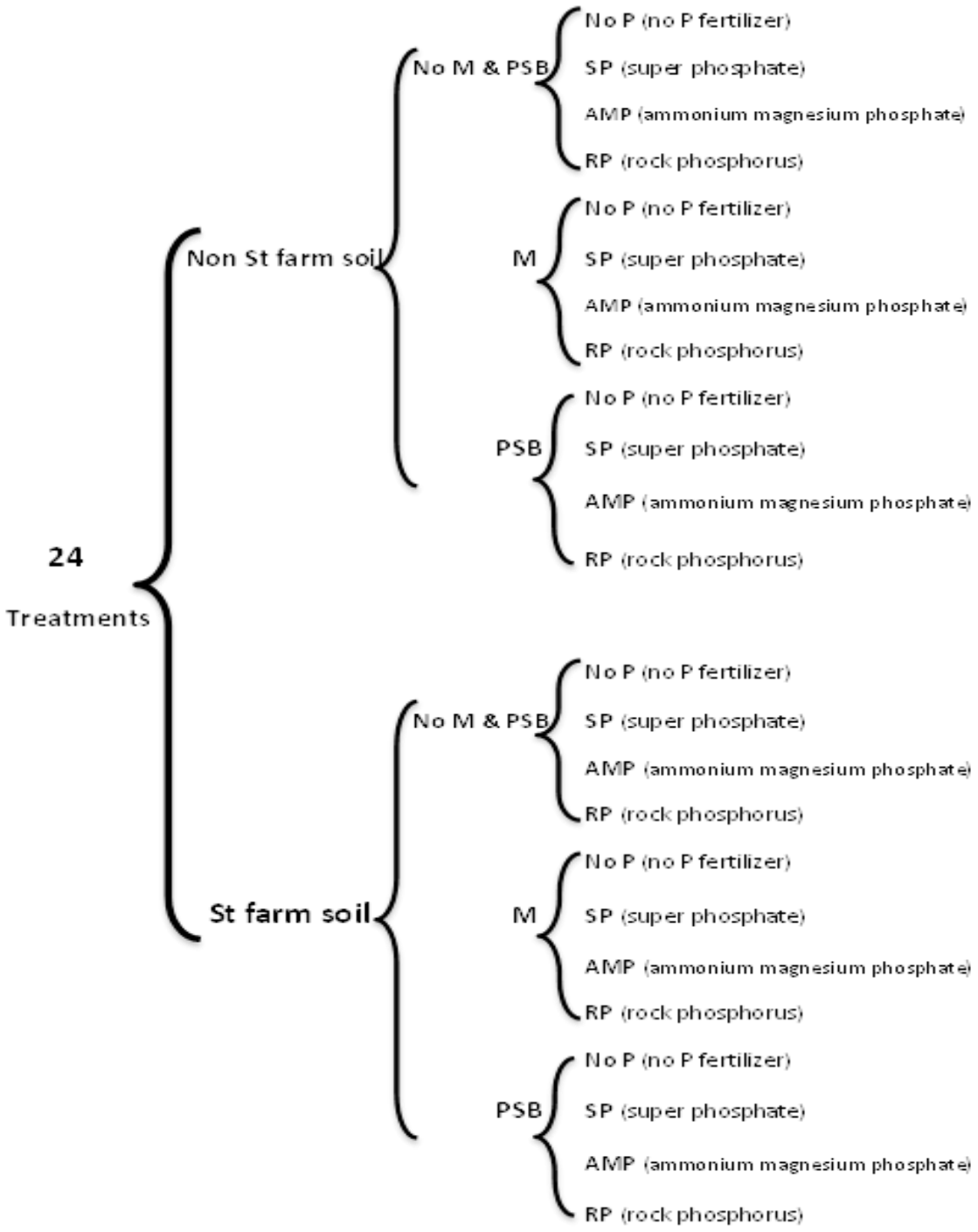
heating soil at 90˚C for 1 h (Trevors, 1996) to avoid oxidation of any organic matter.Sterilised soil could be useful for understanding the effects of dead/life native microorganisms on plant growth.

4.2.2.3. 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 (Table 4.2.1.4) 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 seeds (cv. Static) were 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 (vesicular arbuscular mycorrhizal(M) called *Biagro* from Glenside Company Starling that located in Scotland), phosphorus solubilising bacteria: *Bacillus Spez (PSB*) from GreenMax Agro Tech Company located in Germany and no *M&PSB*: (uninoculated), four non-biological P treatments (no P (control), super phosphate(SP),ammonium magnesium phosphate = Struvite(AMP*)*, rock phosphate(RP).Non-biological P fertilizers were applied to each pot and mixed with soil before sowing in the plastic cylindrical pots (height 30 cm, diameter 11 cm, volume of 2.55 L with the 100 µm nylon mesh at the bace). Barley seeds (cv. Static) were inoculated with M (1 kg ha^{-1})) in the appropriate treatments for each pot before sowing or PSB (2 L ha^{-1}) applied before sowing at 7-8 cm pot depth in the sandy loam soil.

Table 4.2.1.4. Treatments (24) of the farm soil experiment with 6 replications.



4.3. Results

4.3.1. First Pot Experiment (Horticultural sand) results

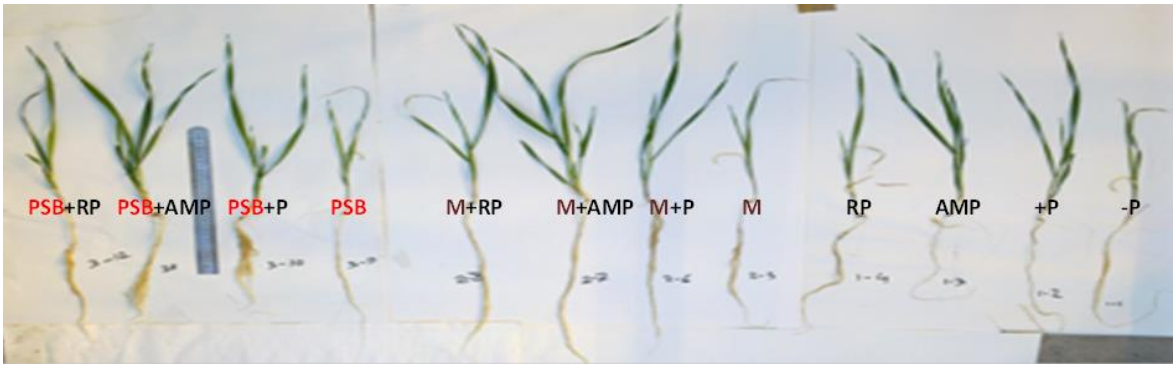


Fig. 4.3.1.1. Firs Sample: Roots and shoots, 22 das (vegetative stage).

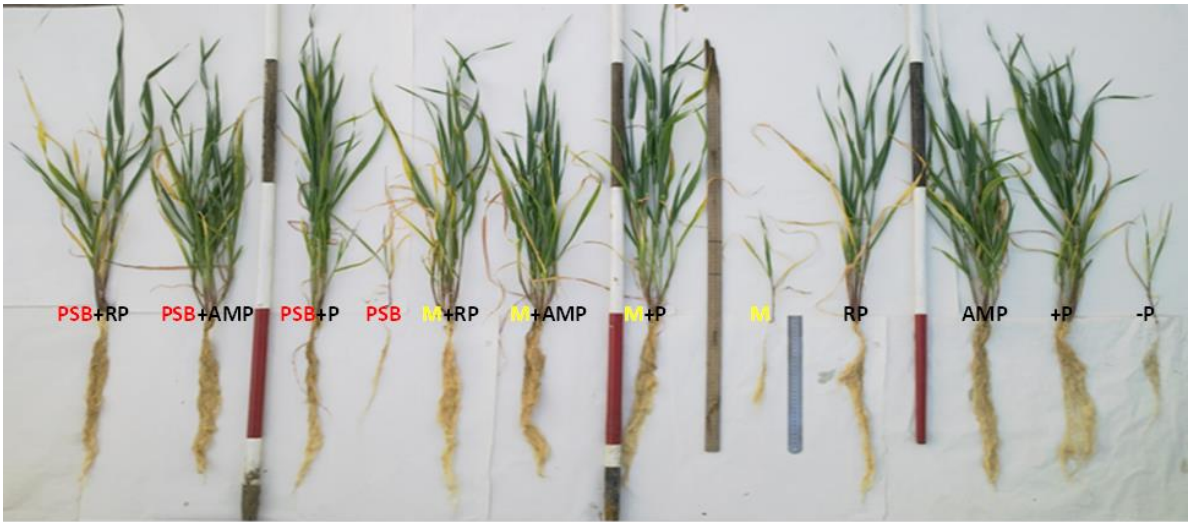


Fig. 4.3.1.2. Second sample: Roots and shoots, 57 das (Ear emergence).

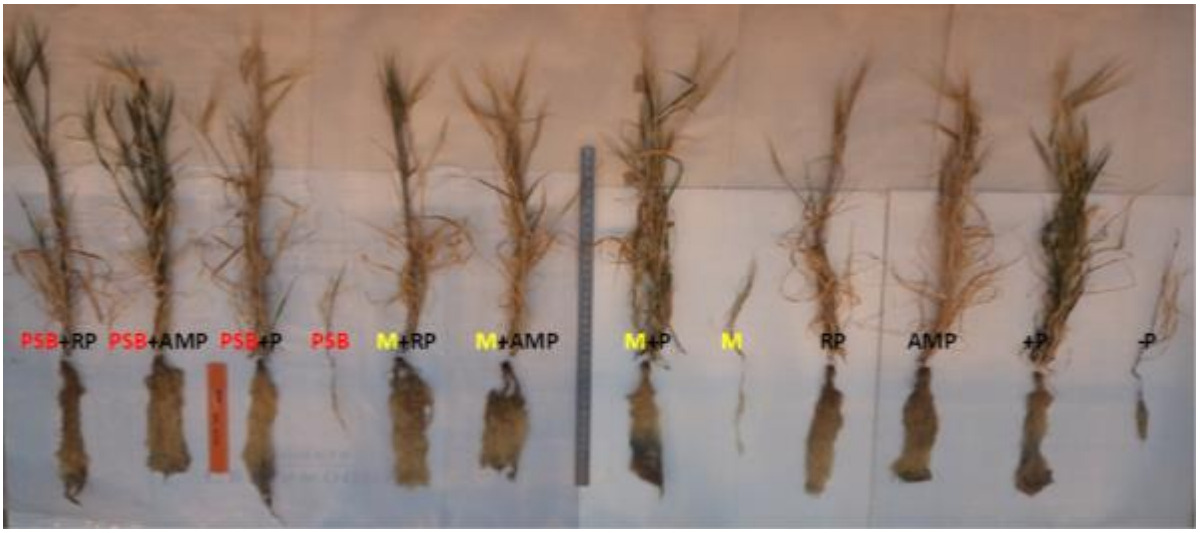


Fig. 4.3.1.3. Third (final) sample: Whole plant, 126 das (Ripe stage).

4.3.1.1. Yield and yield components of barley

In this study application of non-biological P (NBP) significantly increased number of ears per plant and number of grain per plant. SP and AMP were better than RP and also 1000-seed weight and grain yield significantly affected by NBP, biological P (BP) and interactions of NBP and BP (Table 4.2.1.8). The significant numbers of ears per plant and grain yield were produced by application of PSB with SP (Fig 4.3.1.4). PSB were more affected then M for yield due to higher ears/plant. Also rock phosphate was not very effective without inoculation.

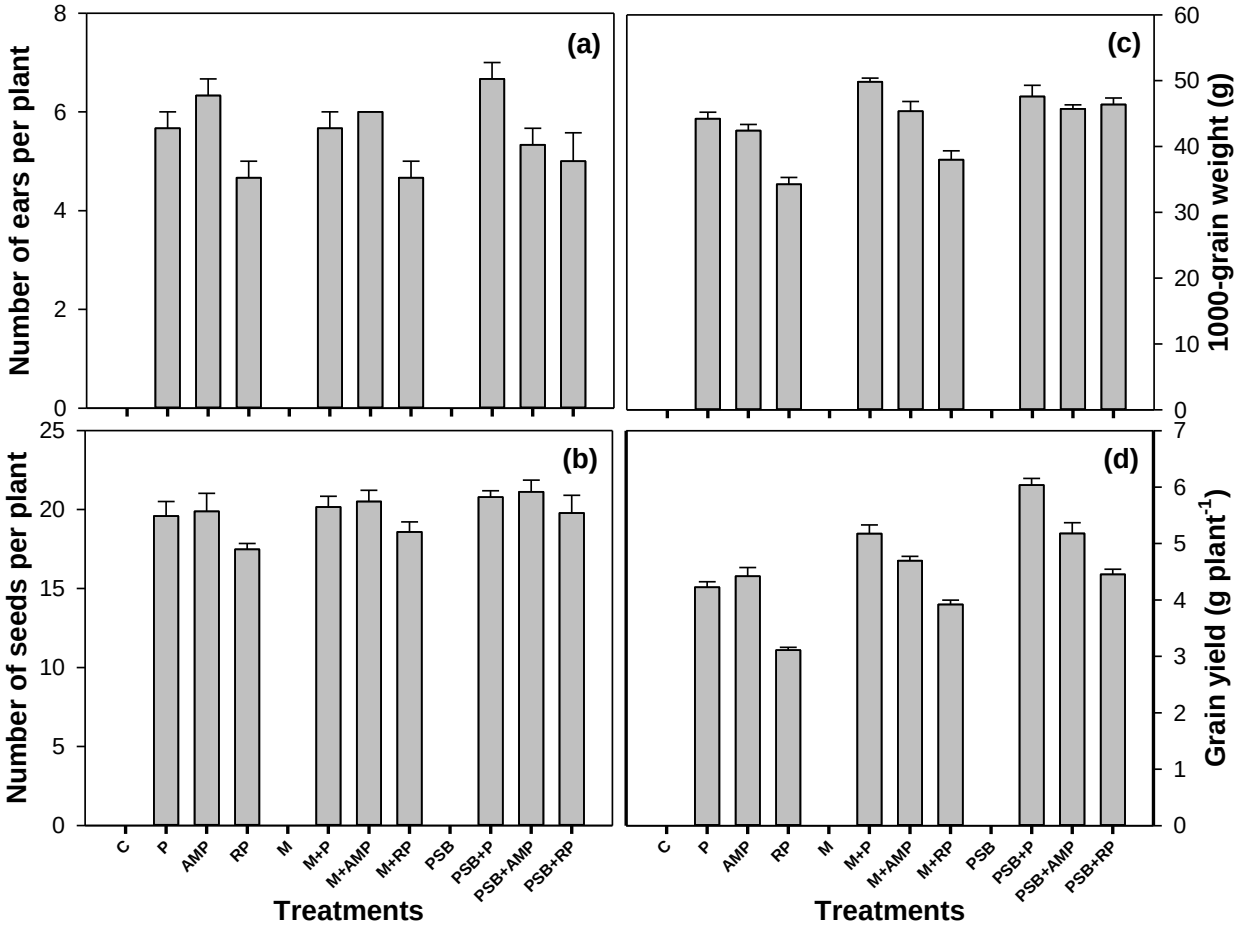


Fig. 4.3.1.4. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on number of ear per plant(a), number of grain per ear(b), 1000-seed weight(c) and grain yield(d) at final sample stage. Control plant (C) had no ears and seeds. Error bars show standard error of means (n=3).

Table 4.3.1.8. Table of standard errors of difference and P values for data presented in Figure 4.3.1.4. Significant levels of P are in bold script.

Treatment	Yield components of barley							
	Nu. of ears per plant (a)		Nu. of seeds per plant (d)		1000-seed weight (g) (b)		Grain yield (g)	
	SED	P	SED	P	SED	P	SED	P
Non-Biological P	0.2462	**<.001**	0.571	0.0841	0.816	**<.001**	0.0841	**<.001**
Biological P	0.2132	0.74	0.495	0.0728	0.706	**<.001**	0.073	**<.001**
Non-Biological P* Biological P	0.4264	0.081	0.989	0.1456	1.413	**<.001**	0.146	**<.001**

4.3.1.2. P concentration

Analysis showed that the P concentration in root and shoot were significantly affected by non-biological phosphorus (NBP), biological phosphorus (BP) and there were interactions of NBP and BP at all sample stages (Table 4.3.1.9). The highest P concentrationsin first sample (22 das) in root and shoot were the AMP+PSB treatments. The highest P concentration inthe second sampling (57 das) in root and shoot was in the SP+M treatment. The highest P concentration in third sample (126 das) in root and shoot were in the SP+PSB and SP+M treatments (Fig 4.3.1.5).

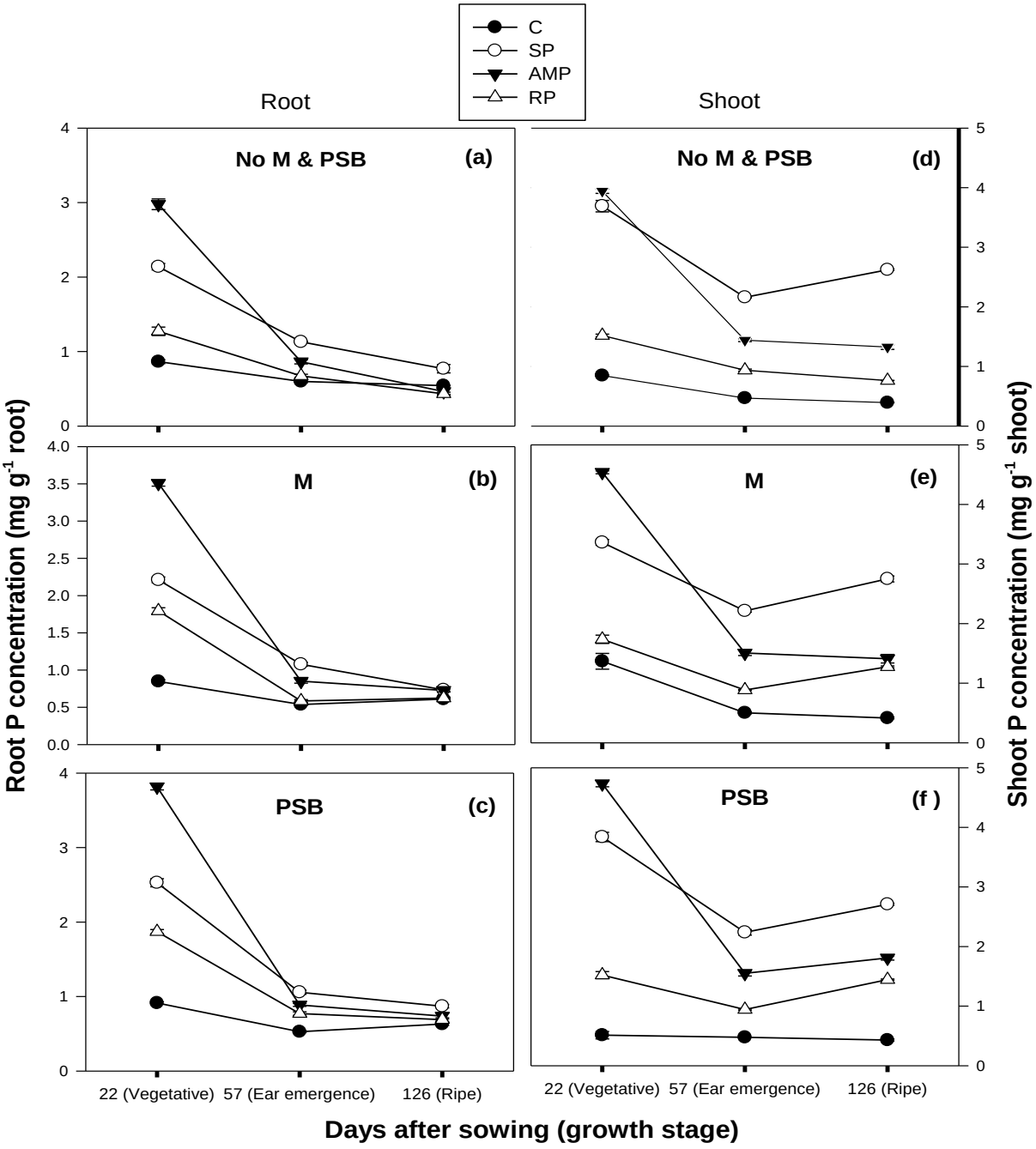


Fig. 4.3.1.5. Mean values of P concentration at vegetative stage (22 das), ear emergence (57 das), and ripe stage stage (126 das). (a) Effect of non-biological Phosphorus (NBP) on root P concentration; (b) Effect of NBP with mycorrhiza inoculation (M) on root P concentration; (c) Effect of NBP with phosphorus solubilizing bacteria (PSB) on root P concentration; (d) Effect of NBP on shoot P concentration; (e) Effect of NBP with M on shoot P concentration; (f) Effect of NBP with PSB on shoot P concentration.Error bars show standard error of means (n=3).

Table 4.3.1.9. Table of standard errors of difference and P values for data presented in Figure 4.3.1.5. Significant levels of P are in bold script.

Treatment	P concentration in root (mg g^{-1} root)					
	Sample 1 (vegetative stage)		Sample 2 (Ear emergence)		Sample 3 (Ripe stage)	
	SED	P	SED	P	SED	P
Non-Biological P	0.01973	**<.001**	0.01348	**<.001**	0.02274	**<.001**
Biological P	0.01708	**<.001**	0.01168	**<.001**	0.01969	**<.001**
Non-Biological P* Biological P	0.03417	**<.001**	0.02336	**<.001**	0.03939	**<.001**

Treatment	P concentration in shoot (mg g^{-1} shoot)					
	Sample 1 (vegetative stage)		Sample 2 (Ear emergence)		Sample 3 (Ripe stage)	
	SED	P	SED	P	SED	P
Non-Biological P	0.0536	**<.001**	0.02032	**<.001**	0.02448	**<.001**
Biological P	0.0464	**<.001**	0.0176	**0.03**	0.0212	**<.001**
Non-Biological P* Biological P	0.0928	**<.001**	0.0352	0.138	0.0424	**<.001**

4.3.1.3. Total P uptake

Analysis showed that the total P uptake in root and shoot was significantly affected by NBP, BP and there were interactions of NBP and BP at all three sample stages (Table 4.2.1.10). The highest amount of P uptake in first sample (22 das) in root and shoot was in the AMP+PSB treatment. The highest amount of P uptake in second sample (57 das) in root and shoot was in the AMP+M and SP+M treatments. The highest amount of P uptake in third sample (126 das) in root and shoot was in the AMP+PSB and SP+M treatments (Fig 4.3.1.6).

