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Growth and nutrient uptake in maize and faba bean plants inoculated with arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* DR54 and fertilised with rock phosphate

DISSERTATION

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Abbreviations

AB-DTPA	Ammonium bicarbonate diethylene triamine penta acetic acid
Al	Aluminum
AMF	Arbuscular mycorrhizal fungi
Atmo	Atmosphere
Ca	Calcium
$Ca_{10}F_2(PO_4)_6$	Flour apatite
$Ca_5(PO_4)_3OH$	Hydroxyl apatite
Cd	Cadmium
CEC	Cation exchange capacity
CFU	Colony-forming unit (is a measure of viable bacterial or fungal numbers)
Cit-A	Citric acid
Co_2	Carbon dioxide
$CuSO_4$	Copper sulphate
DM	Dry matter
Fe	Iron
Glu-A	Gluconic acid
$H_2PO_4^-$	Dihydrogen phosphate ion
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HNO_3	Nitric acid
HPLC	High Performance Liquid Chromatography technique
HPO_4^{2-}	Phosphate ion
hrs	hour
K	Potassium
K_2SO_4	Potassium sulphate
Lac-A	Lactic acid
Mal-A	Malic acid

AMF-Maize	
plant	Maize roots colonised by mycorrhiza fungi
Mg	Magnesium
Mn	Manganese
N	Nitrogen
NaH_2PO_4	Monosodium Phosphate
ND	Not detected
NH_4NO_3	Ammonium nitrate
OM	Organic matter
Oxa-A	Oxalic acid
P	Phosphorus
Pdl	Double lactate soluble phosphorus
PF	Pseudomonas fluorescens DR54
PGPR	Plant-growth promoting rhizobacteria
PSM	Phosphate solubilizing microorganisms
RAE	Relative agronomic effectiveness
RP	Rock phosphate
RPs	Rocks phosphate
R2A	Low nutrient medium for enumeration and cultivation of bacteria from potable water
Suc-A	Succinic acid
Tio2	Titanium dioxide
TSB	Tryptic soy broth
UV	Ultraviolet
W/V	Mass per volume
Zn	Zink
α KG-A	α- ketoglutaric acid

1. Introduction

One of the major limiting factors to food production is the deficiency of plant nutrients, especially available phosphorus (P), in the soil. The most essential macronutrient restricting agricultural production worldwide is P (Schachtman *et al*., 1998). P is absorbed by plant roots in the ionic form of either $H_2PO_4^-$ or HPO_4^{2-}.

In acidic soil P is taken up mainly as $H_2PO_4^-$, whereas in alkaline soil it is mostly absorbed as HPO_4^{2-}. In soils P exists in organic and inorganic forms. The organic form of P occurs in organic materials and humus. Inorganic P can be adsorbed onto the surface of some clay particles and forms complexes or reacts with aluminium (Al), iron (Fe), and calcium (Ca) to form insoluble precipitates (Fageria *et al*., 2003).

The direct application of rock phosphate (RP) as a fertiliser is an easy and comparatively cheap way of adding P to soil. However, RP is an insoluble inorganic form and its solubility is related to many factors, including soil properties, plant species and soil microorganisms.

It is known that nutrient cycles in the soil are largely mediated by the soil microflora. Cakmakci *et al*. (2005) reported that the use of microbial inoculants improves nutrient supply to crops. For instance, RP becomes soluble to plants by microorganism activity in the soil through production of organic acids (Kang *et al*., 2002; Maliha *et al*., 2004).

1.1 Factors affecting RP solubilisation

Blatt and Tracy (1996) reported that phosphate originates from different natural sources. For example, fluorapatite ($Ca_{10}F_2(PO_4)_6$) can be created from hydrothermal veins while hydroxylapatite ($Ca_5(PO_4)_3OH$) is dissolved from vertebrate bones and teeth.

RP is a non-detrital sedimentary rock that contains high amounts of phosphate-bearing minerals (van Straaten, 2002). The phosphate content of RP is at least 20% (Blatt and Tracy, 1996). RP is used as a raw material in the production of P fertilisers and certain other chemicals. For instance, RP and sulphuric acid are used in the production of single

superphosphate and phosphoric acid, and then phosphoric acid is used to obtain triple superphosphate and ammonium phosphate (Engelstad and Hellums, 1993; EFMA, 2000). The availability of P from RP is influenced by many factors including plant species, soil properties, management practices, source of the RP and microbial efficiency (Chien and Menon, 1995).