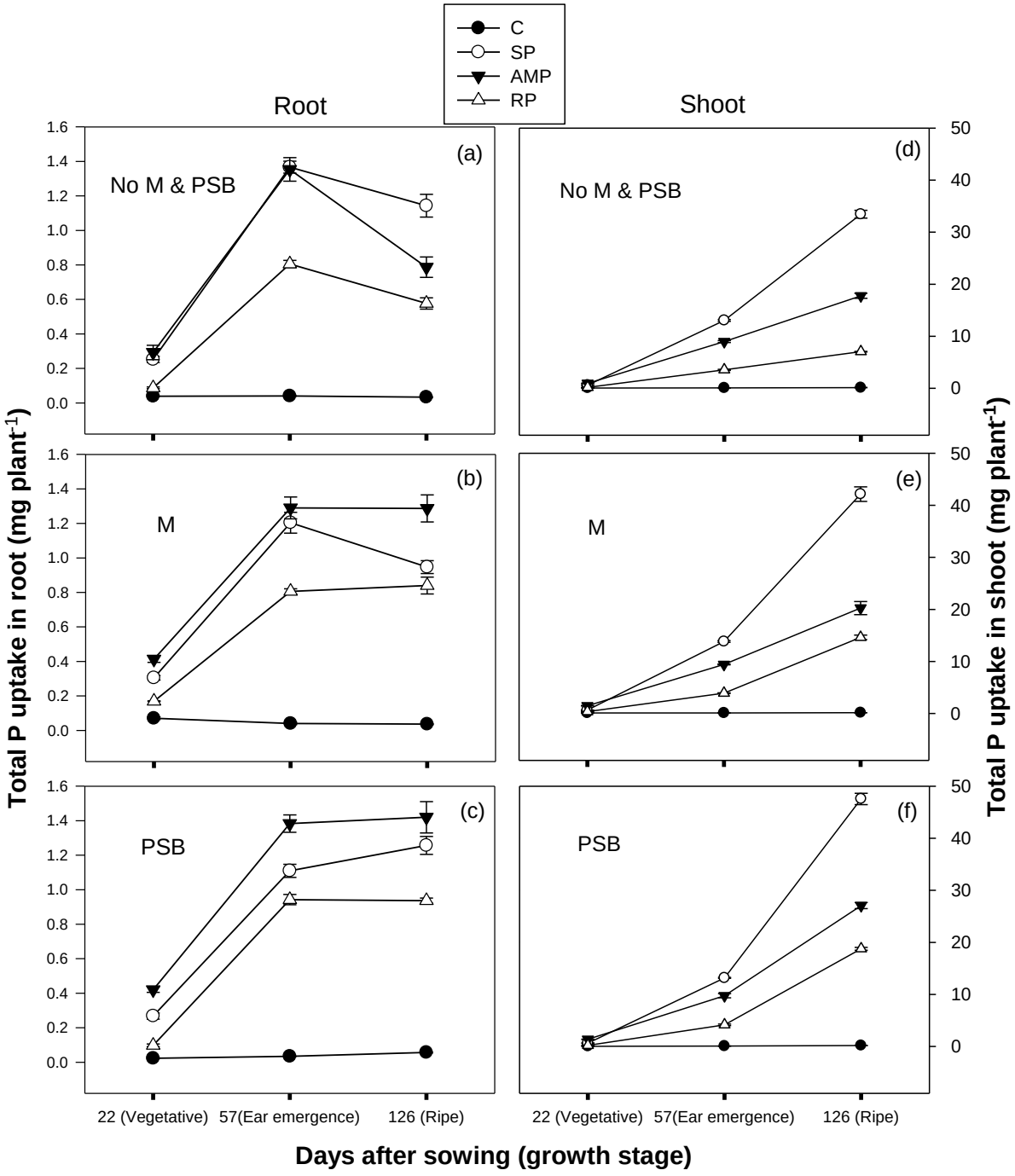


Fig. 4.3.1.6. Mean values of P uptakeat vegetation stage (22 das), ear emergence (57 das), and ripe stage (126 das). (a) Effect of non-biological Phosphorus (NBP) on total P uptake of the roots; (b) Effect of NBP with mycorrhiza inoculation (M) on total P uptake of the roots; (c) Effect of NBP with phosphorus solubilizing bacteria (PSB) on total P uptake of the roots; (d) Effect of NBP on total P uptake of the roots; (e) Effect of NBP with M on total P uptake of the roots; (f) Effect of NBP with PSB on total P uptake of the roots. Error bars show standard error of means (n=3).

Table 4.3.1.10. Table of standard errors of difference and P values for data presented in Figure 4.3.1.6.Significant levels of P are in bold script.

Treatment	Total P uptake in root (mg plant^{-1})					
	Sample 1 (vegetation stage)		Sample 2 (Ear emergence)		Sample 3 (Ripe stage)	
	SED	P	SED	P	SED	P
Non-Biological P	0.01243	**<.001**	0.02469	**<.001**	0.0398	**<.001**
Biological P	0.01077	**<.001**	0.02139	**0.047**	0.0345	**<.001**
Non-Biological P* Biological P	0.02154	**0.003**	0.04277	**<.001**	0.0689	**<.001**

Treatment	Total P uptake in shoot (mg plant^{-1})					
	Sample 1 (vegetation stage)		Sample 2 (Ear emergence)		Sample 3 (Ripe stage)	
	SED	P	SED	P	SED	P
Non-Biological P	0.01915	**<.001**	0.1282	**<.001**	0.608	**<.001**
Biological P	0.01659	**<.001**	0.111	**0.002**	0.527	**<.001**
Non-Biological P* Biological P	0.03317	**<.001**	0.222	**0.03**	1.053	**<.001**

4.3.1.4. Root length

Analysis showed that the total root length was significantly affected by NBP, BP and interactions of NBP and BP in all of the three samples except for interaction of NBP and BP in the second sample (Table 4.3.1.11). The highest root length of first sample (22 das) was in the RP+M treatment. The highest root length of second sample (57 das) was in the SP treatment and the highest root length of third sample (126 das) was affected by SP treatment (Fig 4.3.1.7).

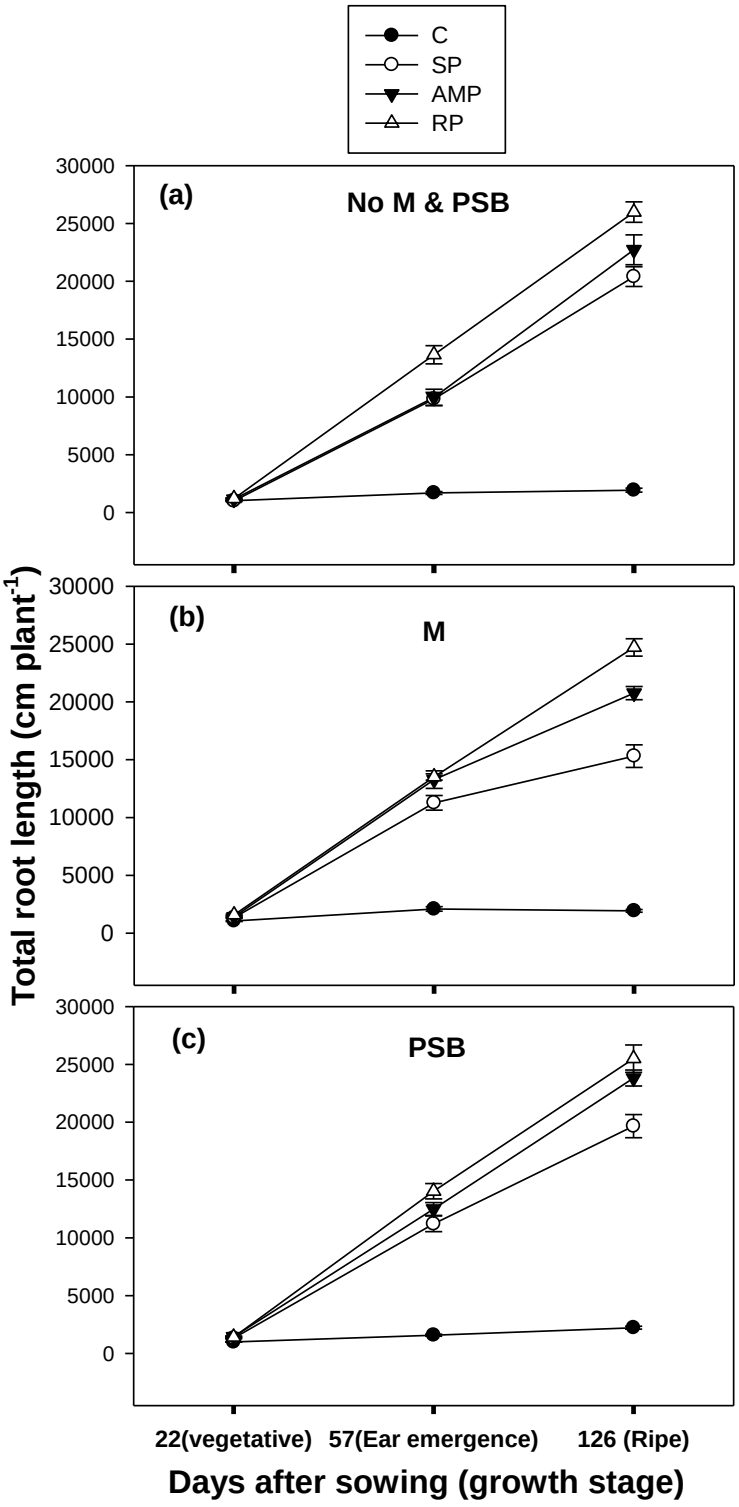


Fig. 4.3.1.7. Mean values of total root length at vegetative stage (22 das), ear emergence (57 das), and ripe stage (126 das). (a) Effect of non-biological Phosphorus (NBP) on total root length; (b) Effect of NBP with mycorrhiza inoculation (M) on total root length; (c) Effect of NBP with phosphorus solubilizing bacteria (PSB) on total root length.Error bars show standard error of means (n=3).

Table 4.3.1.11. Table of standard errors of difference and P values for data presented in Figure 4.3.1.7. Significant levels of P are in bold script.

Treatment	Root length of whole plant (cm plant^{-1})					
	Sample 1 (Vegetation stage)		Sample 2 (Ear emergence)		Sample 3 (Ripe stage)	
	SED	P	SED	P	SED	P
Non-Biological P	34.1	**<.001**	444.5	**<.001**	402	**<.001**
Biological P	29.5	**<.001**	385	**0.007**	348.2	**<.001**
Non-Biological P* Biological P	59	**0.001**	770	0.075	696.3	**0.001**

4.3.1.5. Root and shoot dry weight

Analysis showed that the root dry weight and shoot (grain and straw) dry weight were significantly affected by NBP , BP and interactions of NBP and BP in the all of three samples, except root dry weight that was affected only by BP and interactions of NBP and BP in the third sample (Table 4.3.1.12). The highest root and shoot dry weight in first sample (22 das) wasin the AMP+PSB and AMP+M treatments. The highest root and shoot dry weight in second sample (57 das) was in the AMP+PSB treatment. The highest root and shoot dry weight of third sample (126 das) was in the AMP+PSB and SP+PSB treatments (Fig 4.3.1.8).

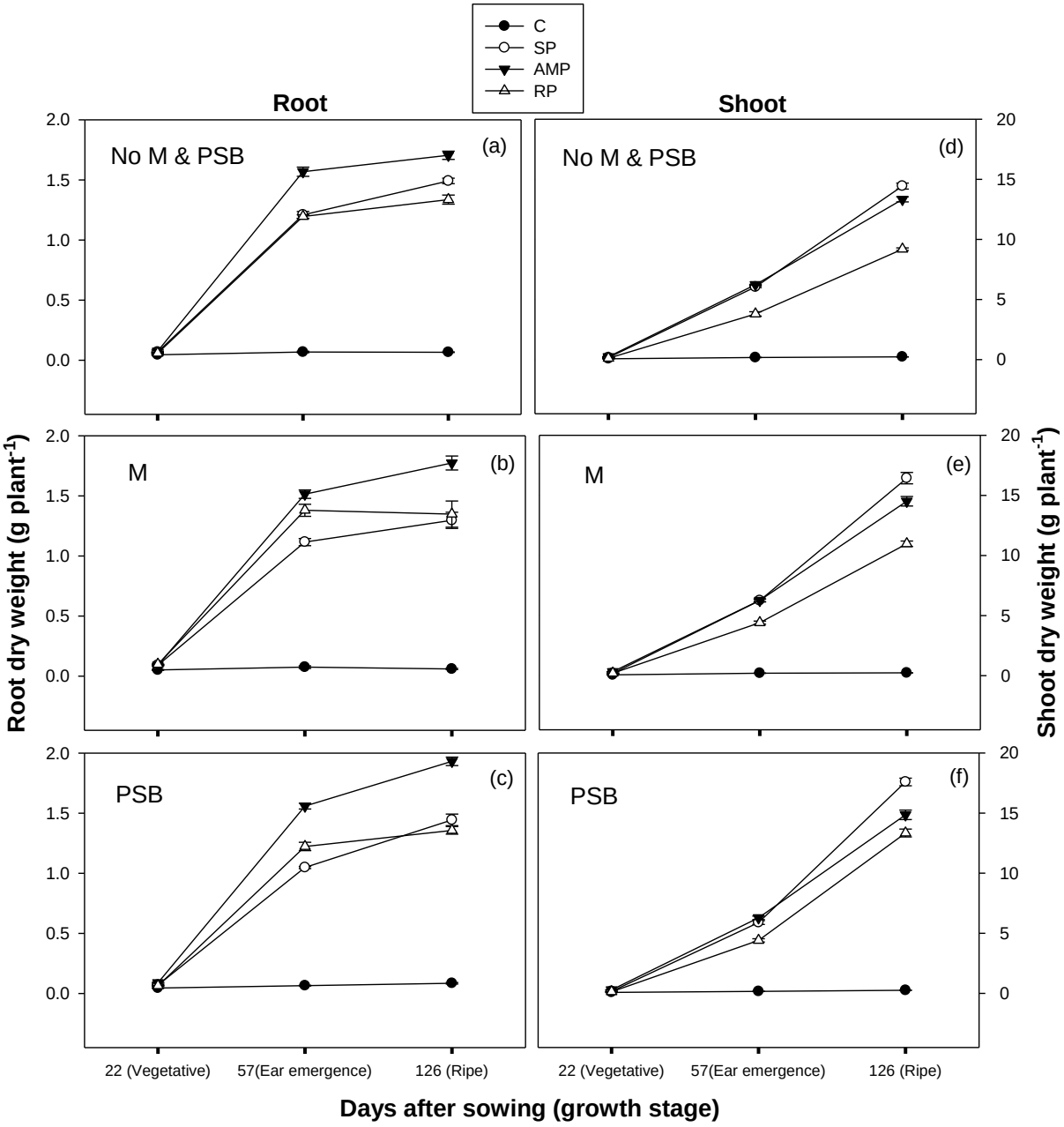


Fig. 4.3.1.8. Mean values of root and shoot dry weight per pot at vegetative stage(22 das), ear emergence (57 das), and ripe stage (126 das). (a) Effect of non-biological Phosphorus (NBP) on root dry weight; (b) Effect of NBP with mycorrhiza inoculation (M) on root dry weight; (c) Effect of NBP with phosphorus solubilizing bacteria (PSB) on root dry weight; (d) Effect of NBP on shoot dry weight; (e) Effect of NBP with M on shoot dry weight; (f) Effect of NBP with PSB on shoot dry weight.Error bars show standard error of means (n=3).

Table 4.3.1.12. Table of standard errors of difference and P values for data presented in Figure 4.3.1.8. Significant levels of P are in bold script.

Treatment	Root dry weight (g plant^{-1})					
	Sample 1 (Vegetation stage)		Sample 2 (Ear emergence)		Sample 3 (Ripe stage)	
	SED	P	SED	P	SED	P
Non-Biological P	0.00322	**<.001**	0.0215	**<.001**	0.0388	**<.001**
Biological P	0.00278	**<.001**	0.01862	**0.047**	0.0336	0.057
Non-Biological P* Biological P	0.00557	**0.002**	0.03724	**<.001**	0.0672	0.056

Treatment	Shoot dry weight (g plant^{-1})					
	Sample 1 (Vegetation stage)		Sample 2 (Ear emergence)		Sample 3 (Ripe stage)	
	SED	P	SED	P	SED	P
Non-Biological P	0.00506	**<.001**	0.0931	**<.001**	0.2341	**<.001**
Biological P	0.00439	**<.001**	0.0806	**0.038**	0.2027	**<.001**
Non-Biological P* Biological P	0.00877	**<.001**	0.1612	**0.034**	0.4054	**<.001**

4.3.1.6. Ratio of root:shoot and whole dry plant weight

Analysis showed that the whole dry plant weight and ratio of root:shoot was significantly affected by NBP , BP and interactions of NBP and BP for the all of three samples, except whole plant dry weight which was in the BP in the second sample and ratio of root:shoot that was in the BP and interaction of NBP and BP in the first and second samples (Tables4.3.1.13). The highest whole plant dry weight and ratio of root:shoot of first sample (22 das) was observed in SP+M and M treatments. The highest whole plant dry weight and root:shoot ratio at the second sample (57 das) was observed in the AMP+M and PSB treatments. The highest whole dry plant weight and ratio of root:shoot in the third sample (126 das) was in the SP+PSB and PSB treatments respectively (Fig 4.3.1.9).

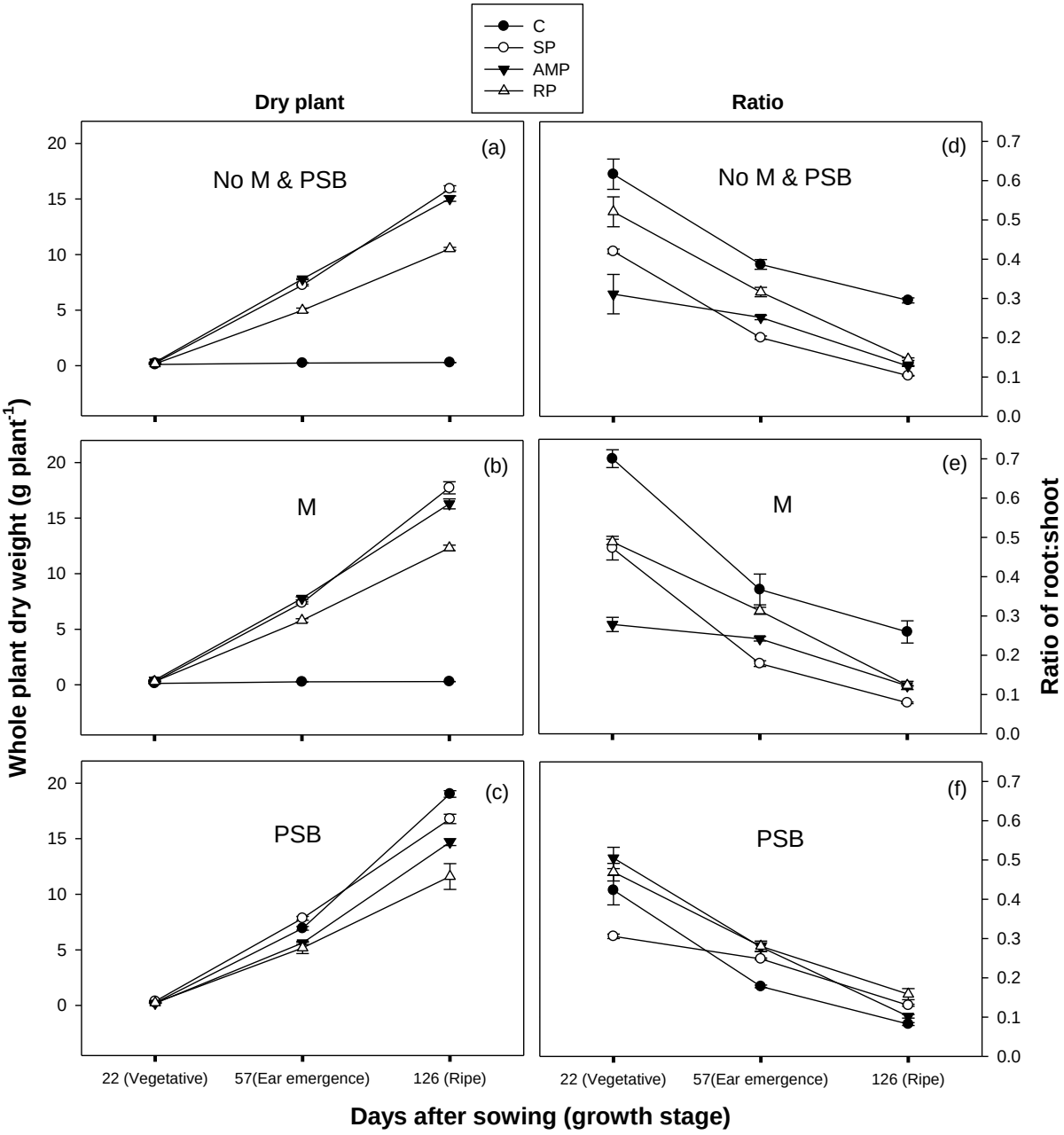


Fig. 4.3.1.9. Mean values of whole plant dry weight and ratio of root:shoot at vegetative stage (22 das), ear emergence (57 das), and ripe stage (126 das). (a) Effect of non-biological Phosphorus (NBP) on whole plant dry weight; (b) Effect of NBP with mycorrhiza inoculation (M) on whole plant dry weight; (c) Effect of NBP with phosphorus solubilizing bacteria (PSB) on whole plant dry weight; (d) Effect of NBP on ratio of root:shoot; (e) Effect of NBP with M on ratio of root:shoot; (f) Effect of NBP with PSB on ratio of root:shoot. Error bars show standard error of means (n=3).

Table 4.3.1.13. Table of standard errors of difference and P values for data presented in Figure 4.3.1.9. Significant levels of P are in bold script.

Treatment	Whole plant dry weight (g plant^{-1})					
	Sample 1 (Vegetation stage)		Sample 2 (Ear emergence)		Sample 3 (Ripe stage)	
	SED	P	SED	P	SED	P
Non-Biological P	0.00536	**<.001**	0.1041	**<.001**	0.2539	**<.001**
Biological P	0.00464	**<.001**	0.0901	0.051	0.2199	**<.001**
Non-Biological P* Biological P	0.00929	**<.001**	0.1802	**0.012**	0.4398	**<.001**

Treatment	Ratio of root:shoot					
	Sample 1 (Vegetation stage)		Sample 2 (Ear emergence)		Sample 3 (Ripe stage)	
	SED	P	SED	P	SED	P
Non-Biological P	0.02533	**<.001**	0.01412	**<.001**	0.00921	**<.001**
Biological P	0.02194	0.405	0.01223	0.511	0.00797	**0.031**
Non-Biological P* Biological P	0.04387	0.259	0.02446	0.541	0.01595	**0.009**

4.3.1.7. Root colonization

Analysis showed that root colonization by mycorrhiza did not occur in any of the treatments not inoculated by mycorrhiza(Table 4.3.1.14).The highest root colonization by mycorrhiza at all sample stages was observed in the RP+M treatment (Fig 4.3.1.10), which showed a marked peak at 57 days.

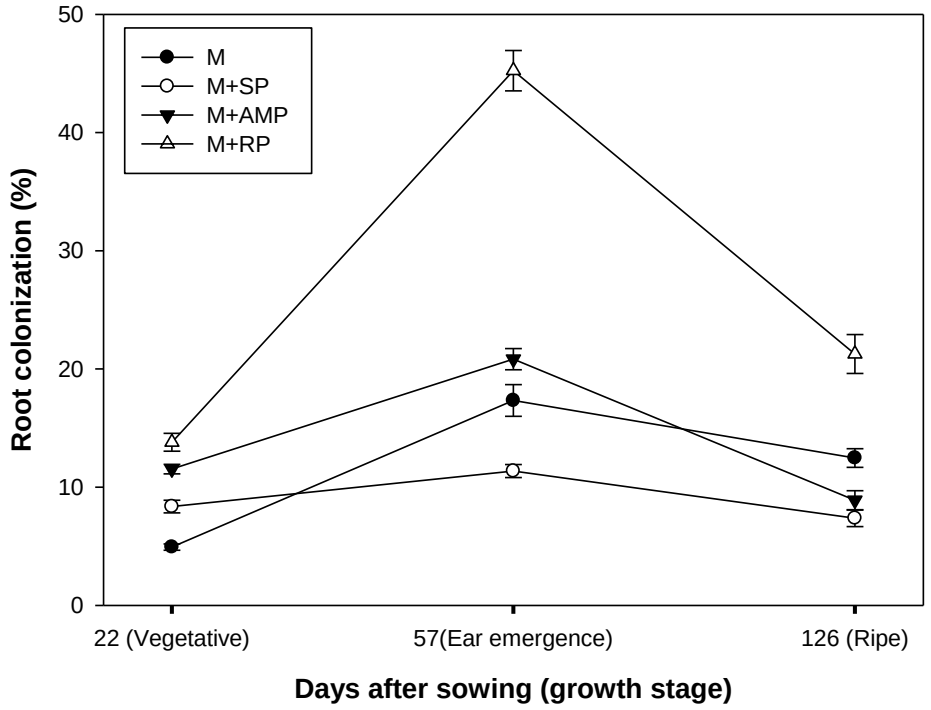


Fig. 4.3.1.10. Mean values of root colonization by mycorrhiza at vegetative stage(22 das), ear emergence (57 das), and ripe stage (126 das). Error bars show standard error of means (n=3).

Table 4.3.1.14. Table of standard errors of difference and P values for data presented in Figure 4.3.1.10. Significant levels of P are in bold script.

Treatment	Root colonization (%)					
	Sample 1 (Vegetationstage)		Sample 2 (Ear emergence)		Sample 3 (Ripe stage)	
	SED	P	SED	P	SED	P
Non-Biological P	0.2556	**<.001**	0.584	**<.001**	0.482	**<.001**
Biological P	0.2213	**<.001**	0.506	**<.001**	0.417	**<.001**
Non-Biological P* Biological P	0.4427	**<.001**	1.011	**<.001**	0.835	**<.001**

4.3.1.8. Leaf area index (LAI)

Leaves had senesced by 126 days. Analysis showed that the LAI was significantly affected by NBP, BP and interaction of NBP and BP in the first and second samples (Table 4.3.1.15). The highest LAI of first samples (22 das) and second sample (57 das) was observed in the AMP+M and AMP+PSB treatments (Fig 4.3.1.11).

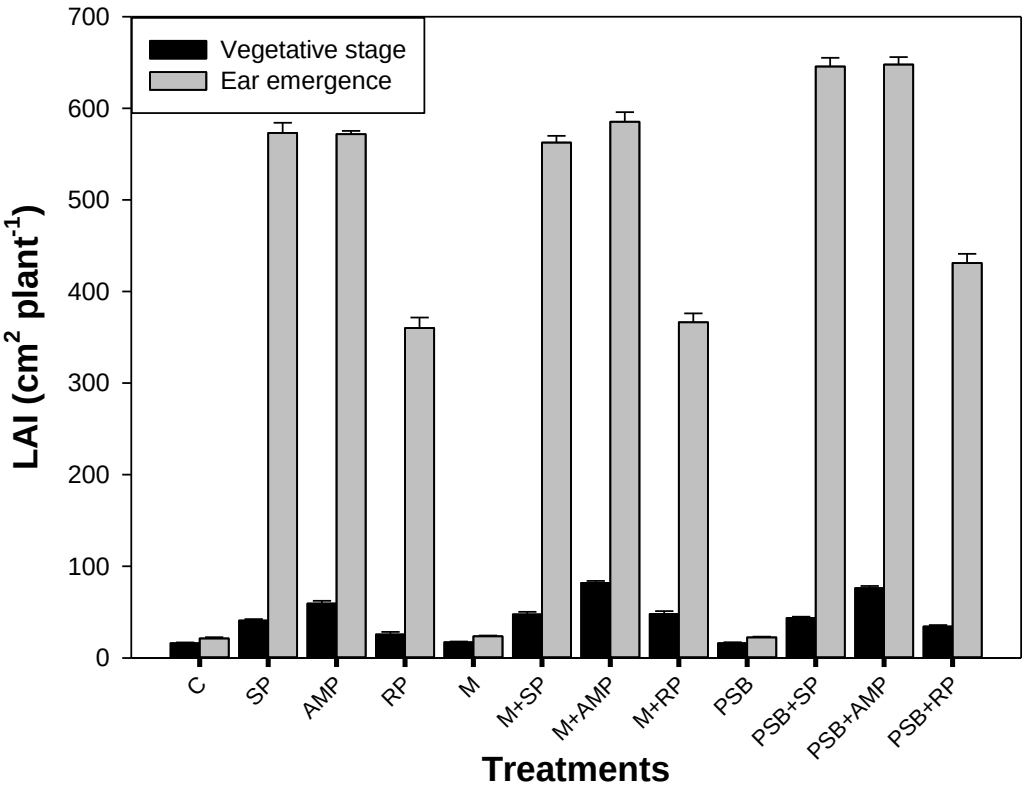


Fig. 4.3.1.11. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on LAI. Error bars show standard error of means (n=3).

Table 4.3.1.15. Table of standard errors of difference and P values for data presented in Figure 4.3.1.8. Significant levels of P are in bold script.

	LAI ($cm^2 plant^{-1}$)			
Treatment	**SED**	**P**	**SED**	**P**
Non-Biological P	34.1	**<.001**	444.5	**<.001**
Biological P	29.5	**<.001**	385	**0.007**
Non-Biological P* Biological P	59	**<.001**	770	0.075

4.3.1.9. Total P uptake

Analysis showed that the total P uptake in plant was significantly affected by NBP, BP and interactions of NBP and BP in all of the three samples (Tables 4.3.1.16).The highest total plant P uptake in first sample (22 das) in plant was greatest in RP+M and AMP+M treatments. The highest total P uptake in second sample (57 das) in plant was in the SP and SP+M treatments. The highest total P uptake in third sample (126 das) in plant was in the SP and SP+PSB treatments (Fig 4.3.1.12).

At the final sample, plants were dissected in to constituent parts and P uptake was measured. Application of NBP, BP and interaction of NBP and BP significantly increased total P uptake in root, stem, leaf and grain (Table 4.3.1.17). The highest P uptake in root, stem, leaf and grain were in treatments which used soluble P fertilizer(Fig 4.3.1.13).

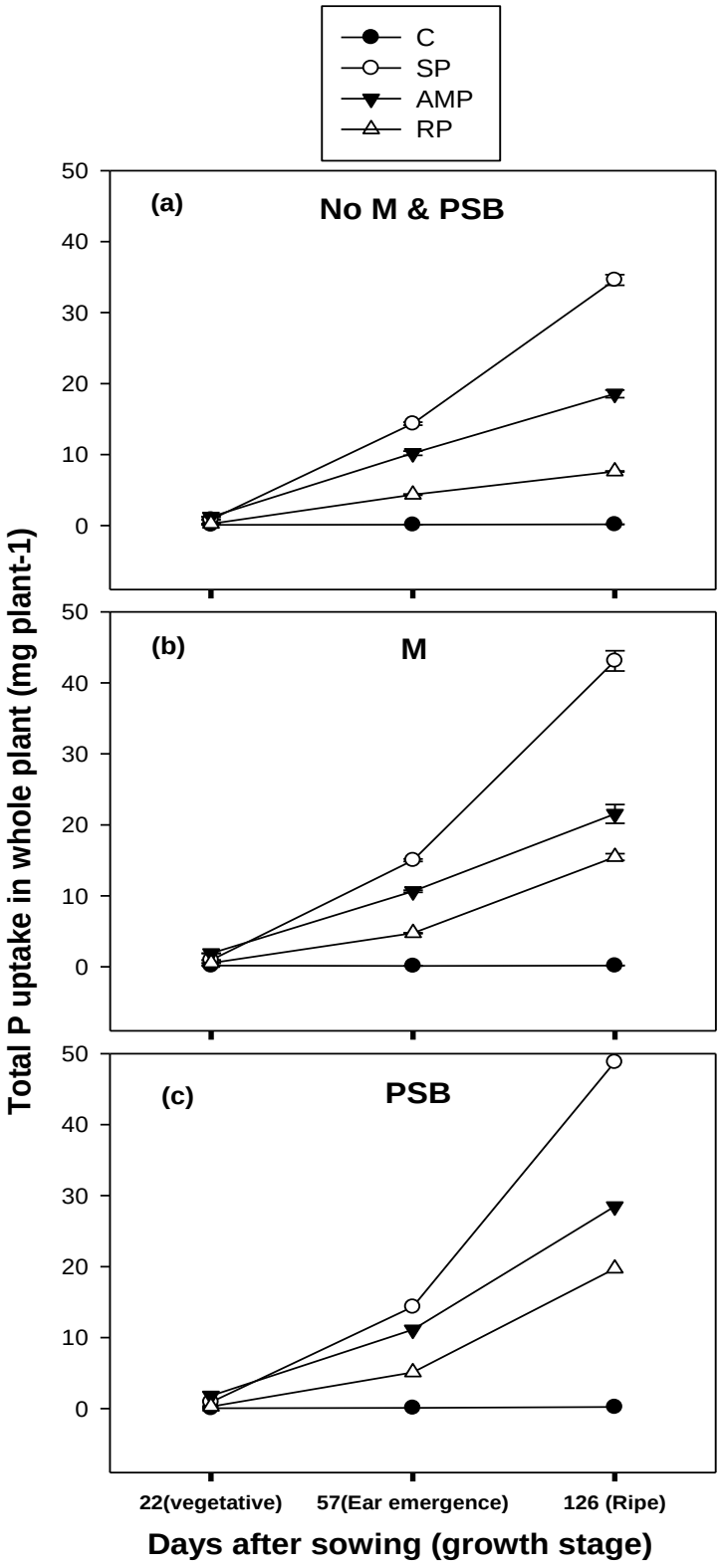


Fig. 4.3.1.12. Mean values of total plant P uptakeat vegetative stage(22 das), ear emergence (57 das), and Ripe stage (126 das). (a) Effect of NBP on total P uptake of plant; (b) Effect of NBP with M on total P uptake of plant; (c) Effect of NBP with PSB on total P uptake of plant. Error bars show standard error of means (n=3).

Table 4.3.1.16. Table of **s**tandard errors ofdifference and P values for data presented in Figure 4.3.1.12. Significant levels of P are in bold script.

Treatment	Total P uptake in whole plant (mg plant^{-1})					
	Sample 1 (Vegetation stage)(a)		Sample 2 (Ear emergence)(b)		Sample 3 (Ripe stage)(c)	
	SED	P	SED	P	SED	P
Non-Biological P	0.0203	**<.001**	0.1463	**<.001**	0.62	**<.001**
Biological P	0.0176	**<.001**	0.1267	**0.006**	0.537	**<.001**
Non-Biological P* Biological P	0.0352	**<.001**	0.2534	**0.026**	1.074	**<.001**

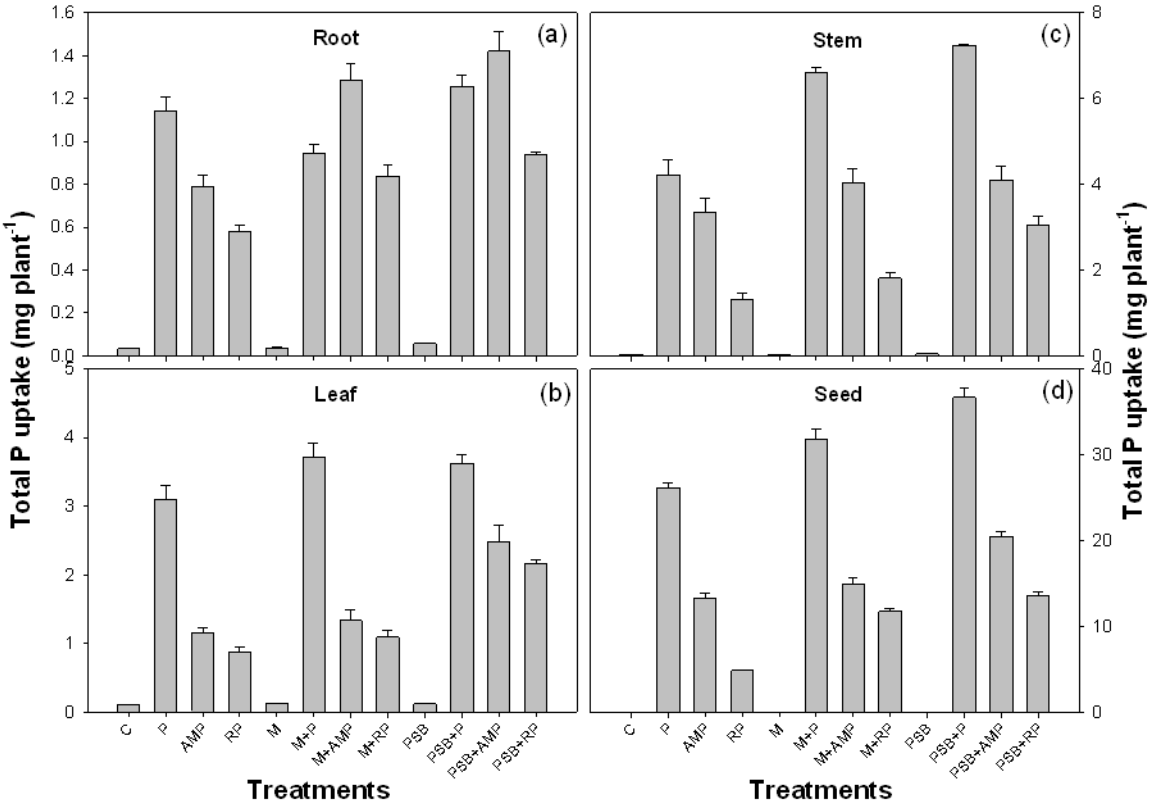


Fig. 4.3.1.13. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on final total P uptake in root(a), final total P uptake in leaf(b),final total P uptake in stem(c) and final total P uptake in seed(d). Error bars show standard error of means (n=3).

Table 4.3.1.17. Table of standard errors of difference and P values for data presented in Figure 4.3.1.13. Significant levels of P are in bold script.

Treatment	Total P uptake in final sample (mg plant^{-1})							
	Root (a)		**Stem** (b)		**Leaf** (c)		**Seed** (d)	
	SED	P	SED	P	SED	P	SED	P
Non-Biological P	0.0398	**<.001**	0.1661	**<.001**	0.1111	**<.001**	0.518	**<.001**
Biological P	0.0345	**<.001**	0.1439	**<.001**	0.0962	**<.001**	0.449	**<.001**
Non-Biological P* Biological P	0.0689	**<.001**	0.2878	**<.001**	0.1925	**<.001**	0.898	**<.001**

4.3.1.10. P& N concentration and content in grain

The final P and N concentration in grain was significantly affected by NBP, BP and interactions of NBP and BP treatments. Also the final P and N content of grain was significantly affected by NBP, BP and interactions of NBP and BP treatments (Table 4.3.1.18).

The highest and lowest P and N concentrationsin grain were in the SP and RP treatments respectively (Fig 4.3.1.14a). Also the highest and lowest P and N content of grain weresimilarly in the SP and RP treatments respectively (Fig 4.3.1.14b).

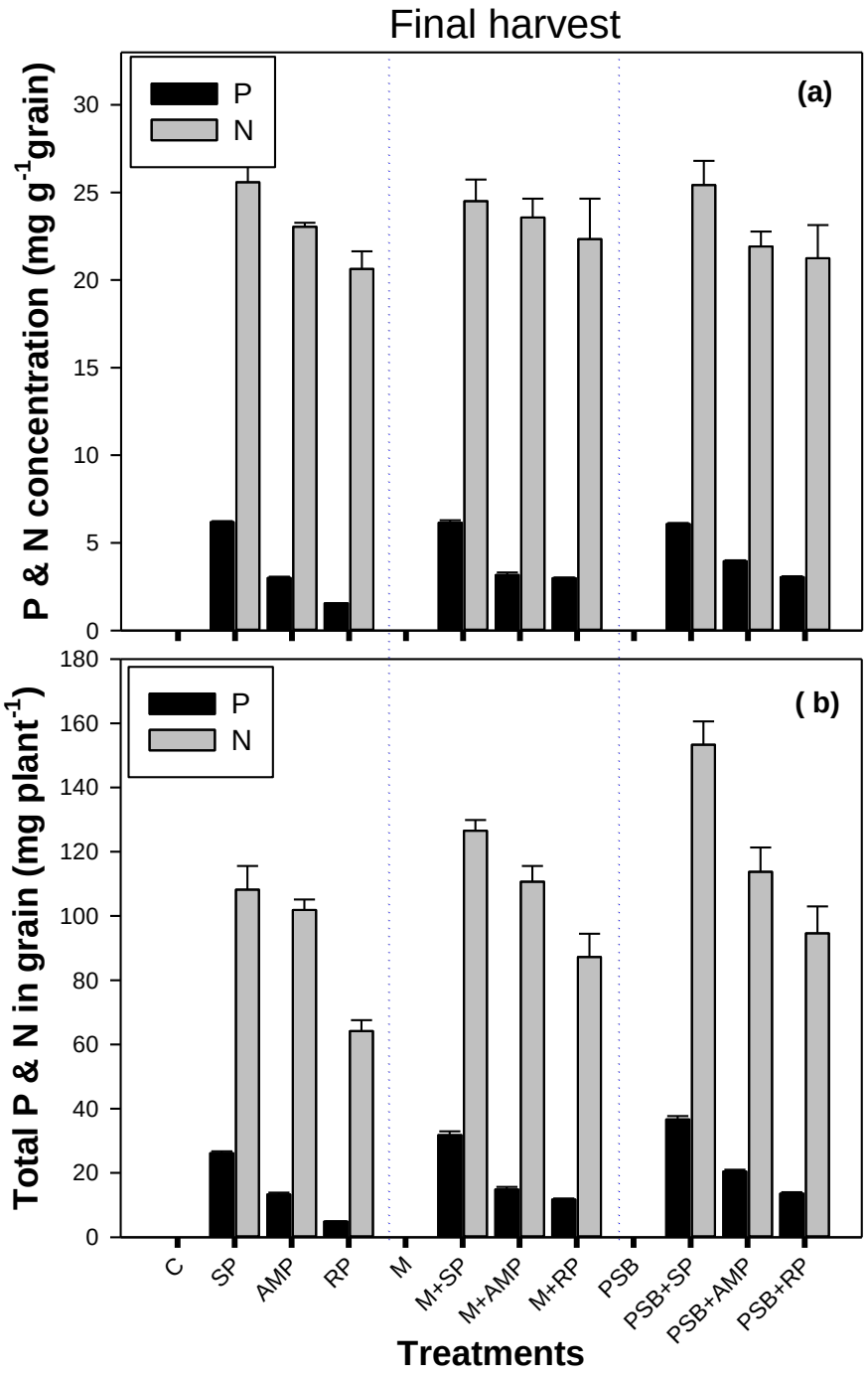


Fig. 4.3.1.14. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on P & N concentration(a) and P &N content(b). Error bars show standard error of means (n=3).

Table 4.3.1.18. Table of standard errors of difference and P values for data presented in Figure 4.3.1.14. Significant levels of P are in bold script.

Treatment	P & N concentration (mg g^{-1} grain) (a)				P &N content in grain (mg $plant^{-1}$) (b)			
	P concentration		N concentration		P content		N content	
	SED	P	SED	P	SED	P	SED	P
Non-Biological P	0.059	**<.001**	1.020	**<.001**	0.518	**<.001**	4.57	**<.001**
Biological P	0.051	**<.001**	0.884	0.874	0.449	**<.001**	3.96	**<.001**
Non-Biological P* Biological P	0.101	**<.001**	1.767	0.909	0.898	**<.001**	7.91	**0.015**

4.3.1.11. 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 (Fig 4.3.1.15).

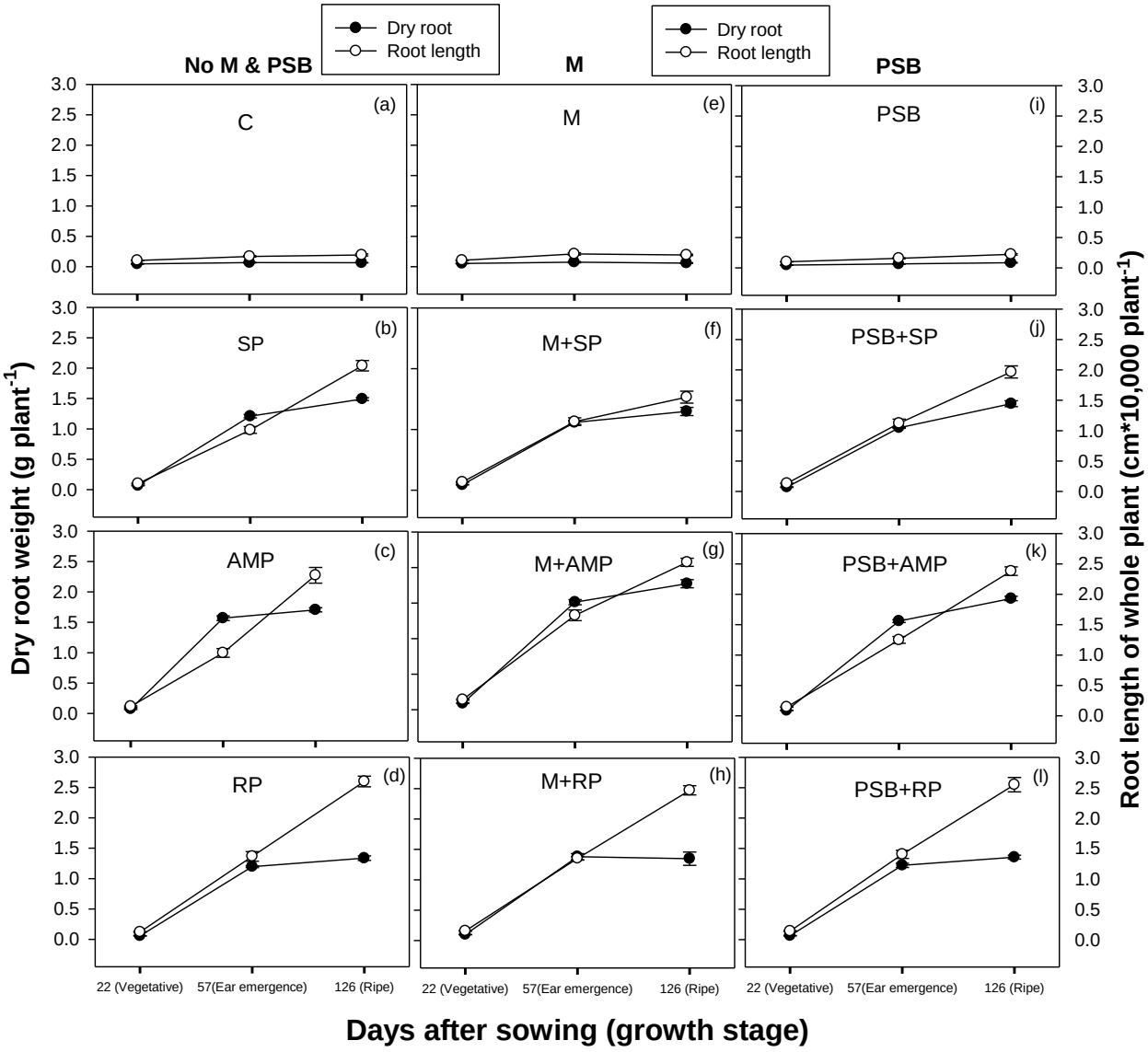


Fig. 4.3.1.15. Mean values of total dry root weight and total root length of plant at vegetative stage (22 das), ear emergence (57 das), and ripe stage (126 das). (a) Effect of no P on dry root weight and root length; (b) Effect of SP on dry root weight and root length; (c) Effect of AMP on dry root 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).