1.1.1 Effect of plant species on solubilisation of RP

Plants have different strategies to mobilise P and may take up P from less soluble sources (Eichler-Löbermann *et al*., 2008). Hinsinger and Gilkes (1997) compared RP dissolution in the rhizosphere of five plant species: oilseed rape, annual medics, annual ryegrass, subterranean clover and tomato. They concluded that these plants have different strategies for obtaining P from acidic, P-fixing soils fertilised with RP. In their study rape was the only species that raised its rhizosphere pH by secreting OH^- or HCO_3^+, while for the other plant species the root-induced dissolution of RP was a result of a combination of Ca uptake and H^+ excretion. Ryegrass was the second most efficient species for dissolving RP and had the lowest requirement for Ca. Some plant species such as lupines (Adams and Pate, 1992), rapeseed (Hoffland, 1992; Montenegro and Zapata, 2002) and pigeon pea (Ae *et al*., 1990) have been studied because of their ability to secrete organic acids that result in enhanced solubilisation of RP by many mechanisms. Such mechanisms include acidification, chelation and exchange reactions (Dakora and Phillips, 2002).

Legumes can be particularly well-suited for the use of RP. Mnkeni *et al*. (2000) reported that legumes are more efficient than cereals in utilising P from RP.

1.1.2 Effect of time on solubilisation of RP

RP is a known low dissolved fertiliser that needs time for the dissolution products to spread away from the RP particles into the soil. Goenadi *et al.* (2000) reported that direct application of RP is often ineffective for most annual crops when it is used in short

intervals. Truong and Fayard (1995) measured the kinetics of RP dissolution over time. They studied the effect of formic acid on the solubilisation of three types of RP. High reactivity was confirmed for Tilemsi and Kodjari RP in the short and long term, while solubilisation of Hahotoe RP increased in the long term.

1.1.3 Effect of soil properties on solubilisation of RP

Soil properties such as the exchangeable Ca^{2+}, soil cation exchange capacity (CEC), soil texture, soil pH, organic matter content, P concentration and P retention capacity are fundamental factors that affect solubilisation of RP **(**Chien and Menon, 1995).

In the case of Ca, solubilisation of RP is achievable when the exchangeable Ca is low, as in tropical acidic soils (Vanlauwe *et al*., 2006). It is possible to achieve this target of solubilisation if Ca is leached away from the site of RP dissolution, or if there are sufficient soil cation exchange sites available to absorb the Ca released from RP (Perrott, 2003).

Selvi *et al*. (2004) studied the dissolution of two RPs in soils of different pH and different texture. They concluded that the lower the pH, the higher is the percentage of solubilisation of RP. In their study the highest RP solubility was recorded in sandy loam soil with a pH of 5.8, followed by clay soil (pH=7.6) and clay loam soil (pH=8.75).

Chien *et al*. (1990) reported that the soil organic matter may contribute to increasing the RP solubilisation and its availability to plants. Organic acids with high CEC tend to increase the Ca retention capacity of soils and build up the Ca-organic matter complexes. This can reduce the Ca concentration in solution and enhance RP solubilisation (Heredia *et al*., 2002).

Rajan and Casanova, (2004) cited that when the P concentration in soil solutions is very low (0.05-0.5 mg/l), it has little influence on the solubilisation of RP

However, Chien *et al*. (1980) reported that the greater the P sorption capacity of soil, which causes a decrease in the soil solution P, the greater the solubilisation of RP.

1.1.4 Effect of N fertiliser on solubilisation of RP

Solubilisation of RP by N fertilisers, whether with NH_4^+ or NO_3^- ions, depends on the ion uptake pattern. For instance, plants that revealed a higher uptake of cations than anions by exuding H^+ from their roots may cause low soil pH, which contributes to RP dissolution (Hinsinger *et al*., 2003). The soil pH with ryegrass (*Lolium perenne*) decreased by 1.6 units when fertilised with NH_4^+, while the pH increased by 0.6 units when fertilised with NO_3^- (Gahoonia *et al*., 1992). Legumes require little N in the form of NO_3^- for nitrification, thus they take up more cations than anions, contributing to more H^+ release (Weil, 2000). Apthorp *et al*. (1987) ordered the forms of nitrogen fertiliser according to the effects of increasing plant yield and P uptake from soil alone and from soil fertilised with RP. This order is as follows: ammonium sulphate > sulphurised urea > ammonium nitrate > urea > potassium nitrate.