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

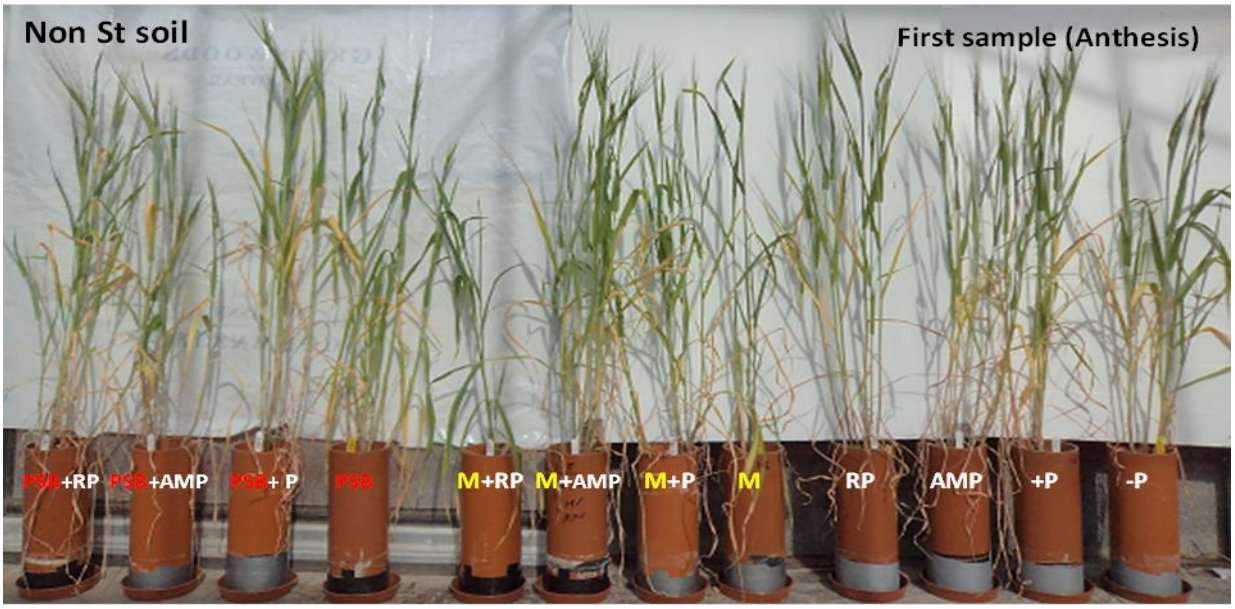


Fig.4.3.2.1. First Sample: Shoots in unsterilized soil, 74 das (anthesis stage).

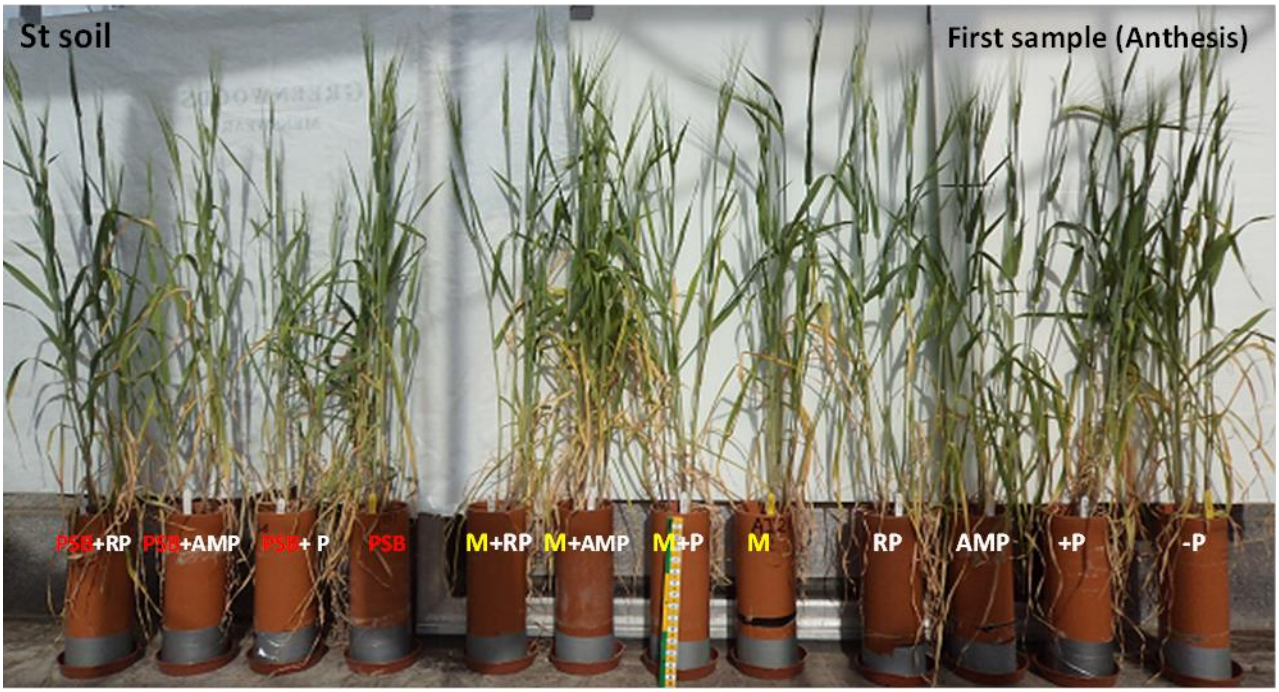


Fig.4.3.2.2. First Sample: Shoots in sterilized soil, 74 das (anthesis stage).

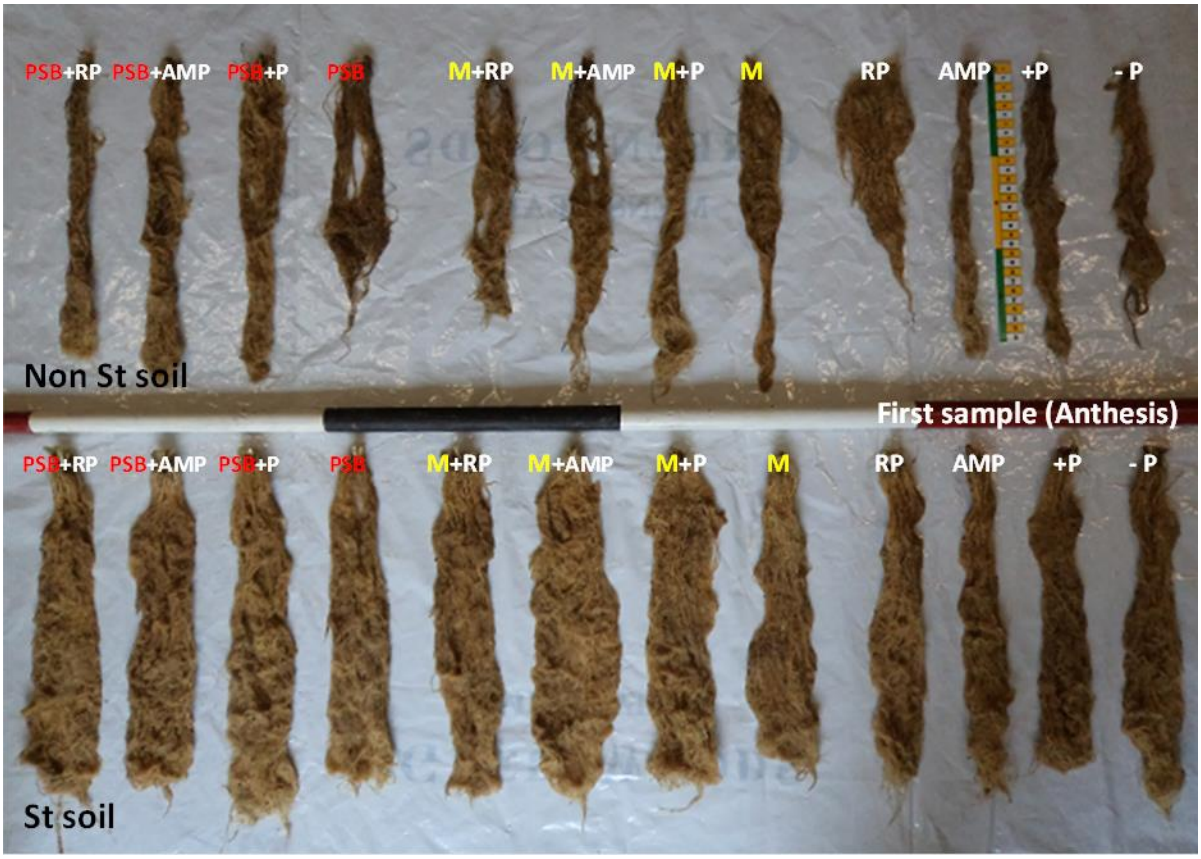


Fig.4.3.2.3. First Sample: Roots in unsterilized and sterilized soil, 74 das (anthesis stage).

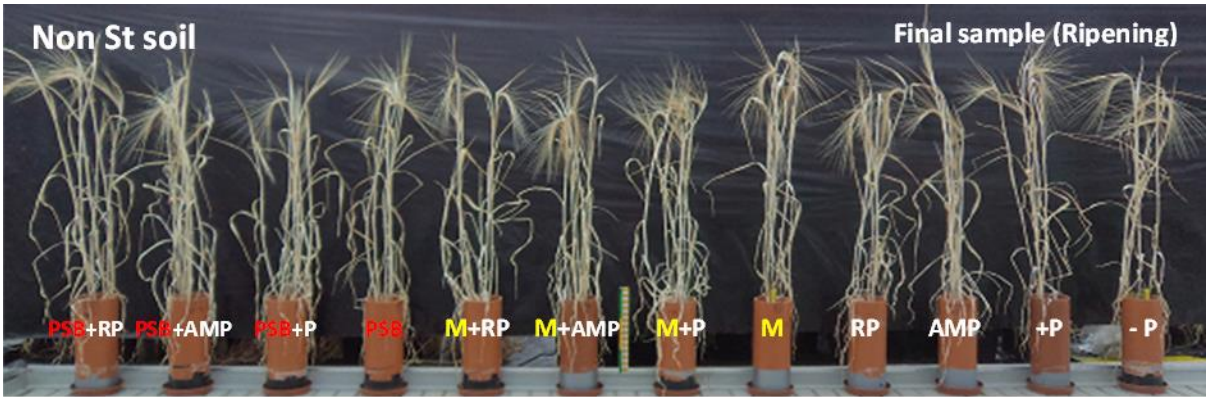


Fig.4.3.2.4. Second Sample: Shoots in unsterilized soil, 120 das (ripe stage).

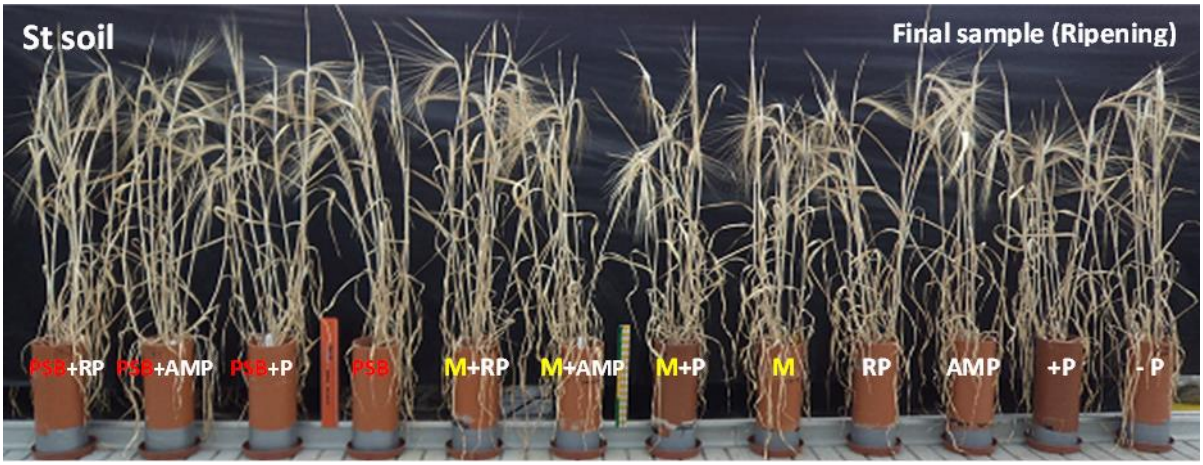


Fig.4.3.2.5. Second Sample: Shoots in sterilized soil, 120 das (ripe stage).

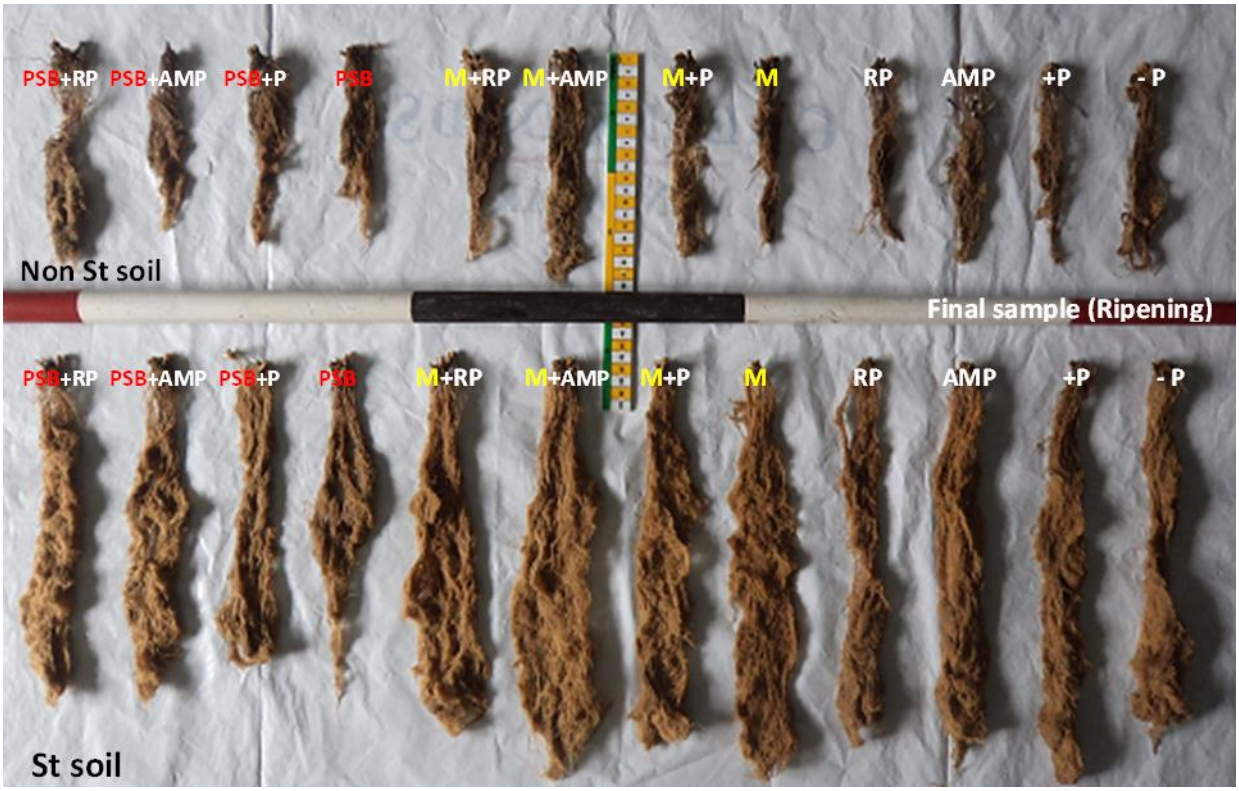


Fig.4.3.2.6. Second Sample: roots in unsterilized and sterilized soil, 120 das (ripe stage).

4.3.2.1. Root and shoot dry matter and root:shoot ratio

The clearest treatment effect was that of sterilizing soil on shoot and root dry weights, as depicted visually in Fig 4.4.2.1, 4.3.2.2 and 4.3.2. 3 (first sample) and also 4.3.2.4 and 4.3.2.6 in the second sample (Fig 4.3.2.7a & b). There were also clear root:shoot effect (Fig 4.3.2.7b). Some non-biological P effects were also evident (Table 4.4.2.1) in sample 1 and 2.

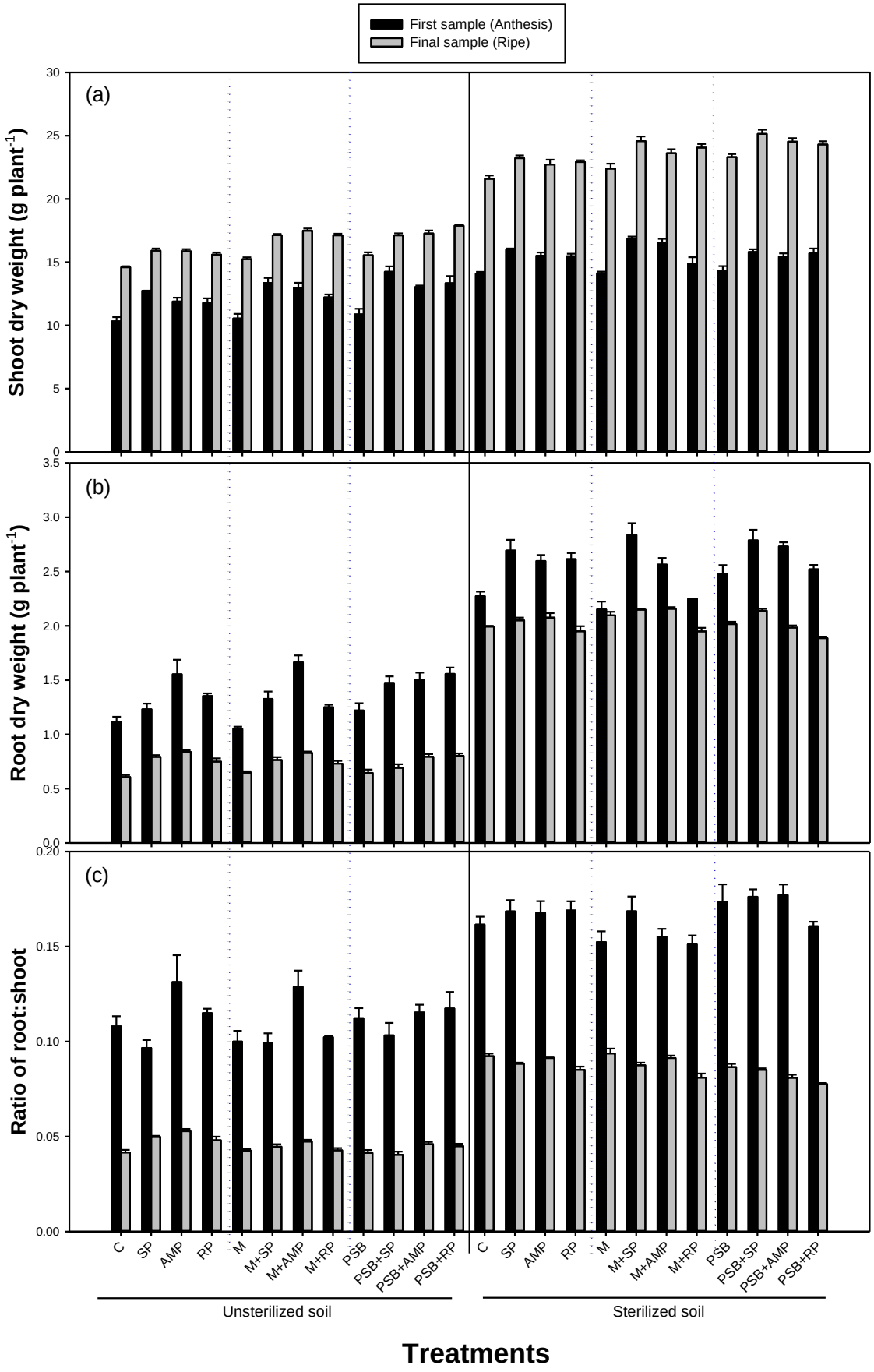

First sample (Anthesis)
Final sample (Ripe)
(a)
Shoot dry weight (g plant-1)
(b)
Root dry weight (g plant-1)
(c)
Ratio of root:shoot
C
SP
AMP
RP
M
M+SP
M+AMP
M+RP
PSB
PSB+SP
PSB+AMP
PSB+RP
Unsterilized soil
Sterilized soil
Treatments

Fig. 4.3.2.7. 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 shoot dry weight(a), root dry weight(b) and ratio of root:shoot(b) in unsterilized soil and sterilized soil in the anthesis and ripe stage. Error bars show standard error of means (n=3).

Table 4.3.2.1. Table of standard errors of difference and P values for data presented in Figure 4.3.2.6. Significant levels of P are in bold script.

Treatment	Shoot dry weight (g plant^{-1}) (a)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.568	**<.001**	1.216	0.467
Biological P	0.492	0.429	1.053	0.308
Soil	0.696	**<.001**	1.489	**<.001**
Non-Biological P* Biological P	0.984	0.961	2.106	1.000
Non-Biological P*Soil	0.984	0.987	2.106	0.998
Biological P*Soil	0.899	0.978	1.922	0.997
Non-Biological P* Biological P*Soil	1.504	0.997	3.217	1.000
Treatment	**Root dry weight (g plant^{-1})** (b)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.207	0.251	0.223	0.939
Biological P	0.179	0.704	0.193	0.971
Soil	0.254	**<.001**	0.273	**<.001**
Non-Biological P* Biological P	0.359	0.992	0.386	1.000
Non-Biological P*Soil	0.359	0.985	0.386	0.998
Biological P*Soil	0.327	0.974	0.352	0.999
Non-Biological P* Biological P*Soil	0.548	1.000	0.589	1.000
Treatment	**Ratio of root:shoot** (c)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.010	0.618	0.007	0.919
Biological P	0.008	0.485	0.006	0.653
Soil	0.012	**<.001**	0.009	**<.001**
Non-Biological P* Biological P	0.017	0.99	0.013	1.000
Non-Biological P*Soil	0.017	0.905	0.013	0.991
Biological P*Soil	0.015	0.914	0.012	0.997
Non-Biological P* Biological P*Soil	0.026	0.994	0.019	1.000

4.3.2.2. Ear, leaf and stem dry matter

The ear dry weight and stem dry weight were significantly increased by NBP and soil treatments in the first sample and also by soil treatments in the second sample (Table 4.3.2.2 e & d). The leaf dry weight was significantly affected by soil treatments in the first and second samples (Table 4.3.2.2f). The clearest treatment effect was that of sterilizing soil on leaf and ear dry weights, as showed visually in Fig 4.3.2.8 (d & e).

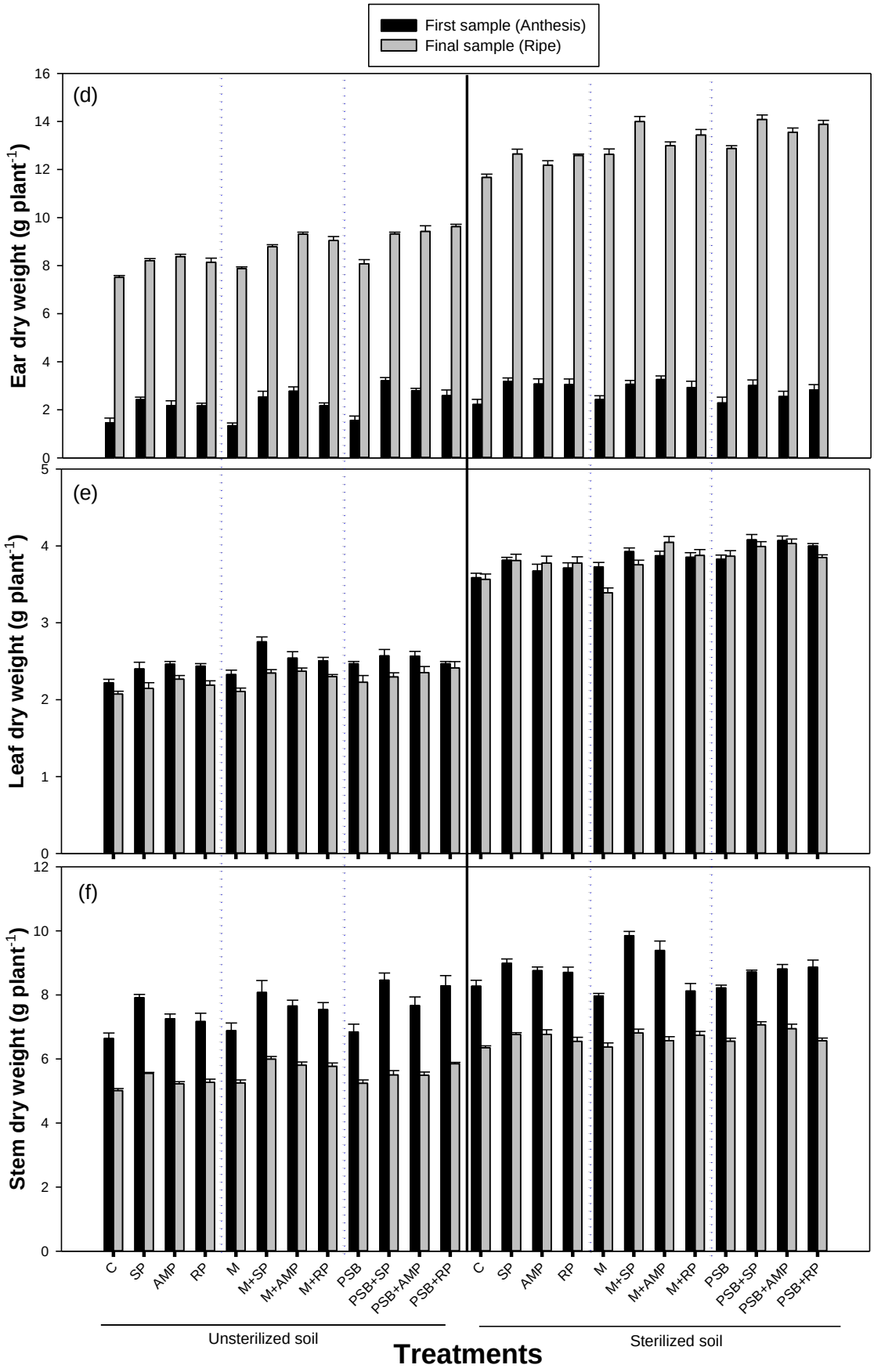

First sample (Anthesis)
Final sample (Ripe)
(d)
Ear dry weight (g plant-1)
(e)
Leaf dry weight (g plant-1)
(f)
Stem dry weight (g plant-1)
C
SP
AMP
RP
M
M+SP
M+AMP
M+RP
PSB
PSB+SP
PSB+AMP
PSB+RP
Unsterilized soil
Sterilized soil
Treatments

Fig. 4.3.2.8. 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 ear dry weight(d), leaf dry weight(e) and stem dry weight(f) in unsterilized soil and sterilized soil in the anthesis and ripe stage. Error bars show standard error of means (n=3).

Table 4.3.2.2. Table of standard errors of difference and P values for data presented in Figure 4.3.2.7. Significant levels of P are in bold script.

Treatment	Ear dry weight (g plant^{-1}) (d)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.1295	**<.001**	0.7560	0.465
Biological P	0.1121	0.475	0.6550	0.185
Soil	0.1586	**0.001**	0.9260	**<.001**
Non-Biological P* Biological P	0.2242	0.503	1.3090	1.000
Non-Biological P*Soil	0.2242	0.723	1.3090	0.996
Biological P*Soil	0.2047	0.572	1.1950	0.987
Non-Biological P* Biological P*Soil	0.3425	0.977	2.0000	1.000
Treatment	**Leafdry weight (g plant^{-1})** (e)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.2356	0.791	0.2663	0.775
Biological P	0.2041	0.556	0.2306	0.742
Soil	0.2886	**<.001**	0.3262	**<.001**
Non-Biological P* Biological P	0.4081	1.000	0.4612	0.999
Non-Biological P*Soil	0.4081	0.998	0.4612	0.997
Biological P*Soil	0.3726	0.896	0.4211	0.980
Non-Biological P* Biological P*Soil	0.6234	1.000	0.7046	1.000
Treatment	**Stem dry weight (g plant^{-1})** (f)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.2539	**<.001**	0.2093	0.141
Biological P	0.2199	0.432	0.1813	0.376
Soil	0.3110	**0.001**	0.2564	**<.001**
Non-Biological P* Biological P	0.4398	0.614	0.3625	0.999
Non-Biological P*Soil	0.4398	0.994	0.3625	0.997
Biological P*Soil	0.4015	0.966	0.331	0.898
Non-Biological P* Biological P*Soil	0.666	0.977	0.579	0.989

4.3.2.3. Phosphorus concentration in the plant

The most significant effect of treatments on P concentration of plant parts were affected by soil and NBP treatments on leaf and stem in the first sample (Fig 4.3.2.9 b and c) and (Table 4.3.2.3). The results also showed significant effect of NBP, BP and soil treatments on P concentration of root and ear in the second sample (Fig 4.3.2.9a and d) and (Table 4.3.2.3).

On the whole, more effect of biological sources of P than soil sterilization on P concentrations of plant parts.

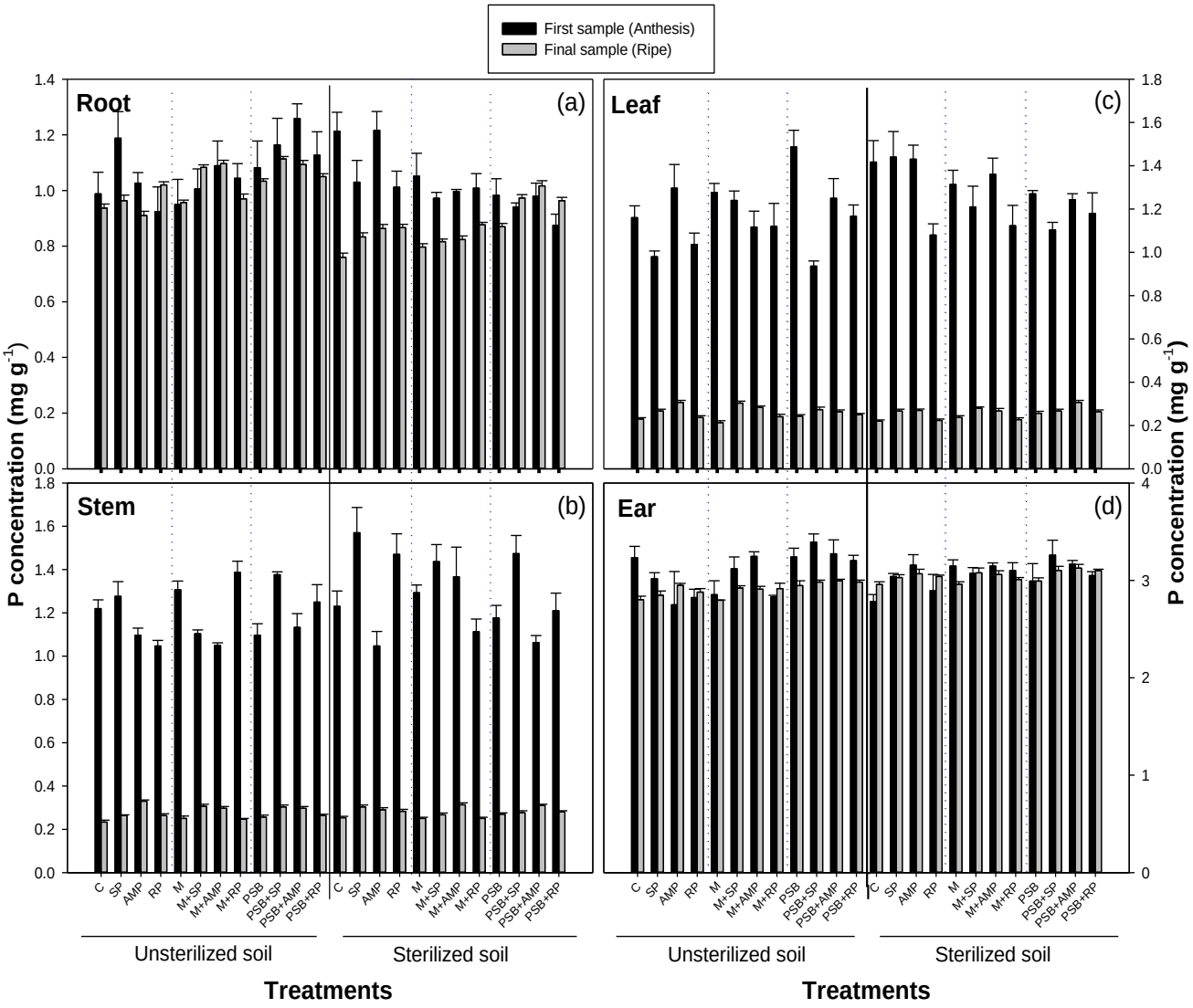


Fig. 4.3.2.9. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on P concentration of root(a), stem(b), leaf(c) and ear(d) in unsterilized soil and sterilized soil in the anthesis and ripe stage. Error bars show standard error of means (n=3).

Table 4.3.2.3. Table of standard errors of difference and P values for data presented in Figure 4.3.2.8. Significant levels of P are in bold script.

Treatment	P concentration in root (mg g^{-1} root)(a)			
	Sample 1 (Anthesis)		Sample 1 (Anthesis)	
	SED	SED	SED	SED
Non-Biological P	0.0449	0.218	0.0296	**0.045**
Biological P	0.0389	0.307	0.0256	**<.001**
Soil	0.0550	0.530	0.0362	**<.001**
Non-Biological P* Biological P	0.0777	0.732	0.0512	0.814
Non-Biological P*Soil	0.0777	0.701	0.0512	0.936
Biological P*Soil	0.0710	0.393	0.0468	0.767
Non-Biological P* Biological P*Soil	0.1187	0.657	0.0783	0.994
Treatment	**P concentration in stem (mg g^{-1} stem)** (b)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.0502	**<.001**	0.00624	**<.001**
Biological P	0.0434	0.724	0.00541	0.219
Soil	0.0614	**0.025**	0.00765	0.828
Non-Biological P* Biological P	0.0869	0.151	0.01082	0.271
Non-Biological P*Soil	0.0709	0.104	0.01082	0.871
Biological P*Soil	0.0614	0.232	0.00987	0.604
Non-Biological P* Biological P*Soil	0.1229	0.676	0.01652	0.373
Treatment	**P concentration in leaf (mg g^{-1} leaf)** (c)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.0480	**<.001**	0.0056	**<.001**
Biological P	0.0416	0.828	0.0049	**0.032**
Soil	0.0588	**0.002**	0.0069	0.339
Non-Biological P* Biological P	0.0832	0.057	0.0098	**0.008**
Non-Biological P*Soil	0.0832	0.909	0.0098	0.062
Biological P*Soil	0.0760	0.200	0.0089	0.242
Non-Biological P* Biological P*Soil	0.1271	0.570	0.0149	0.359
Treatment	**P concentration in ear (mg g^{-1} ear)** (d)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.0715	0.092	0.0303	**0.005**
Biological P	0.0619	**0.002**	0.0262	**0.005**
Soil	0.0875	0.106	0.0371	**0.005**
Non-Biological P* Biological P	0.1238	0.756	0.0524	0.951
Non-Biological P*Soil	0.1238	0.270	0.0524	0.962
Biological P*Soil	0.1130	0.242	0.0479	0.458
Non-Biological P* Biological P*Soil	0.1891	0.388	0.0801	0.828

4.3.2.4. Total P uptake in root, stem and leaf

The total P uptake of roots was significantly increased by soil treatments in the first and secondsamples (Table 4.3.2.4a)and (Fig 4.3.2.10a). The total P uptake of stem was significantly affected by soil and NBP treatments in the first and second samples (Table 4.3.2.4b)and (Fig 4.3.2.9b). The total P uptake of leaf was significantly affected by NBP treatments in the second sample, and significantly affected by soil treatments in the first and second samples (Table4.3.2.4c) and (Fig 4.3.2.10c)

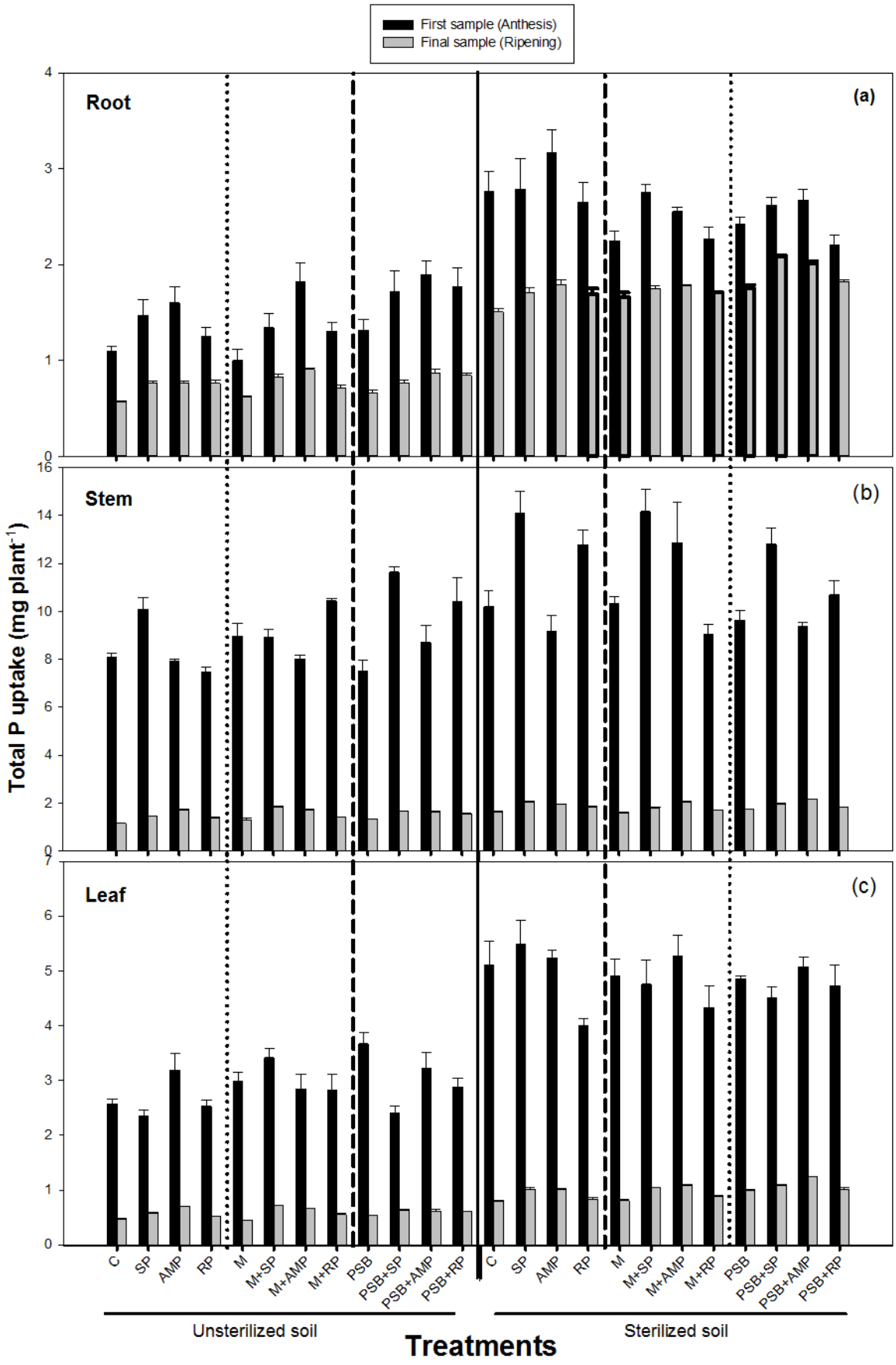

First sample (Anthesis)
Final sample (Ripening)
Root
(a)
Stem
(b)
Leaf
(c)
Total P uptake (mg plant-1)
C
SP
AMP
RP
M
M+SP
M+AMP
M+RP
PSB
PSB+SP
PSB+AMP
PSB+RP
Unsterilized soil
Sterilized soil
Treatments

Fig. 4.3.2.10. 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 root(a), stem(b), leaf(c) in unsterilized soil and sterilized soil in the anthesis and ripe stage. Error bars show standard error of means (n=3).

Table 4.3.2.4. Table of standard errors of difference and P values for data presented in Figure 4.3.2.9. Significant levels of P are in bold script.

Treatment	P uptake in root (mg plant^{-1}) (a)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.2158	0.133	0.1748	0.599
Biological P	0.1869	0.557	0.1514	0.575
Soil	0.2643	**<.001**	0.2140	**<.001**
Non-Biological P* Biological P	0.3738	1.000	0.3027	1.000
Non-Biological P*Soil	0.3738	0.923	0.3027	0.999
Biological P*Soil	0.3412	0.832	0.2763	0.949
Non-Biological P* Biological P*Soil	0.5710	0.995	0.4624	1.000

Treatment	P uptake in stem (mg plant^{-1}) (b)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.626	**<.001**	0.0664	**<.001**
Biological P	0.542	0.792	0.0575	0.325
Soil	0.767	**0.002**	0.0814	**<.001**
Non-Biological P* Biological P	1.085	0.570	0.1151	0.953
Non-Biological P*Soil	1.085	0.358	0.1151	0.995
Biological P*Soil	0.990	0.480	0.105	0.810
Non-Biological P* Biological P*Soil	1.657	0.922	0.1758	0.986

Treatment	P uptake in leaf (mg plant^{-1}(C)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.346	0.352	0.0704	**0.016**
Biological P	0.300	0.921	0.0610	0.283
Soil	0.424	**<.001**	0.0863	**<.001**
Non-Biological P* Biological P	0.600	0.872	0.1220	0.980
Non-Biological P*Soil	0.600	0.959	0.1220	0.981
Biological P*Soil	0.547	0.824	0.1114	0.896
Non-Biological P* Biological P*Soil	0.916	0.982	0.1864	1.000

4.3.2.5. Total P uptake in ear, shoot and whole plant

The total P uptake of the ear was significantly increased by NBP treatments in the first sample, and significantly affected by soil treatments in the first and second samples (Table 4.3.2.5d). The total P uptake of the shoot was significantly affected by the soil treatments in the first and second samples (Table 4.3.2.5e). The total P uptake of the whole plant was significantly affected by NBP treatments in the first sample, and significantly affected by soil treatments in the first and second samples (Fig 4.3.2.11f). The highest ear P uptake of the first sample and second sample was increased most by USS+M+AMP and USS+PSB+ SP treatments (Fig 4.3.2.11d). The highest shoot P uptake of the first sample and second sample was affected by USS+SP and USS+PSB+SP treatment (Fig 4.3.2.11e). The highest leaf P uptake of first sample and second sample was affected by USS+SP and USS+PSB+AMP treatments respectively (Fig 4.3.2.11f).

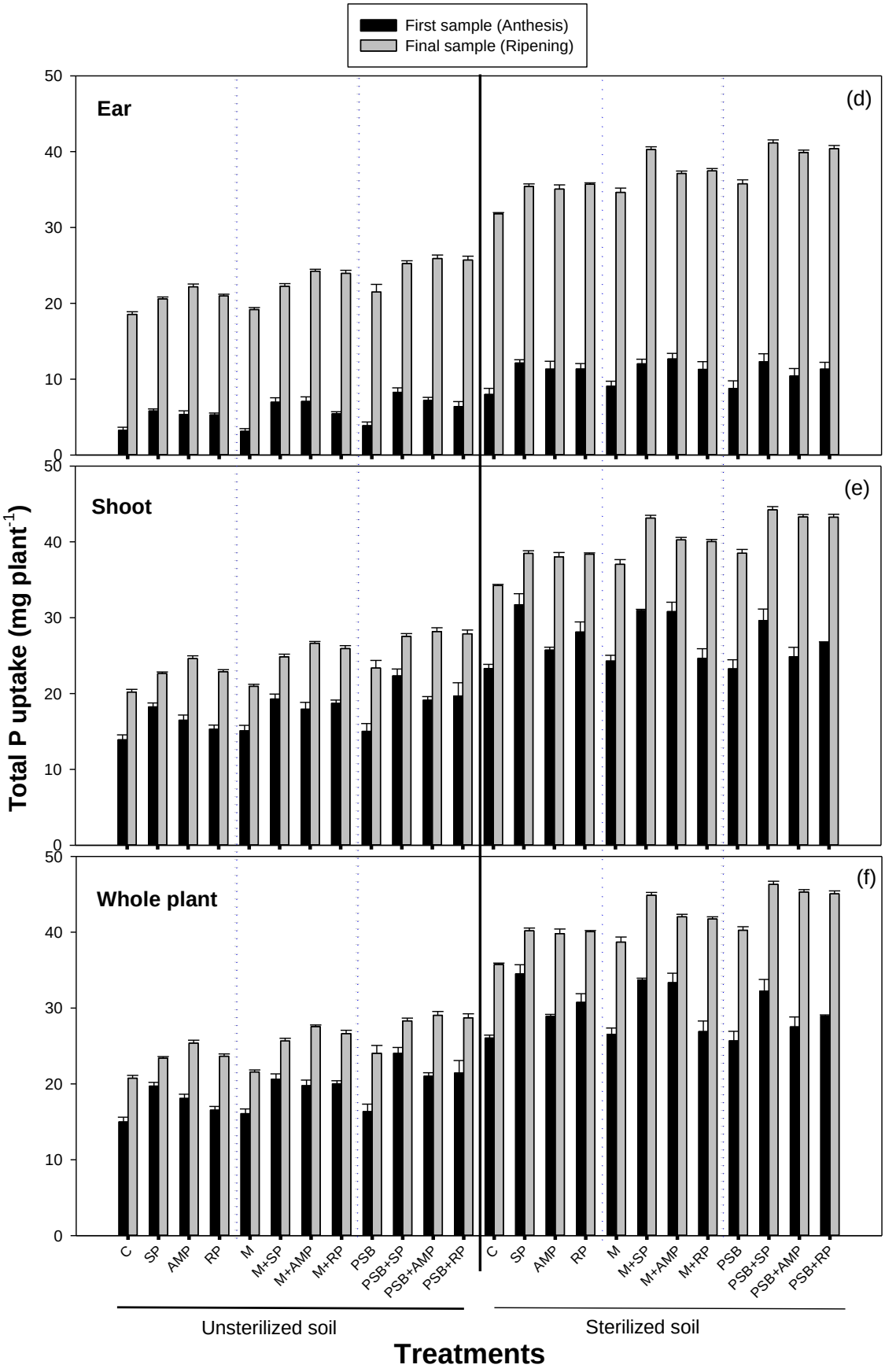

First sample (Anthesis)
Final sample (Ripening)
Ear
(d)
Shoot
(e)
Whole plant
(f)
Total P uptake (mg plant-1)
0
10
20
30
40
50
C
SP
AMP
RP
M
M+SP
M+AMP
M+RP
PSB
PSB+SP
PSB+AMP
PSB+RP
Unsterilized soil
Sterilized soil
Treatments

Fig. 4.3.2.11. 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).

Table 4.3.2.5. Table of standard errors of difference and P values for data presented in Figure 4.3.2.10. Significant levels of P are in bold script.

Treatment	P uptake in ear (mg plant^{-1}) (d)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.930	**0.002**	2.520	0.333
Biological P	0.806	0.600	2.182	0.141
Soil	1.139	**<.001**	3.086	**<.001**
Non-Biological P* Biological P	1.611	0.987	4.364	1.000
Non-Biological P*Soil	1.611	0.998	4.364	0.998
Biological P*Soil	1.471	0.991	3.984	0.987
Non-Biological P* Biological P*Soil	2.461	0.999	6.666	1.000

Treatment	P uptake in shoot (mg plant^{-1}) (e)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	1.683	**0.007**	2.643	0.275
Biological P	1.457	0.707	2.289	0.144
Soil	2.061	**<.001**	3.237	**<.001**
Non-Biological P* Biological P	2.915	0.970	4.578	1.000
Non-Biological P*Soil	2.915	0.950	4.578	0.999
Biological P*Soil	2.661	0.906	4.179	0.989
Non-Biological P* Biological P*Soil	4.452	0.999	6.993	1.000

Treatment	P uptake in whole plant (mg plant^{-1}) (f)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	1.876	**0.012**	2.816	0.293
Biological P	1.625	0.799	2.438	0.161
Soil	2.298	**<.001**	3.448	**<.001**
Non-Biological P* Biological P	3.250	0.983	4.877	1.000
Non-Biological P*Soil	3.250	0.962	4.877	0.999
Biological P*Soil	2.967	0.945	4.452	0.993
Non-Biological P* Biological P*Soil	5.076	1	7.96	1

4.3.2.6. Root colonization

There was no root colonization by mycorrhizae in the non-inoculated sterilized soil, whereas all treatments showed colonization of barley roots in non-sterilized soil.Results of root staining showed that the highest percentage of root colonization was achieved by control (no P fertilization) and M+RP treatment in the growth stages of plant. By contrast, the lowest percentage of root colonization were observed in the M with SP and AMP treatments.