1.1.5 Effect of microorganisms on solubilisation of RP

Similar to plants, microorganisms can also improve the availability of sparingly soluble inorganic and organic phosphates by releasing substances such as organic ions, enzymes and protons. Recognition that microorganisms are important for P mobilisation in soil has led to use two strategies to improve soil phosphorus availability and plant production:

1. Management of the existing soil microbial populations to optimise their capacity of mobilising P (Khan *et al*., 2010). In this strategy, the knowledge of how soil management practices such as soil amendments, crop rotations, cultivation impact on microbial multitude, diversity and presence of various functional groups and how these relate to the magnitude and availability of different soil P fractions can achieve the success with this approach (Oberson *et al.,* 2001).
2. The use of specific microbial inoculants (Mäder *et al*., 2011; Krey *et al*., 2011). An extent of soil microorganisms able to solubilise precipitated forms of P or mineralise organic P has been characterized. In addition to fungi, the application of rhizobacteria has also been proposed as a component of sustainable nutrient

management systems (Taurian *et al*., 2010). As many attempts to improve plant nutrients using microbial inoculants have focused mainly on fungi (Wakelin *et al*., 2004; Relwani *et al*., 2008). Some species of *Pseudomonas* and *Bacillus* bacteria and *Aspergillus* (Tye *et al*., 2002; Richardson *et al*., 2011) and *Penicillium* fungi are commonly associated with the rhizosphere and improved growth and P nutrition (Kucey *et al*., 1989; Rodriguez and Fraga, 1999; Whitelaw, 2000).

1.2 Bio-fertilisers

Modern agriculture has relied greatly on the stabilised supply of synthetic inputs, mainly fertilisers. However, excessive and imbalanced use of chemical fertilisers may lead to harmful effects. As a result, the need for healthy alternative inputs such as bio-fertilisers is prudent. Bio-fertilisers are living microorganisms that colonise the rhizosphere of plants and promote plant growth by increasing the supply or availability of nutrients to the host plant (Vessey, 2003). They can be applied in different ways; mixed with seeds or sprayed on plants or soil surfaces. The use of bio-fertilisers in crop production might be a wise choice to protect soil health and crop products.

Information provided by recent research has shown that bio-fertilisers have a lot of advantages from different points of view:

- **From an agricultural point of view**

Bio-fertilisers can improve soil fertility and plant growth in the long run. They are associated with the plant root in a symbiotic manner. Involved-microorganisms can safely and readily change complex organic material into simple compounds that can be easily taken up by plant roots. Microorganisms maintain the natural habitat of the soil and they can provide plants with protection against some soil-borne diseases and drought. They improve crop yield by 20-30% and motivate plant growth. They may replace chemical N and P by 25% (Venuturupalli, 2010; Wani and Lee, 1995).

- **From an economic point of view**

Bio-fertilisers may reduce the usage of chemical fertilisers—mainly N and P—up to 50% of the recommended fertiliser amount and would subsequently reduce agricultural costs. The use of decomposed farm waste has become worthwhile as it is a cheap organic

fertiliser. It was reported by Vessey (2003) that decomposed farm waste may increase crop yield by 20-30%.

- **From an ecological point of view**

Bio-fertilisers are friendly substances to surrounding environments (i.e., soils, water sources, and microorganisms) and lead to powerful and healthy plants. Bio-fertilisers do not contain any chemicals; hence, they tend to reduce precipitation of chemicals in soil and enrich the fertility of soils by supplying organic nutrients through microorganisms and their by-products. Rhizobium and *azotobacter* are two examples of bio-fertilisers that add nutrients through the natural process of N fixation for legume and non-legume crops. *Bacillus* and *Pseudomonas* are effective bacteria for solubilising phosphate. An arbuscular mycorrhizal fungus is an example of a fungus that stimulates plant growth through the synthesis of growth promoting substances (Al-Zoubi, 2006).

1.2.1 Arbuscular mycorrhizal fungi (AMF)

Arbuscular mycorrhiza (AM) is a fungus of endo-mycorrhizae that penetrates the cortical cells of roots in vascular plants (Brundrett, 2002). It plays a role in protecting plants against toxic heavy metals (e.g., Zn, Cd, Mn) by accumulating the heavy metals in a mycorrhizal sheath (Clark and Zeto, 2000). In addition, arbuscular mycorrhiza enhances plant tolerance against pathogens by competing with pathogenic microorganisms for space on plant roots, as well as by supplying nutrients to plants (Turk *et al*., 2006). This finding agrees with the work of Gavito and Varela (1995). It was noticed that plants associated with arbuscular mycorrhizal fungi (AMF) became resistant to drought. Good evidence exists showing that plants colonised with AMF have strong growth as a result of reduced nutrient leaching, suppression of weeds (van der Heijden *et al*., 2008) and eventually improved uptake of low mobility mineral nutrients (predominantly P) from the soil solution. This was even shown in severe mineral-stressed environments (Clark and Zeto, 2000). Plants colonised with AMF respond better to applications of insoluble phosphate forms (e.g., tricalcium phosphate and RP) in comparison with non-mycorrhizal roots (Medina *et al*., 2006; 2007). Furthermore, AMF seems to improve soil structure and soil aggregation

by assisting in binding the soil together and increasing the carbon storage (Kabir *et al*., 1997a, Treseder and Allen, 2000).