The root colonization was significantly increased by NBP, BP and soil treatments in the first and second samples (Table 4.3.2.6). The highest root colonization of the first and second samples was affected by USS+M+RP and USS+M treatments respectively (Fig 4.3.2.12).

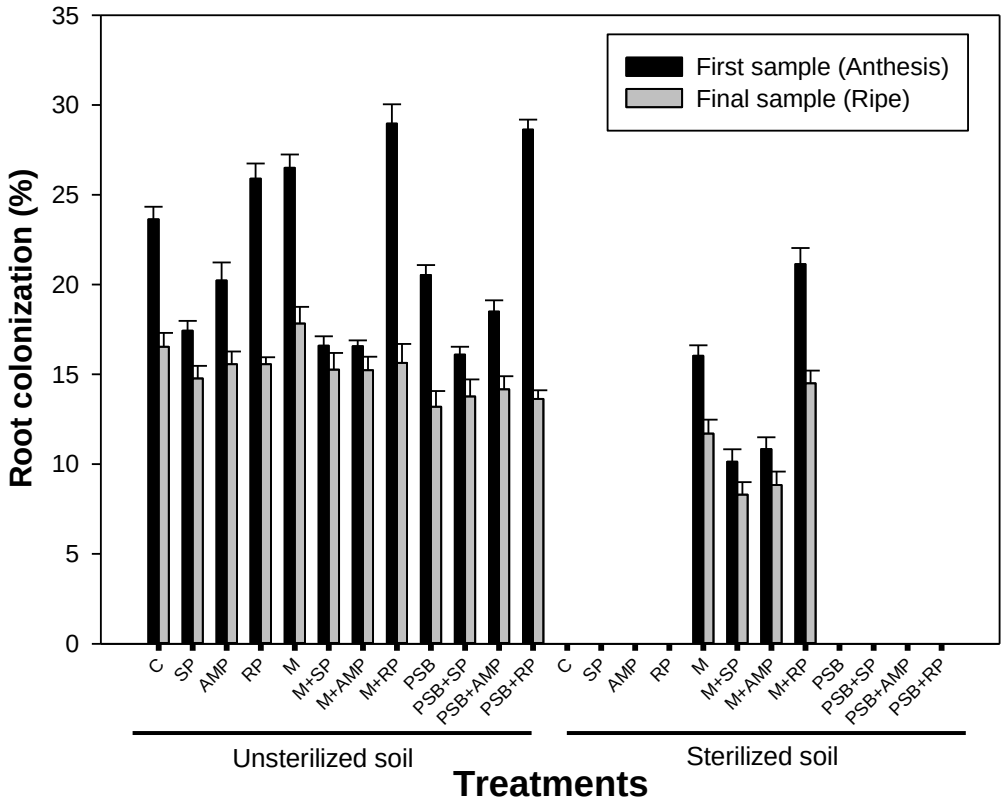


Fig. 4.3.2.12. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on root colonizationin unsterilized soil and sterilized soil in the anthesis and ripe stage. Error bars show standard error of means (n=3).

Table 4.3.2.6. Table of standard errors of difference and P values for data presented in Figure 4.4.2.11. Significant levels of P are in bold script.

Treatment	Root colonization (%) (a)			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	0.325	**<.001**	0.329	**<.001**
Biological P	0.281	**<.001**	0.285	**<.001**
Soil	0.23	**<.001**	0.233	**<.001**
Non-Biological P* Biological P	0.563	**<.001**	0.57	**<.001**
Non-Biological P*Soil	0.46	**<.001**	0.465	**0.014**
Biological P*Soil	0.398	**<.001**	0.403	**<.001**
Non-Biological P* Biological P*Soil	0.796	**<.001**	0.806	**0.002**

Treatment	Root colonization (%) (Unsterilized soil)			
	Sample 1 (Anthesis)	Sample 2 (Ripe stage)	Sample 1 (Anthesis)	Sample 2 (Ripe stage)
	SED	P	SED	P
Non-Biological P	0.554	**<.001**	0.549	0.16
Biological P	0.480	**0.052**	0.475	**<.001**
Non-Biological P*Biological P	0.960	**<.001**	0.951	0.222

Treatment	Root colonization (%)(Unsterilized soil)			
	Sample 1 (Anthesis)	Sample 2 (Ripe stage)	Sample 1 (Anthesis)	Sample 2 (Ripe stage)
	SED	P	SED	P
Non-Biological P	0.717	**<.001**	0.699	**<.001**
Soil	0.507	**<.001**	0.495	**<.001**
Non-Biological P * Soil	1.015	**0.025**	0.989	**0.003**

4.3.2.7. Grain yield

The grain yield was significantly increased only by soil treatments (Table 4.3.2.7). The highest and lowest grain yield were observed in the SS+PSB+SP and USS=control treatments respectively (Fig 4.3.2.13).

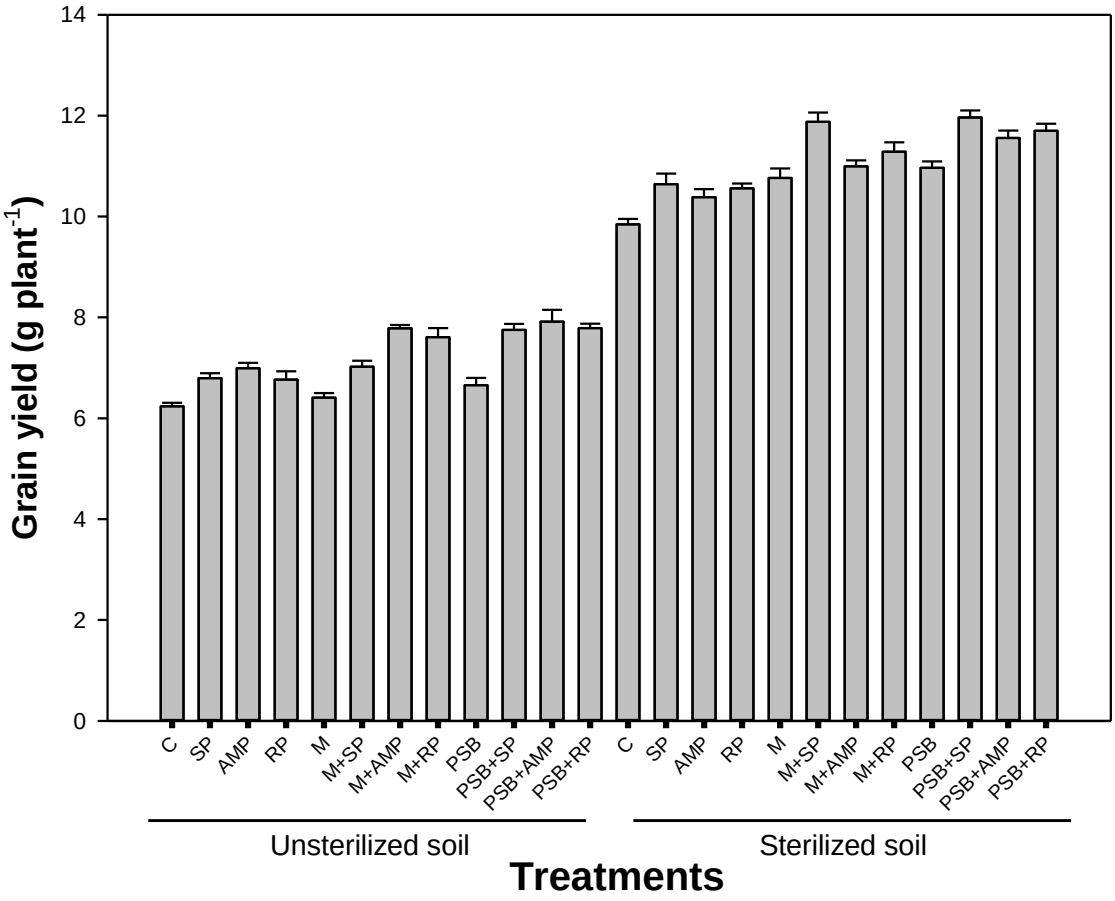


Fig. 4.3.2.13. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on grain yieldin unsterilized soil and sterilized soil.Error bars show standard error of means (n=3).

Table 4.3.2.7. Table of standard errors of difference and P values for data presented in Figure 4.3.2.12. Significant levels of P are in bold script.

Treatment	Grain yield (g plant^{-1})	
	SED	P
Non-Biological P	0.674	0.532
Biological P	0.584	0.220
Soil	0.826	**<.001**
Non-Biological P* Biological P	1.168	1.000
Non-Biological P*Soil	1.168	0.997
Biological P*Soil	1.066	0.976
Non-Biological P* Biological P*Soil	1.784	1.000

4.3.2.8. Leaf area index

The Leaf area index (LAI) was significantly increasedonly by soil treatments (Table 4.3.2.8). The highest and lowest LAI were found in theSS+AMP and USS+AMP treatments (Fig 4.3.2.14).

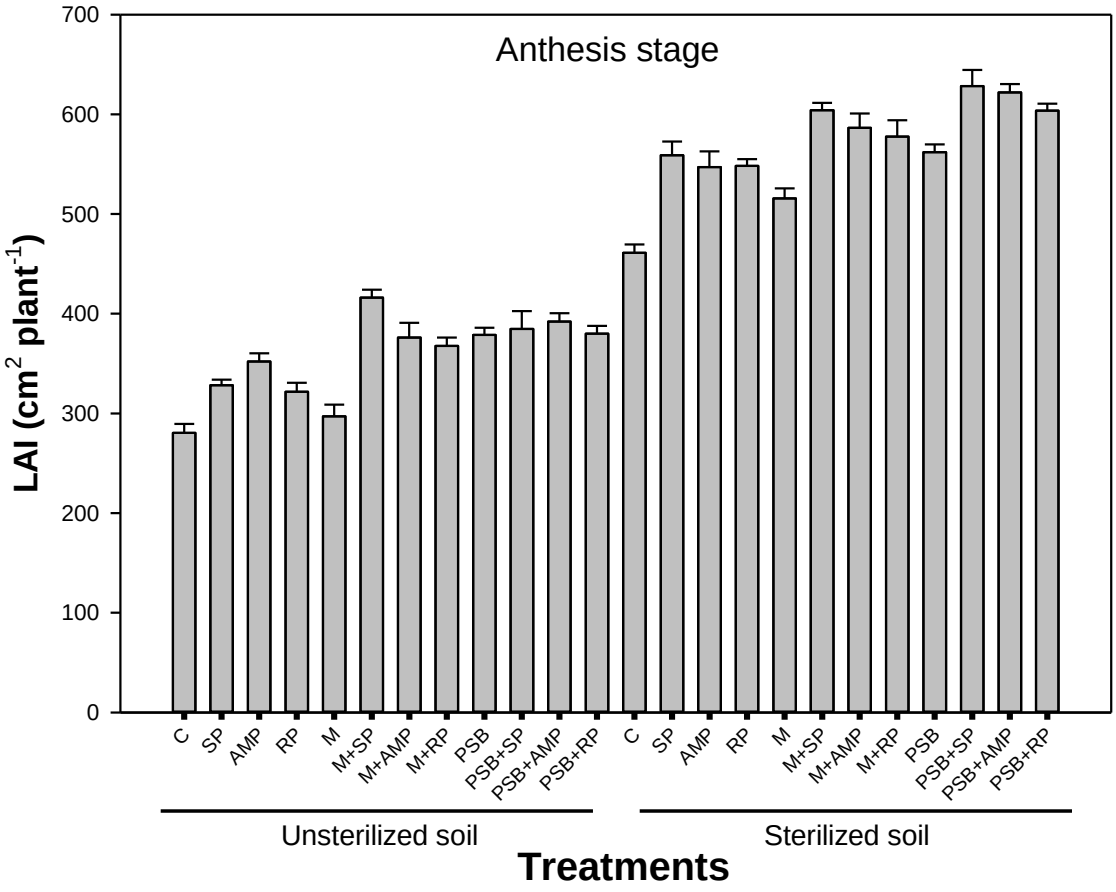


Fig. 4.3.2.14. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on LAI in unsterilized soil and sterilized soil. Error bars show standard error of means (n=3).

Table 4.3.2.8. Table of standard errors of difference and P values for data presented in Figure 4.3.2.13. Significant levels of P are in bold script.

Treatment	LAI (cm^2 $plant^{-1}$)	
	SED	P
Non-Biological P	36	0.207
Biological P	31.2	0.092
Soil	44.1	**<.001**
Non-Biological P* Biological P	62.3	0.993
Non-Biological P*Soil	62.3	0.997
Biological P*Soil	56.9	0.979
Non-Biological P* Biological P*Soil	95.2	1.000

4.3.2.9. Final P concentration and total P uptake in grain

The final P concentration of grain was significantly increasedby BP and soil treatments, and the final total P uptake of grain was significantly affected by soil treatments (Table 4.3.2.9). The highest and lowest P concentration of grain were in the SS+PSB+AMP and USS (control) treatments (Fig 4.3.2.15a), and the highest and lowest P uptake of grain was in the USS+PSB+SP and SS (control) treatments respectively (Fig 4.3.2.15b).

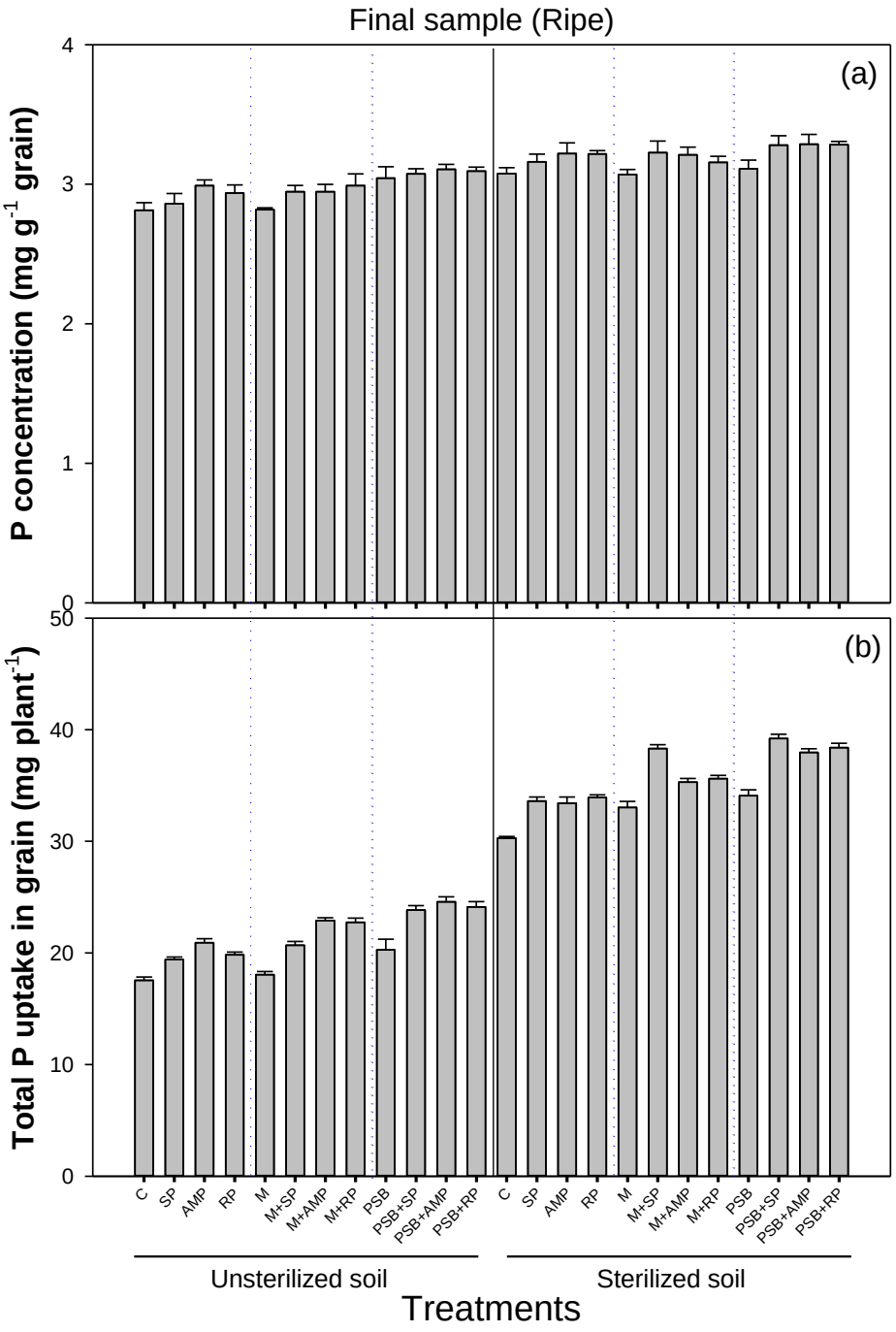


Fig. 4.3.2.15. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on P concentration of grain(a) and total P uptake of grain(b) in unsterilized soil and sterilized soil. Error bars show standard error of means (n=3).

Table 4.3.2.9. Table of standard errors of difference and P values for data presented in Figure 4.4.2.13. Significant levels of P are in bold script.

Treatment	P in Grain (final harvest)			
	P concentration (mg g^{-1}grain)(a)		Total P uptake (mg $plant^{-1}$) (b)	
	SED	P	SED	P
Non-Biological P	0.0506	**0.037**	2.434	0.359
Biological P	0.0438	0.011	2.108	0.150
Soil	0.062	**0.004**	2.982	**<.001**
Non-Biological P* Biological P	0.0876	0.989	4.217	1.000
Non-Biological P*Soil	0.0876	0.901	4.217	0.998
Biological P*Soil	0.08	0.395	3.849	0.987
Non-Biological P* Biological P*Soil	0.1338	0.865	6.441	1.000

4.4.2.10. Final N concentration and total N in grain

The final N concentration total N uptake in grain was significantly increasedby the soil treatments (Table 4.3.2.10). The highest and lowest N concentration of grain were in the SS+M+AMP and USS+M+AMP treatments (Fig 4.3.2.16a), and the highest and lowest total N in grain was affected by USS+PSB+AMP and SS (control) treatments respectively (Fig 4.3.2.16b).

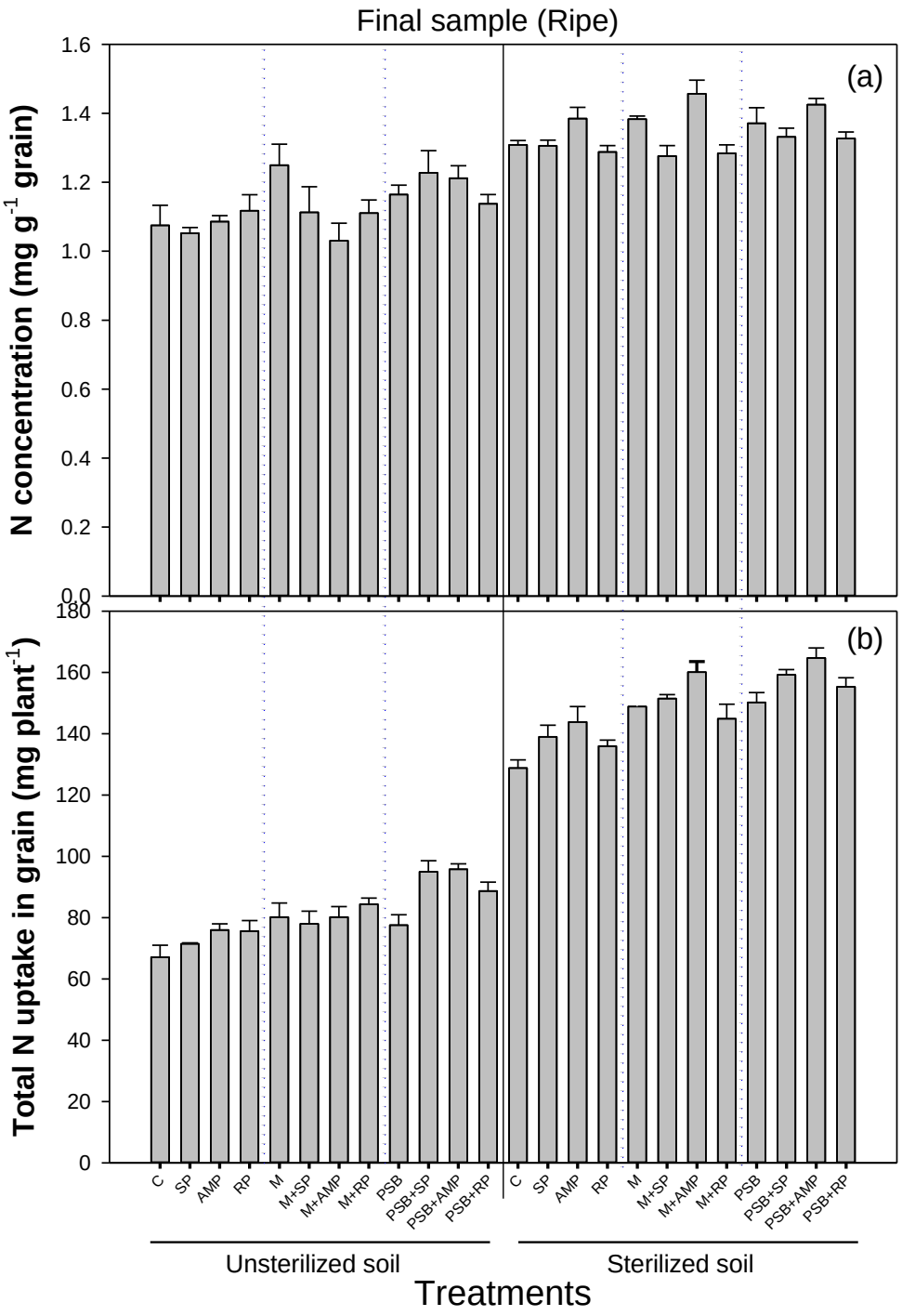


Fig. 4.3.2.16. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on N concentration of grain(a) and total N uptake of grain(b) in unsterilized soil and sterilized soil. Error bars show standard error of means (n=3).

Table 4.3.2.10. Table of standard errors of difference and P values for data presented in Figure 4.3.2.14. Significant levels of P are in bold script.

Treatment	N in Grain (final harvest)			
	N concentration (mg g^{-1}grain)		Total N uptake (mg $plant^{-1}$)	
	SED	P	SED	P
Non-Biological P	0.0442	0.497	11.73	0.813
Biological P	0.0382	0.179	10.16	0.194
Soil	0.0541	**<.001**	14.37	**<.001**
Non-Biological P* Biological P	0.0765	0.827	20.32	1.000
Non-Biological P*Soil	0.0765	0.667	20.32	0.988
Biological P*Soil	0.0698	0.923	18.55	0.995
Non-Biological P* Biological P*Soil	0.1168	0.94	31.03	1.000

4.3.2.11. Final P concentration in the soil

After harvesting the analysis of the remaining soil showed that the Olsen P concentration of the treatments soil was significantly increasedby the non-biological phosphorus (NBP) and interaction of non-biological phosphorus (NBP) with biological phosphorus (BP) treatments (Table 4.4.2.11). The highest and lowest Olsen P concentrations in pots soil were USS+PSB+RP and SS+PSB treatments respectively (Fig 4.4.2.17).

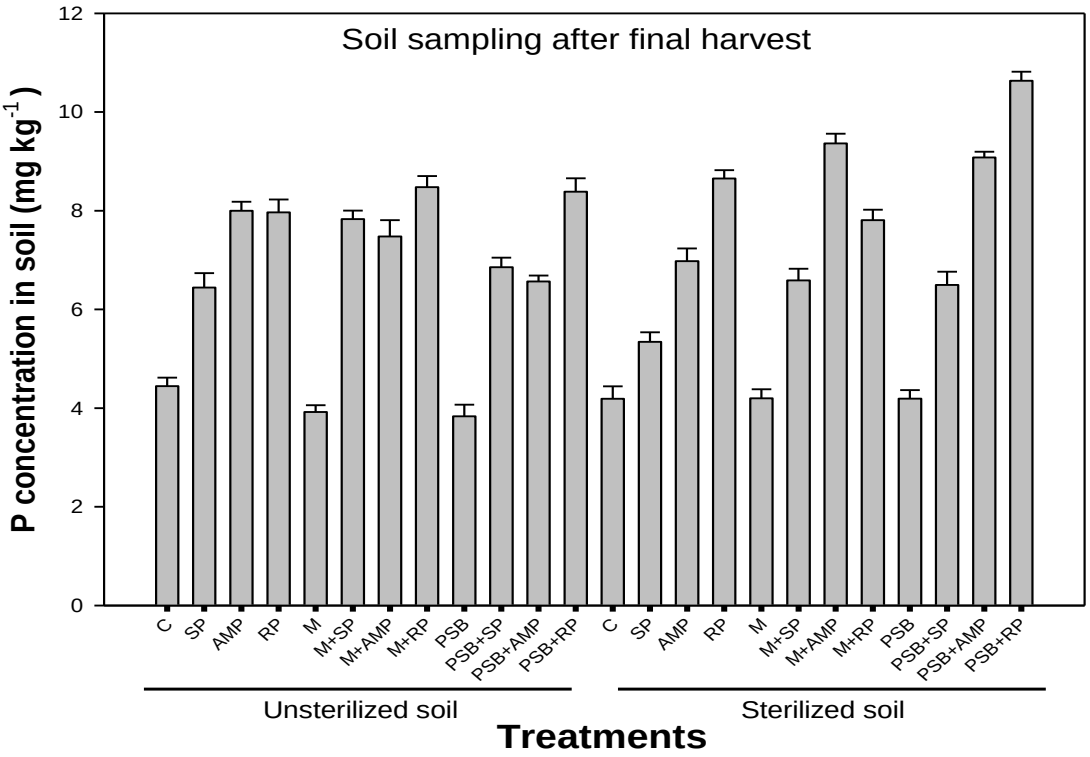


Fig. 4.3.2.17. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on P concentration of soil in unsterilized soil and sterilized soil after final plant harvesting. Error bars show standard error of means (n=3).

Table 3.2.2.11. Table of standard errors of difference and P values for data presented in Figure 4.4.2.15. Significant levels of P are in bold script.

Treatment	P concentration in soil (mg kg^{-1} soil)	
	SED	P
Non-Biological P	0.2802	**<.001**
Biological P	0.2426	0.083
Soil	0.3431	0.296
Non-Biological P* Biological P	0.4853	**0.027**
Non-Biological P*Soil	0.4853	0.637
Biological P*Soil	0.443	0.699
Non-Biological P* Biological P*Soil	0.7412	0.959

4.4.2.12. Yield components

The number of ears per plant was significantly increasedby soil treatments, and the number of seeds per ear and low seed weight were significantly affected by BP and soil treatments (Table 4.3.2.12). The highest and lowest number of ears per plant were observed in the SS+PSB+AMP and USS+PSB treatments respectively (Fig 4.3.2.18a), The highest and lowest number of seeds per ear were in the USS+PSB and USS+AMP treatments, respectively (Fig 4.3.2.18b), and the highest and lowest 1000-seed weight were found in the USS+PSB+RP and C (control) treatments, respectively (Fig 4.3.2.18c).

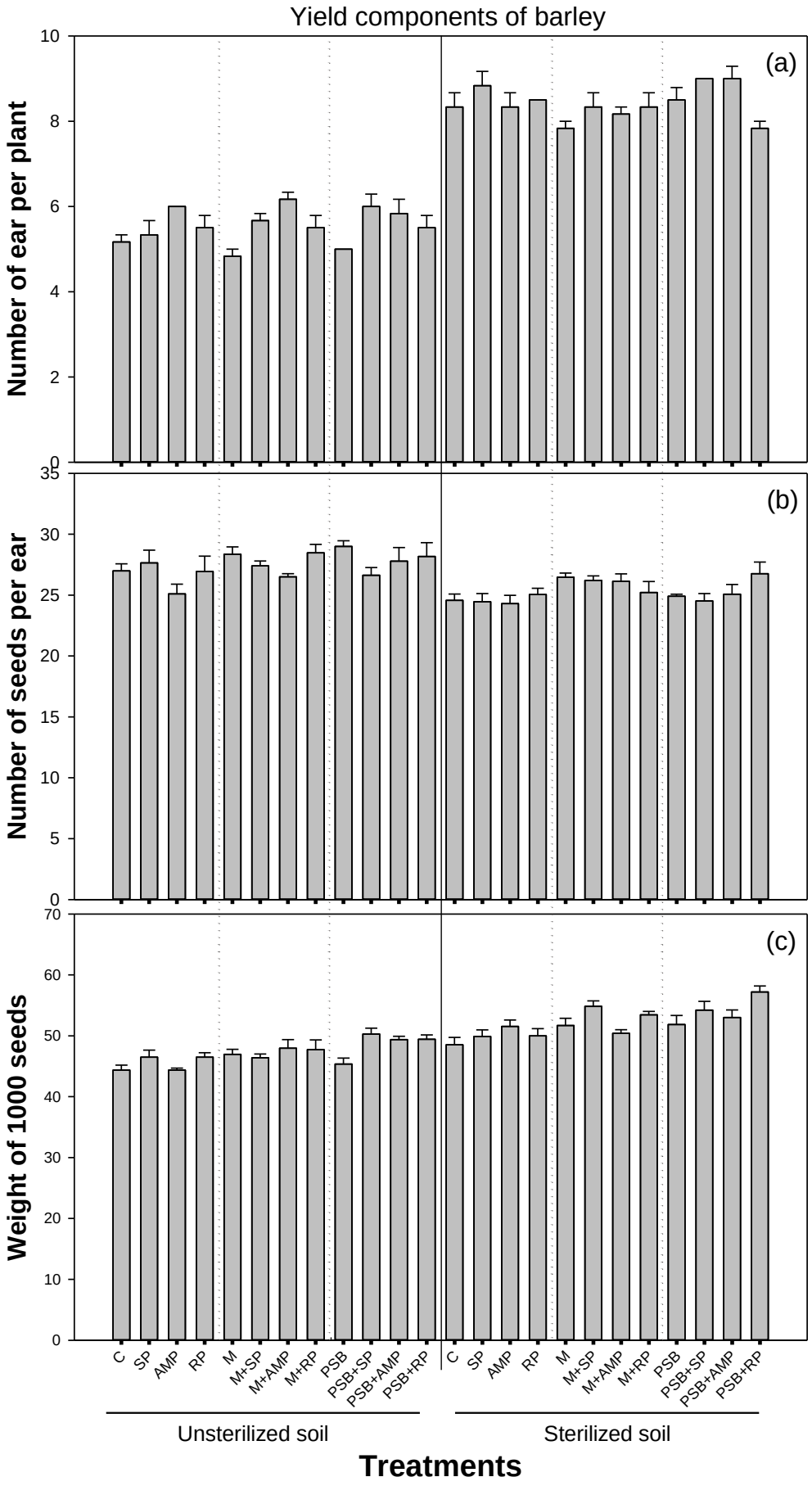

Yield components of barley
(a)
Number of ear per plant
0
2
4
6
8
10
(b)
Number of seeds per ear
0
5
10
15
20
25
30
35
(c)
Weight of 1000 seeds
0
10
20
30
40
50
60
70
C
SP
AMP
RP
M
M+SP
M+AMP
M+RP
PSB
PSB+SP
PSB+AMP
PSB+RP
Unsterilized soil
Sterilized soil
Treatments

Fig. 4.3.2.18. 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 number of are per plant(a), Number of seeds per ear(b), and 1000-seed weight(c) in unsterilized soil and sterilized soil. Error bars show standard error of means (n=3).

Table 3.3.2.12. Table of standard errors of difference and P values for data presented in Figure 4.4.2.12. Significant levels of P are in bold script.

Treatment	Yield components(final harvest)					
	Number of ear per plant(a)		Number of seeds per ear(b)		1000-seeds weight(g)(c)	
	SED	P			SED	P
Non-Biological P	0.518	0.576	0.552	0.26	1.068	0.087
Biological P	0.449	0.875	0.478	**0.035**	0.925	**0.001**
Soil	0.635	**<.001**	0.676	**<.001**	1.307	**<.001**
Non-Biological P* Biological P	0.897	0.995	0.955	0.676	1.849	0.865
Non-Biological P*Soil	0.897	0.959	0.955	0.907	1.849	0.925
Biological P*Soil	0.819	0.948	0.872	0.659	1.688	0.765
Non-Biological P* Biological P*Soil	1.371	1.000	1.459	0.917	2.824	0.991

4.4.2.13. Total root length

The root length was significantly increased by soil treatments in the first and second samples (Table 4.3.2.13). The highest and lowest root length of the first sample were observed in the USS+PSB+SP and SS+M+SP treatments respectively and also the highest and lowest root length of the second sample were in the USS+C and USS+PSB+SP treatments, respectively (Fig 4.3.2.19).

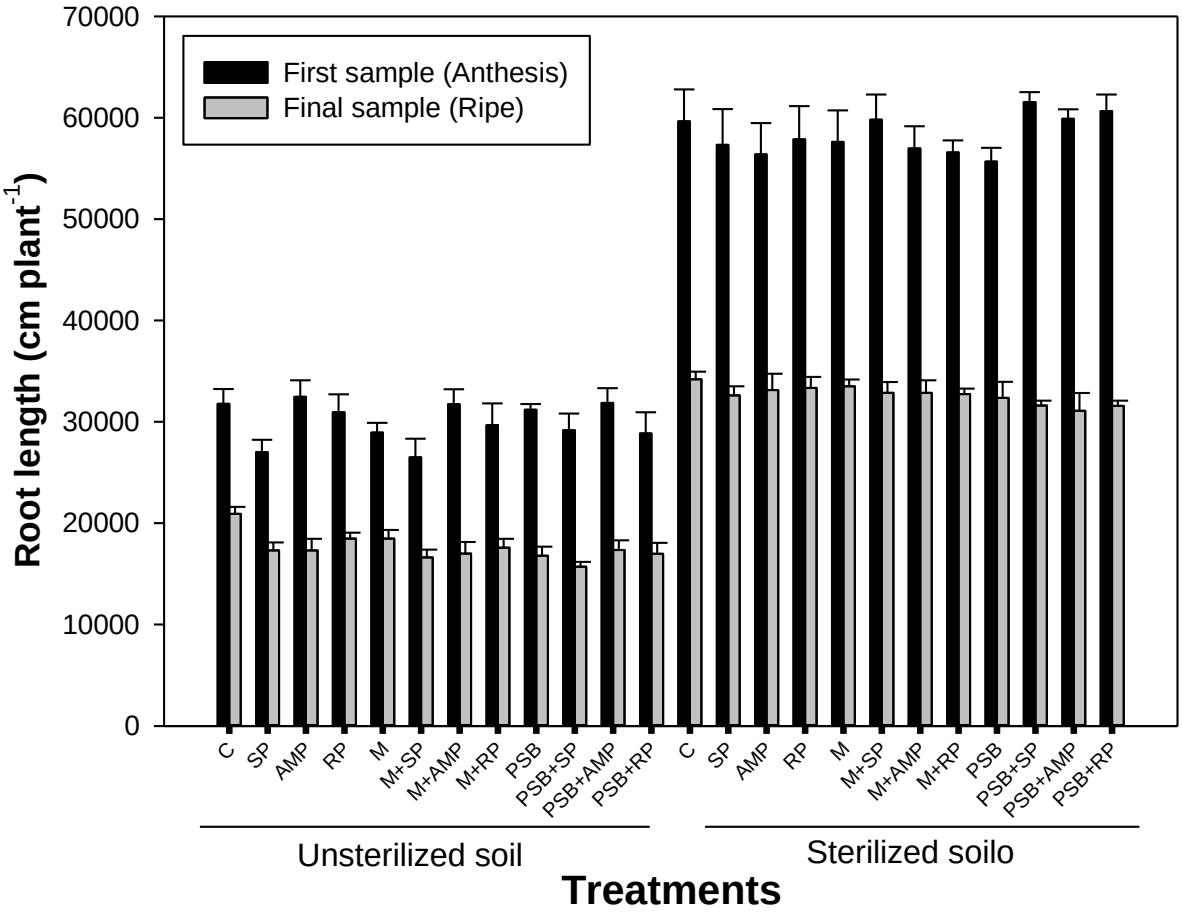


Fig. 4.3.2.19. Effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilising bacteria (PSB) and no M & PSB (C)) on root length in unsterilized soil and sterilized soil in the anthesis and ripe stage. Error bars show standard error of means (n=3).

Table 4.4.2.13. Table of standard errors of difference and P values for data presented in Figure 4.4.2.17. Significant levels of P are in bold script.

Treatment	Root length (cm plant^{-1})			
	Sample 1 (Anthesis)		Sample 2 (Ripe stage)	
	SED	P	SED	P
Non-Biological P	5107.1	0.995	2627.2	0.938
Biological P	4422.9	0.953	2275.2	0.747
Soil	6254.9	**<.001**	3217.7	**<.001**
Non-Biological P* Biological P	8845.8	1.000	4550.5	1.000
Non-Biological P*Soil	8845.8	0.994	4550.5	0.980
Biological P*Soil	8075.1	0.997	41540	0.955
Non-Biological P* Biological P*Soil	13512.2	1.000	69510	1.000

4.3.2.14. 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 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 (Fig 4.3.2.20).

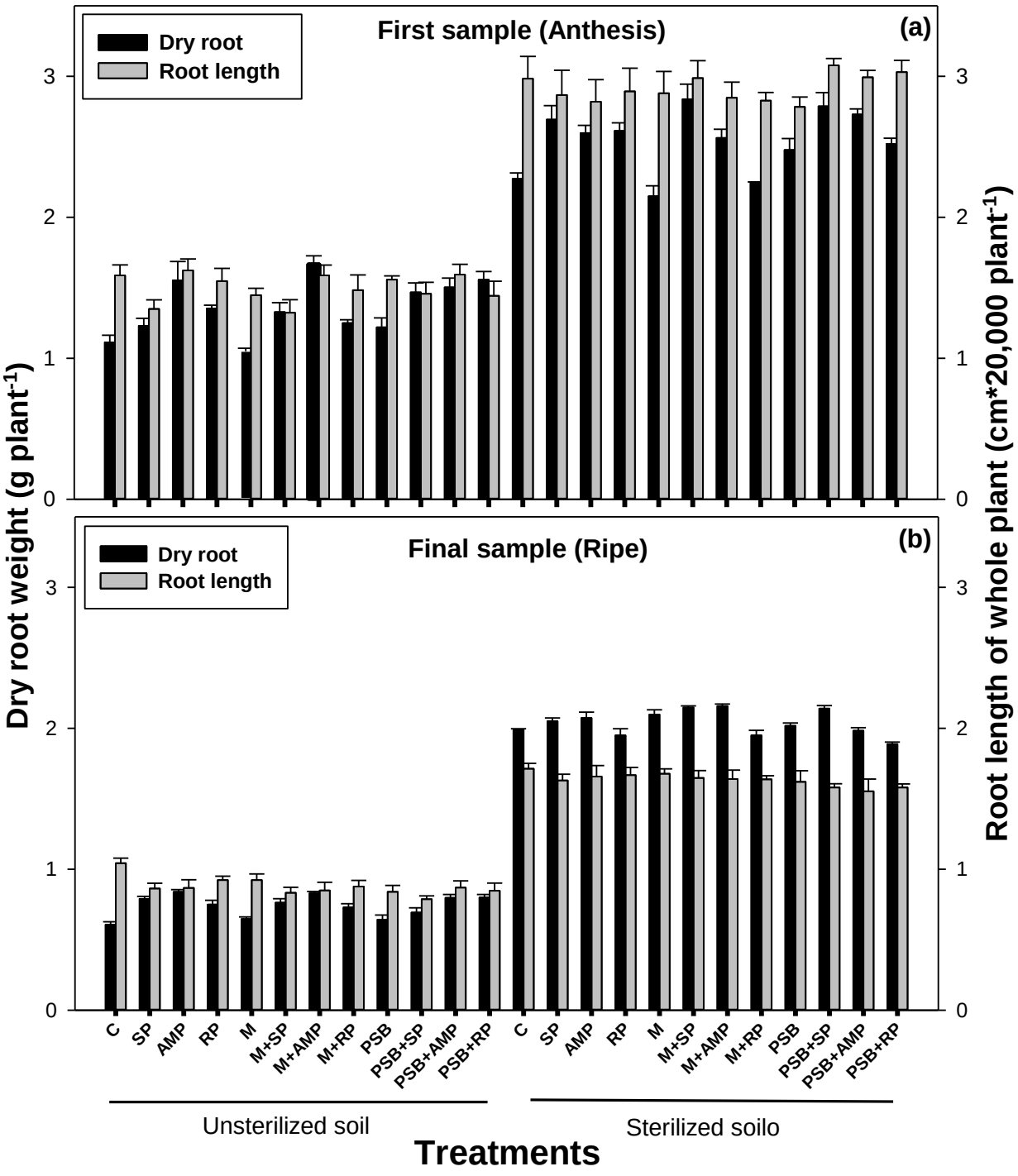


Fig. 4.3.2.20. Mean values of effect of non-biological phosphorus fertilizers (Super-phosphate (SP), ammonium magnesium phosphate (AMP) and powdered rock phosphate (RP)) and biological phosphorus fertilizers (vesicular arbuscular mycorrhizal (M), phosphorus solubilizing bacteria (PSB) and no M & PSB (C)) on total dry root weight and total root length of plant in unsterilized soil and sterilized soil in the anthesis (a) and ripe stage (b).Error bars show standard error of means (n=3).

4.4. Discussions

4.4.1. Dry matter

The present study showed that the root dry weight, leaf dry weight, shoot dry weight and whole plant dry weight were significantly increased by NBP , BP and interaction of NBP and BP in all three sampling occasions compared to the control. In this study concluded that application of PSB (microbial inoculation) and/or vesicular-arbuscular mycorrhizal fungi (mycorrhiza colonization) are playing positive roles in the solubilisation of inorganic phosphate, promoting growth and dry matter production, and increasing of nutrient levels.

Several studies reported that application of PSB (microbial inoculation) and/or vesicular-arbuscular mycorrhizal fungi (mycorrhizael colonization) play significant roles in the optimization of P solubilization (solubilization of inorganic phosphate), promotion of growth and dry matter production, increase of nutrient levels and mineralization of RP in a phosphorus deficient soil amended with non-biological P fertilizers (Gaskins et al., 1985;Toro et al., 1997;Widada et al., 2003; Anon 2006;Adesemoye & Kloepper, 2009;Heydari et al. 2009; Brook et al. 2011;Singh et al., 2011;Groppa et al., 2012;Fernández Bidondo et al., 2012). Thus, the use of biologic phosphorus fertilizers (PSB and VAM) could allow the achievement of satisfactory yield with the minimum amounts of non-biological phosphorus fertilizers, and also decrease fertilization costs and environmental pollution.

This study showed that the root dry weight, leaf dry weight, ear dry weight, shoot dry weight and grain yield significantly increased (p 0.05) in the sterilized soil when compared to the unsterilized soil. It suggests that in unsterilized soil, microorganisms may be competing with the plant for the same and limiting resources (Seastedt et al. 1988; Nehl et al. 1996) Alternatively, or in addition, the availability of nutrients may have been increased in the soil

sterilization process (Jakobsen & Andersen, 1982;Turkington & Klein, 1991;Trevors, 1996;Zhang et al., 2011). Several mechanisms for negative effects of rhizosphere microorganisms and arbuscular mycorrhizal fungus on plants growth are possible such as competition with plants for nutrients, immobilizing essential nutrients, production of phytotoxins and pathogens, decreased microbial respiration and biomass C by mycorrhiza(Turkington & Klein, 1991; Filion et al., 1999; Langley et al., 2005;Raiesi & Ghollarata, 2006; Gianinazzi-Pearson et al., 2007).

4.4.2. Phosphorus concentration and total P uptake in plant

Results showed that the phosphorus concentrations and P uptake in the root, stem, leaf, and grain were significantly increased by NBP, BP, soil and interactions of the factors. Maximum phosphorus concentrations and P uptake of the root, stem, leaf, and grain were achieved by application of PSB or/and M with SP and/or AMP treatments. This investigation suggested that the highest P content of root and shoot in the treatments with PSB and M may be related to the P uptake of plants from solubilization of inorganic phosphate (AMP or SP) and mineralization of RP by significant role of PSB and M. This response was consistent with the results of (Zaidi et al., 2004; Khan et al., 2009; Galavi et al. 2011; Gupta et al., 2012).

In the first sample (anthesis) P concentration of plant parts (root, stem, leaf, and ear) were higher than at the second sampling (Ripe stage). From anthesis to Ripe stage P concentration decreased in all of the plant parts (root, stem, leaf, and ear) because of P remobilization, from vegetative plant parts to the ear in the grain filling period. Also the amount of total P uptake (accumulation) of the plant from sowing to anthesis was higher than the amount of total P uptake of the plant from anthesis to ripe stage. The highest P concentration and P uptake were in the seeds and lowest P concentration and P uptake were

in the root, leaf and stem. From anthesis to ripe stage (in grain filling) P content and P content decreased in the roots, leaves and stems due to P remobilization from vegetative plant parts to grain and to decreased P accumulation from soil to plant. This results is in accordance with the results of (Papakosta, 1994;Masoni et al., 2007; Dordas, 2009; Ramaekers et al., 2010).

This investigation showed that the highest P content of root, stem, leaf and ear in the treatments with sterilized soil may be related to the uptake of P from solubilization of inorganic phosphate (AMP or SP) and mineralization of RP by significant role of PSB alone and without competition and negative effects of native microorganisms and fungi in the sterilized soil. This response was consistent with the results of (Piccini & Azcon, 1987;Zaidi et al., 2004;Khan et al., 2009;Galavi et al. 2011; Gupta et al., 2012). Inorganic P could solubilized by the role of organic acids and inorganic acid exuded by PSB and also reduction of pH in the soil (Toro et al., 1997b; Kpomblekou & Tabatabai, 2003; Vassilev et al., 2006).

4.4.3. Root colonization an leaf area index

Results of root staining showed that the highest percentage of root colonization was achieved by control (no P fertilization) and M+RP treatment in the growth stages of plant. By contrast, the lowest percentage of root colonization were observed in the M with SP and AMP treatments. It seems that the high colonization levels associated with low P (C and RP) in the soil. Several studies have reported that high levels of P fertilization reduced mycorrhizal inoculization in wheat (Rubio et al. 2003;(Covacevich et al. 2007), tomato (Manian et al. 1995), corn (Miransari et al. 2009;Zhang et al. 2011) and barley (Khaliq & Sanders 2000).

The leaf area index (LAI) of barley showed that the highest and lowest LAI were in the M+AMP and C treatments respectively. Studies (Rasmussen, 1997;Knyazikhin et al., 1998;Yao et al., 2008) show that LAI is an important parameter that plays a significant role in crop production and yield estimation.