AMF can be beneficial in different cereal crops. Inoculation of maize with AMF significantly increases the biomass of roots and shoots compared to non-inoculated maize plants (Liang *et al*., 2009). Similar results were reported by Subramanian *et al*. (2009). The application of AMF in maize in the presence of different Zn concentrations in the soil improves the root architectural tangs and availability of soil nutrients.

In the case of legume plants the obvious benefits from AMF are increasing vegetative growth and seed yield; also, nodulation on their root system is improved (Lambert and Weidensaul, 1991; Mathur and Vyas, 2000). It was found that chickpea plants inoculated with the mycorrhizal fungus *Glomus versiforme* had a higher number of nodules, P content in shoots, shoot dry weight and grain yield than un-inoculated plants (Alloush *et al*. 2000). The synergistic interaction between phosphorus solubilising organisms (PSM) and AMF under different experimental conditions has been studied by many researchers. Zaidi *et al*. (2003) observed that in P-deficient soil, dual inoculation of PSM and AMF stimulated plant growth to a greater extent than single inoculation. This is in complete agreement with results reported by Babana and Antoun (2005). Their results showed that inoculating wheat seeds with both RP and AMF increased grain yield in contrast to those where P fertiliser was applied alone. Moreover, root colonisation by indigenous AMF may be affected by the applied *Pseudomonas fluorescens* strain. However, Azcon-Aguilar and Barea (1985) observed that colonisation of a plant by an AMF (*G. mosseae*) was stimulated by a strain of *Pseudomonas* sp. Gryndler and Vosatka (1996) found that *Pseudomonas putida* stimulated maize root colonisation by *Glomus fistulosum*, and that the dual inoculation had a synergistic effect on plant growth. Similar results are observed in other studies (Azcon-Aguilar et al., 1986; Azcon, 1987; Linderman and Paulitz, 1990).

1.2.2 Plant growth promoting rhizobacteria (PGPR)

Pseudomonas spp. are one of the most efficient phosphate solubilising bacteria, plant growth promoting rhizobacteria and are considered as bio-inoculants due to their various

activities in simulating plant growth regulators and exerts beneficial influences on crop growth through several mechanisms such as phyto-hormone and organic acid production, stimulation of nutrient uptake and bio-control of deleterious soil bacteria and phyto-pathogenic fungi (Weller, 1988; Sindhu *et al*., 1999; Ganeshan and Kumar, 2005), improving the availability of soil nutrients, and suppressing soil-borne pathogens (Vyas *et al*., 2009).

Pseudomonas fluorescens (PF) is a gram-negative, rod shaped bacteria occurring in the soil and on plant and water surfaces (Palleroni, 1984). Di-Simine *et al*. (1998) reported that PF produces mainly gluconic acid during phosphate solubilisation. In another study conducted by Hoberg *et al*. (2005), it was declared that the major organic acid produced by PF in media is citric acid. Vyas and Gulati (2009) concluded that each strain of *Pseudomonas* has its own ability of producing organic acids during solubilisation of inorganic phosphates.

Some bacteria are called mycorrhiza helpers, including PF, as they promote mycorrhization. These bacteria utilise several mechanisms to achieve this. Firstly, they stimulate hyphal growth and improve mycorrhizal root colonisation; secondly, they encourage AMF spore germination (Garbaye, 1994; Gryndler *et al*., 2000). Garbaye (1994) suggested that the mechanism of mycorrhiza helpers in modified rhizospheric soil is by either changing the soil pH or altering the ion complexes. One of these mechanisms increases the root cognizance and accession by the fungus. The latter action is achieved either by production of plant hormones or cell wall softening enzymes. Another suggested mechanism is the promotion of fungal growth and then germination of fungal propagules that lead to the production of amino and organic acids, carbon dioxide, vitamins, etc.

1.3 Objectives and hypotheses

Deficiency in the available P for plant is considered to be a major limiting factor to food production in many agricultural soils. Present progression in sustainability include a reasonable investment of soil microbial activities and the use of less expensive,

through less availability sources of plant nutrients such as RP, which may be made available by soil microorganisms. These microorganisms are important in agriculture in order to support the plant nutrients circulation and reduce the need for chemical fertilisers as much as possible (Egamberdiyeva, 2007).