4.4.4. Yield and yield components

Results of final harvesting in the first experiment showed that the grain yield and 1000-seeds weight was significantly increased by NBP, BP and interaction of NBP with BP. Also, in the second experiment results showed the number of seeds per ear 1000-seeds weight were significantly affected by BP and soil treatments. Maximum grain yield was recorded in the PSB+SP treatment. Similar results were found in the increased grain yield of barley achieved arising from the increase in the number of ears per plant, number of seeds per ear and the weight of grain as reported by (Rodríguez & Fraga 1999; Salantur et al. 2005;Takahashi & Anwar, 2007).

The increases in grain yield with PSB inoculation may be described with possible mechanisms of phosphorus solubilization, siderophore, IAA production capabilities of bacteria strains, alone and/or in combination, as described by other researchers e.g. (Cáceres et al., 1996;Kim et al. 1998;Rodríguez & Fraga 1999; Gholami et al., 2009;Salantur et al., 2005;Yadav et al., 2011). In this experiment, the indigenous bacteria (non-sterilized soil) and combination of indigenous bacteria (non-sterilized soil) with PSB inoculation or M colonization led to a significant decrease of yield compared to sterilized soil, This may be explained in part by the negative effects of microorganisms on crop yield such as competition of microorganisms with the plant and/or with themselves for the same and limiting resources.

The results observed in this study were similar to those reported by(Seastedt et al., 1988; Nehl et al., 1996;Bhattarai & Hess, 1993).

4.4.5. Final P & N concentration and content in grain

Results of final harvesting showed that the phosphorus concentration of grain was significantly increased by BP, and the total phosphorus uptake in grain was significantly affected by soil. Results of grain analysis showed that the highest P content of grain by BP treatments may be related to the capability of M and PSB to increase of P availability in the soil and P uptake of plant by expansion of hyphae and solubilisation of AMP or SP and mineralization of RP and then P remobilization, from root, stem and leaf to the grain in grain filing period as reported by other researchers (Zaidi et al., 2004;Khan et al., 2009).

Grain analysisshowed that the N concentration and total N uptake in grain was significantly increased by the soil and NBP treatments. It seems that increased total N uptake in grain was affected by increases in the LAI, N stored in vegetative organs stage and uptake of N from soil to plant in the post-anthesis period (N sources increasing). Several researchers (Paul & Pellny, 2003;Pan et al., 2006;Triboi et al., 2006;Sandaña et al., 2009) reported the same results.

4.4.6. 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) (Fig 4.3.1.15 and 4.3.2.20). 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 & Ridge 2000;Toro et al. 1997a; Wang, et al. 2010).

4.4.7. Soil P concentration

The comparison of the soil analysis from before cultivation with that from after harvesting showed that the use of PSB in the unsterilized and sterilized soil increased the amount of available P in soil (Mittal et al. 2008;Rahi et al. 2010). The maximum amount of available P was observed in treatment of RP with PSB in sterilized soil. The results showed the important role of PSB for solubilizing available P for plant uptake.

The reasons of high Olsen P in the soil after harvesting in the PSB+RP treatment could be some points:

1. Release more available P from RP, after anthesis stage.
2. Majority of P uptake from soil to plant was happen in the vegetative stage (before anthesis stage) of barley.

4.5. Conclusion

The present study showed that the growth of plant, dry matter (root, shoot and grain), P and N concentration, P and N uptake and total root length of the plant were significantly increased in sterilized soil compared to unsterilized soil. It seems that the soil sterilization process, eliminated the native soil microorganisms which are competing with the bio-inoculated and plant for the same and limiting resources (Seastedt et al. 1988; Bhattarai & Hess, 1993; Nehl et al. 1996), increased the availability of nutrients (Fig. 4.3.2.15) and released nutrients from the decomposition of dead microbial cells(Tanaka et al., 2003) and decomposition of soil components and changes in the soil structure, control of other negative effects on plant such as weed seeds and mosses (Fig 4.5.1) in the soil following sterilization process (Jakobsen & Andersen, 1982;Turkington & Klein, 1991;Trevors, 1996; Zhang et al., 2011).

Several researchers reported that the increased concentrations of Ca, Na, K, N, NH_4^+ and NO_3^- in soil following sterilization were assumed to be from the decomposition of dead microbial cells contents following sterilization process. (Powlson & Jenkinson, 1976;Marumoto et al., 1982;Lensi et al. 1991; Tanaka et al., 2003;Ekschmitt, et al., 2005;Kuzyakov, 2010).

The present study showed that the application of M and PSB alone were not significant but interaction of NBP (SP or AMP or RP) with BP (M or PSB) significantly increased growth of plant, dry matter (root, shoot and grain), P and N concentration, P and N uptake and total root length of the plant.

Several researchers (Abbott & Robson, 1982;Pan et al., 1998;Augé, 2001;Mehrotra, 2005;Pellerin et al., 2007) have concluded that mycorrhizaare capable of taking up, translocation 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 external hyphae.

PSB are able to decrease the pH of the surroundings, solubilize inorganic phosphate, mineralize mineral phosphate and increase the availability of P for plants in the rhizosphere by manufacturing organic and inorganic acids, (Kim et al., 1998; Stevenson et al., 2005; Abou-el-Seoud & Abdel-Megeed, 2012), alkaline and acid phosphatises (Rao et al. 1999;Rodríguez & Fraga, 1999) as well as through the production of H^+(Illmer & Schinner, 1992;Smith, 2002).

Application of PSB (microbial inoculation) and/or M (mycorrhiza colonization) are playing significant roles in the optimization of P solubilization (solubilisation of inorganic phosphate), promoting of growth and dry matter production, increase of nutrient levels and mineralization of mineral phosphate (RP) (Toro et al., 1997;Widada et al., 2003; Anon 2006;Adesemoye & Kloepper, 2009;Heydari et al. 2009; Brook et al. 2011;Singh et al., 2011;Groppa et al., 2012;Fernández Bidondo et al., 2012). Thus, the use of biologic phosphorus fertilizers (PSB and M) could be allow the achievement of satisfactory yield with the minimum amounts of non-biological phosphorus fertilizers, and also decrease of fertilization costs and environmental pollution.

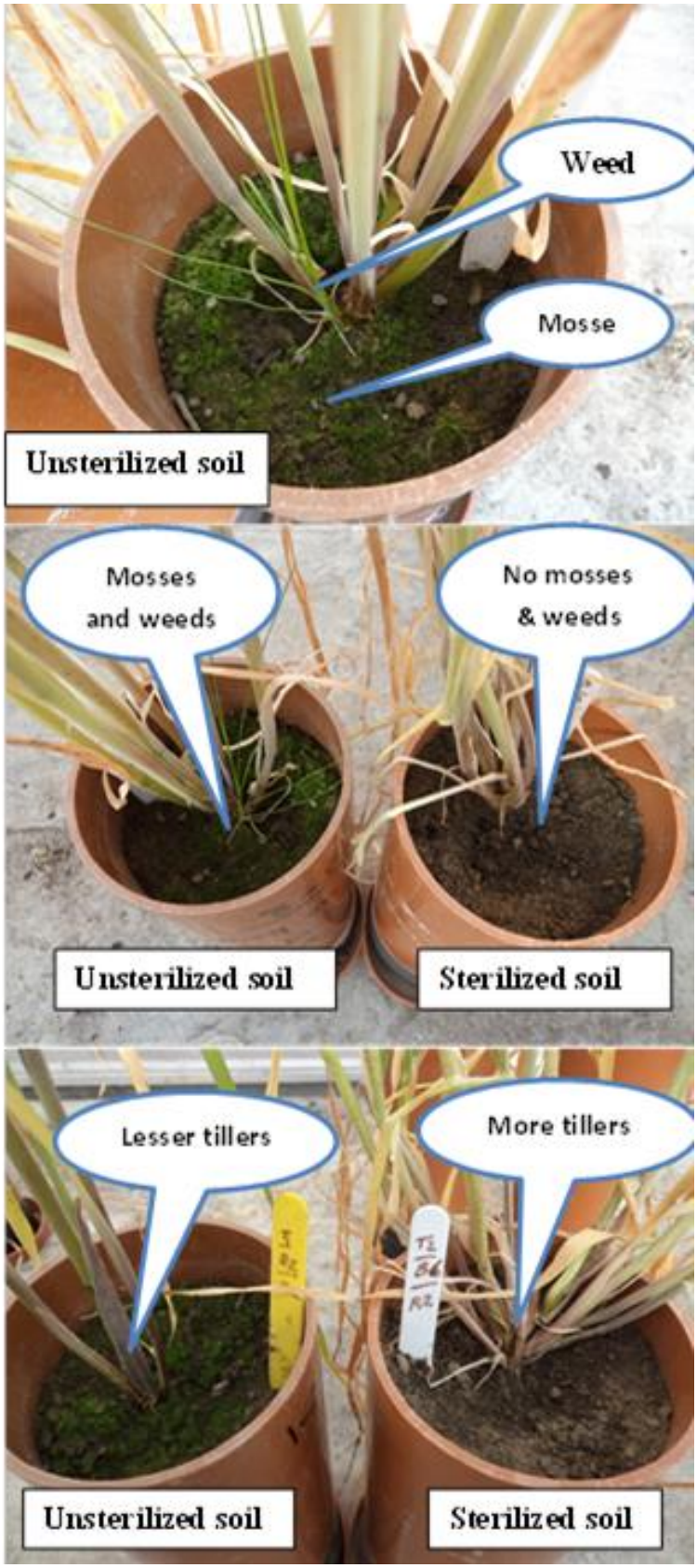


Fig. 4.5.1 Tillers, weeds and mosses in unsterilized soil and sterilized soil.

Some additional results that reported out of the hypothesise research such as:

1- Results of root staining showed that the highest percentage of root colonization was achieved by M+RP treatment in the growth of plant. By contrast, the lowest percentage of root colonization was observed in the treatments of M in the growth stage and M+SP in the growth of plant. It seems that the high colonization levels associated with low (with basic P such as RP to mycorrhizal activities and root colonization) P in the soil.

2- 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) (Fig 3.5.1 and 3.5.2). 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.

Chapter 5: Final discussion

5.1. The key objectives of this study were:

1. To advance understanding of the effects of fungal (mycorrhizae, M) and bacterial (phosphorus solubilising bacteria, PSB) bio-inoculants, on the availability of soil and applied fertiliser phosphorus (P) to barley and assess their potential role in sustainable agriculture.
2. To measure the root, shoot and yield response of barley to bio-inoculants (M & PSB) and different inorganic P fertilisers in field and greenhouse studies.
3. To assess the effectiveness of a novel recycled fertiliser (Struvite (ammonium magnesium phosphate, AMP) to supply P to barley and increase the growth of root, straw, and yield of barley with/without bio-inoculants.
4. To compare rock phosphate (RP) and different manufactured inorganic P fertilizers (e. g. Triple super phosphate and single super phosphate) as sources of P to barley in the absence or presence of bio-inoculants to optimize barley production.

5.2 Experiments results and justification

The first field experiment showed the positive effects of bio-inoculants (PSB and M) in solubilizing and mobilizing P from soil by enhancing crop P concentration and P uptake and increasing the growth and yield of barley cultivated in P deficient conditions. PSB and M therefore have a role in mobilizing and transferring unavailable P sources in soil and increasing agricultural production on soils low in P.

After demonstrating the positive effects of bio-inoculants on barley growth and production, the second field experiment replicated significant positive effects of M and PSB on crop

growth and production but using P fertilizers of varying solubility. This is of particular interest to low input sustainable crop production systems that make use of recycled fertilisers (e.g. Struvite) and reduced quantities of P fertilizer with potential reductions in fertiliser manufacturing costs and rates of exploitation of finite rock phosphate reserves.

Building on the results obtained from the two field experiments, a pot experiment in horticultural sand confirmed a beneficial effect of the bio-inoculants on barley growth and yield without the presence of native M and PSB. This experiment gave the advantage of easy root extraction from pots, separation of roots, scanning and identification of root components as well as controlling the external factors such as temperature, irrigation regimes and light.

A second set of pot experiments under the same experimental and laboratory conditions further examined bio-inoculant effectiveness using and unsterilized and sterilized field soil. The results showed significant effects in terms of root length and shoot growth, P uptakeand yield production with different P fertilizers combined with bio-inoculants; however results in all treatments of the sterilized soil pots were higher than in unsterilized soil pots.

Fig 5.1 Effects of different P levels and bio-inoculants on grain yield, dry matter and P uptake of barley plant in terms of increase (%) over the zero external P control treatment.

Experiment	Bio-inoculante	Treatments	Grain yield	Increase (%)	Plant dry weight	Increase (%)	P uptake in plant	Increase (%)
Field 1								
			(g m^{-2})		**(g m^{-2})**		**(mg m^{-2})**	
	-	Control	444.3	0.0	775.4	0.0	1470.8	0.0
	M	M	480.8	8.2	840.1	8.3	1685.3	14.6
	PSB	PSB	472.3	6.3	845.3	9.0	1621.4	10.2
	PSB+M	M+PSB	487.9	9.8	860.9	11.0	1720.8	17.0
	-	RP	483.6	8.9	859.3	10.8	1679.3	14.2
	M	M+RP	490.0	10.3	883.3	13.9	1898.7	29.1
	PSB	PSB+RP	486.3	9.5	879.6	13.4	1845.6	25.5
	PSB+M	M+PSB+RP	505.8	13.8	921.0	18.8	2026.4	37.8
Field 2								
	-	Control	471.6	0.0	945.7	0.0	1750.0	0.0
	-	RP	492.5	4.4	978.8	3.5	1884.8	7.7
	-	TSP	540.6	14.6	1077.6	13.9	2203.8	25.9
	-	AMP	547.3	16.1	1072.6	13.4	2324.3	32.8
	M	M	489.1	3.7	1007.3	6.5	1923.3	9.9
	M	M+RP	511.9	8.6	1037.7	9.7	2109.5	20.5
	M	M+TSP	568.9	20.6	1105.6	16.9	2464.0	40.8
	M	M+AMP	570.7	21.0	1110.6	17.4	2437.8	39.3
Pots 1								
(Hort. sand)		*	**(g $plant^{-1}$)**		**(g $plant^{-1}$)**		(mg $plant^{-1}$)	
	-	RP, AMP, SP	11.8	0.0	41.5	0.0	60.8	0.0
	M	RP, AMP, SP	13.8	17.4	46.3	11.7	80.1	31.8
	PSB	RP, AMP, SP	15.7	33.3	50.5	21.7	97.0	59.6
Pots 2								
Non St soil								
	-	Control	6.2	0.0	15.2	0.0	20.8	0.0
	-	SP	6.8	8.9	16.7	9.8	23.4	12.7
	-	AMP	7.0	12.1	16.7	9.9	25.4	22.2
	-	RP	6.8	8.5	16.4	7.5	23.6	13.9
	M	M	6.4	2.8	15.9	4.5	21.6	3.9
	M	M+SP	7.0	12.6	17.9	17.7	25.7	23.6
	M	M+AMP	7.8	24.8	18.3	20.5	27.5	32.7
	M	M+RP	7.6	22.0	17.8	17.4	26.6	28.3
	PSB	PSB	6.7	6.7	16.2	6.5	24.0	15.8
	PSB	PSB+SP	7.8	24.3	17.8	17.2	28.3	36.3
	PSB	PSB+AMP	7.9	26.9	18.1	18.8	29.0	39.9
	PSB	PSB+RP	7.8	24.9	18.7	22.9	28.7	38.3
St soil	-							
	-	Control	9.8	0.0	23.6	0.0	35.7	0.0
	-	SP	10.6	8.1	25.3	7.2	40.2	12.4
	-	AMP	10.4	5.5	24.8	5.1	39.8	11.4
	-	RP	10.6	7.2	24.9	5.4	40.1	12.1
	M	M	10.8	9.3	24.5	3.8	38.7	8.3
	M	M+SP	11.9	20.7	26.7	13.2	44.9	25.6
	M	M+AMP	11.0	11.7	25.8	9.3	42.0	17.6
	M	M+RP	11.3	14.6	26.0	10.3	41.7	16.8
	PSB	PSB	11.0	11.4	25.3	7.4	40.3	12.6
	PSB	PSB+SP	12.0	21.5	27.3	15.7	46.3	29.6
	PSB	PSB+AMP	11.6	17.4	26.5	12.4	45.3	26.7
	PSB	PSB+RP	11.7	18.9	26.2	11.1	45.1	26.1

*****Because plant growth in C = 0, only main effect means presented for inoculants groups.

(Data consolidated from the Tables of chapter 3 & 4).

5.3. This PhD study has obtained some main results that include:

Across all experiments bio-inoculants (M & PSB) enhanced grain and shoot yield and P uptake. This is important in organic agriculture, where only RP or P in manure or compost can be used.

This research demonstrated that M, both as inoculate or native, effectively colonized barley roots, in both field and pot experiments, but not in sterile media (horticultural sand and sterilized field soil) (Fig. 4.3.2.11). It seems that the soil sterilization process, eliminated the native soil microorganisms that compete with the added bio-inoculants and the plant for the same and limiting resources (Seastedt et al. 1988; Bhattarai & Hess, 1993; Nehl et al. 1996). It is postulated that the large increases in root and shoot growth in sterilised soils compared with unsterilized soils were due to the increased the availability of nutrients (Ca, Na, K, N, P, NH_4^+ and NO_3^-) released from the decomposition of dead microbial cells (Tanaka et al., 2003; Kuzyakov, 2010), to the decomposition of soil components, to changes in soil structure (Anderson, 2005: Baker et al. 2010;Griffiths, 1987), and to the control of other negative effects on plant of weed seeds and mosses (Fig 4.5.1),(Jakobsen & Andersen, 1982;Turkington & Klein, 1991;Trevors, 1996; Zhang et al., 2011). Therefore mycorrhizal inoculation and M activity were decreased by the increasing amount of available P and nutrition in the soil. Several studies have reported that high levels of P fertilization reduced mycorrhizal inoculization in wheat (Rubio et al. 2003;(Covacevich et al. 2007), tomato (Manian et al. 1995), corn (Miransari et al. 2009;Zhang et al. 2011) and barley (Khaliq & Sanders 2000).

Application of M increased P uptake of plants either in the absence of non-biological inorganic fertilizers (from 3.9% to 15.8%) or in combinations with non-biological P (from 23.6% to 40.8%) and also P concentration in the plant parts (Figs.

4.3.2.8, 4.3.1.5, 3.3.2.8 and). However, increased P uptake resulted in limited increases in grain yield (from 3.8% to 9.3% alone) and combinations with non-biological P (from 8.2% to 26.9%), especially in the field (Fig. 5.1). Several researchers (Pan et al., 1998;Augé, 2001;Mehrotra, 2005;Pellerin et al., 2007) have concluded that mycorrhiza are capable of taking up, translocation 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 a depletion zone that from the root surface around the root system through the external hyphae.

PSB inoculation increased P uptake from soil to plants (from 12.6% to 15.8% alone) and combinations with non-biological P (from 29.6% to 59.6%), dry matter of plant and grain yield alone (from 6.7% to 11.4%) and with non-biological P (from 21.5% to 33.3%) (Fig. 5.1). Researchers reported that PSB are able to decrease the pH of the rhizosphere, solubilize inorganic phosphate, mineralize mineral phosphate and increase the availability of P for plants by manufacturing organic and inorganic acids, (Kim et al., 1998; Stevenson et al., 2005; Abou-el-Seoud & Abdel-Megeed, 2012), alkaline and acid phosphatases (Rao et al. 1999;Rodríguez & Fraga, 1999) as well as through the production of H^+(Illmer & Schinner, 1992;Smith, 2002).

Combinations of M and PSB significantly increased grain yield, plant dry matter and P uptake from soil to plants to a much greater extent than when applied alone in all of the experiments (Fig. 5.1). Bio-inoculants (M and PSB) significantly increased the efficiency of chemical fertilizer (SP), AMP and RP and the combination of P fertilizers (RP, SP and AMP) with bio-inoculants (M and PSB) significantly increased yield and yield components, P concentration and total P uptake, plant dry matter, LAI and concentration of P in the grain compared to fertilizers alone.

When applied alone, AMP significantly increased yield, dry weight of plant and plant P uptake but greater increases in barley P uptake, growth and yield were achieved with bio-inoculants (Fig. 5.1). AMP could be a useful replacement for traditional chemical P fertilizers because it is a new fertilizer material recycled from domestic wastewater at sewage treatment works. It is a slow-release fertilizer but with greater solubility than RP over a range of soil types and environmental conditions and has lower impact on environment than chemical P fertilizers.

Results of root staining showed that the highest percentage of root colonization was achieved by M+RP treatment. In contrast, the lowest percentage of root colonization was observed in treatment M+SP. Higher colonization levels were associated with low (with basic P such as RP to mycorrhizal activities and root colonization) P in the soil.

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) (Fig 4.1, 4.2 and 4.3). It seems that under lack of P, plants increased root length and decreased root diameter thereby achieving a greater surface area and increasing the volume of soil that is explored by the roots. This effect has not previously been recorded in the literature.

Finally, the pot and field studies have demonstrated the large potential benefits of using bio-inoculants (PSB and M) in solubilizing and mobilizing P from soil, enhancing P concentration and P uptake to increase growth and yield of barley cultivated in P deficient conditions and the role of PSB and M in mobilizing and transferring unavailable or slowly available P sources for plant uptake. Furthermore, the use of bio-inoculants could be helpful

for satisfactory crop growth and production with the minimum application of chemical phosphorus fertilizers, and also lead to a decrease in fertilization costs and environmental pollution. Therefore application of M and/or PSB with non-biological phosphorus fertilizers could be encouraged in barley growing areas.

5.4 Suggestions for future research:

I used only one standard variety and M may be ever more effective in other varieties but this is a topic for future research. Also, this M inoculum was selected from pasture grasses, not specifically for barley. So possibly M inoculum may be refined to work better by investigating

1. Varying edaphic concentrations of P from low P to high P for understanding the effect on root and shoot growth.
2. The effect of P in the soil on root diameter and total root length of root cluster.
3. Using bigger and deeper pots to simulate quasi field situations.
4. Using another variety(s) of barley.
5. Plant tissue analysis of some important macro and micro nutrients, during different stages of plant growth.
6. Pre and post soil analysis of some important soil properties (such as pH, N, P and K) for better understanding of the effect of bio-inoculants on soil quality..
7. Increase the frequency of soil sampling and soil analysis from rhizosphere during plant growth.
8. Use a Rhizotron for monitoring root growth.
9. Increasing treatment variability through the combination of P and N with bio-inoculants.

10. Long term studies looking at P uptake efficiency as a function of crop yield and sustainable crop production. To improve the quality of grain for human and animal consumption, it could be better to use a variety that can take up more P as well as high yield. If we are interesting for sustainable crop production with less application of P fertilizers, we could use a variety can produce optimum yield with less P uptake.

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Appendix 1

Hoagland's Solution (Plant Nutrient Solution)

Component	Stock Solution	mL Stock Solution/1L
2M KNO_3	202g/L	2.5
2M $Ca(NO_3)_2$ x $4H_2O$	236g/0.5L	2.5
Iron (Sprint 138 iron chelate)	15g/L	1.5
2M $MgSO_4$ x $7H_2O$	493g/L	1
1M NH_4NO_3	80g/L	1
Minors:		1
H_3BO_3	2.86g/L	
$MnCl_2$ x $4H_2O$	1.81g/L	
$ZnSO_4$ x $7H_2O$	0.22g/L	
$CuSO_4$	0.051g/L	
H_3MoO_4 x H_2O or	0.09g/L	
Na_2MoO_4 x $2H_2O$	0.12g/L	
1M KH_2PO_4 (pH to 6.0 with 3M KOH)	136g/L	0.5

1) Make up stock solutions and store in separate bottles with appropriate label.
2) Add each component to 800mL deionized water then fill to 1L.
3) After the solution is mixed, it is ready to water plants.