However, the effects of microorganisms may vary considerably when combined with different cultivated crops and also strongly depend on environmental conditions. Therefore, the main objective of this study was to evaluate RP as a P source for maize (*Zea mays*) and faba bean (*Vicia faba*) in combination with single or dual applications of *Pseudomonas fluorescens* (PF-DR54, hereafter referred to as PF) and AMF (*Glomus etunicatum, Glomus intraradices, and Glomus claroideum*). Special attention was given to the vegetation length, N fertilisation and mineral soil content.

All greenhouse experiments had the same treatments that consisted of RP together with single and dual inoculations with AMF and PF. The control in the first three greenhouse experiments consisted of soil without any applications, while the control of the 2009 experiment was modified with N fertiliser. Three additional treatments were established in the 2009 experiments and include single and dual inoculations with both microorganisms without RP addition in order to investigate the interactive effects of RP and microorganisms on plant P nutrition.

The four experiments were established with N supply (soil was taken from the field fertilised with N in spring). However, additional N was added to the 2009 experiment to study the effect of N fertilisation on plant growth and nutrient uptake in combination with the treatments applied.

The purpose of changing the vegetation interval among experiments (75, 55, 45, 55 days after sowing) was to investigate whether time has an effect on the role of AMF and/or PF in dissolving the RP and utilisation of P from the RP.

AM fungi were chosen because they are known to mobilise P from the soil and from an inorganic insoluble P source such as RP and transport it into the host plant (Cardoso and Kuyper, 2006).

The selected PF strain was chosen due to its effects on soil P pools in pot experiments (Krey et al., 2011). As well, it is harmless to humans and animals.

Organic acid production differs from one strain of PF to another (Vyas and Gulati, 2009). The organic acid production by the PF-DR54 strain has not yet been investigated. To investigate organic acid production by PF-DR54, two experiments in greenhouse and *in vitro* were established. These experiments were also designed to investigate the compatibility in quantity and quality of organic acids produced by PF greenhouse and *in vitro*.

The organic acids that were produced by PF *in vitro* were used to study the effect of organic acids on the solubility of RP.

The hypotheses of the present work were:

1. Combined inoculations of P-solubilising bacteria and AMF can increase the fertilising effects of RP.
2. The P solubilising effects may depend on the cultivated crop.
3. The P concentrations in the soil solution may have an influence on the dissolution of RP.
4. The longer vegetation interval and N fertiliser may have a positive effect on the role of AMF and PF in enhancing plant growth and nutrient uptake.
5. Organic acids excreted by PF may contribute to better P availability from RP.

To prove the abovementioned hypotheses, **four work packages were carried out:**

1. Study the effects of single and dual inoculations with PF and/or AMF on availability of P from RP, plant nutrients and soil properties in three conditions:
    - In soils with different P concentrations.
    - In different vegetation intervals.
    - Under effect additional N fertiliser.

2. Determine the organic acids production by single inoculations with either PF or AMF in addition to RP in different soil types.
3. Determine the organic acids production by PF *in vitro* and investigate their ability to dissolve RP.

4. Study the effects at three different concentrations of three organic acids produced by PF *in vitro* on the solubilisation of RP.

2. Materials and methods

2.1 Greenhouse experiments to estimate plant growth, nutrition and soil parameters

Description

Soil

The soils of all pot experiments were sandy loam and originated from the upper soil layer (0–30 cm) from fields of the experimental station of Rostock University, Germany. The nutrient status of the soils was different in dependence of the field they were taken from. All soils had been fertilised with N in spring. Soil characteristics are given in Table 1. The dominating soil type on the field site was a Stagnic Cambisol.

Table 1 Relevant chemical properties of soil used in pot experiments in the greenhouse

Experiment	Pdl	Mgdl	Kdl	OM	pH
	(mg/kg)			%	($CaCl_2$)
2007	62.0	149	99.2	2.20	5.90
2008/1; 2008/2	48.2	234	61.0	2.80	6.86
2009/1; 2009/2	42.9	220	66.3	1.70	6.40

Pdl=double lactate soluble P, Mgdl=double lactate soluble Mg, Kdl=double lactate soluble K, OM=organic matter.

Microbial Inoculants

The commercial mycorrhizal product consisted of *Glomus etunicatum*, *Glomus intraradices* and *Glomus claroideum* with a spore number of 10^5 per litre. The carrier material was expanded clay with a grain size of 2-4 mm and pH 7.5.

The PF inoculum was prepared by growing the bacteria in liquid R2A medium (low nutrient medium for enumeration and cultivation of bacteria from potable water; Difco) at 25°C for

36 h. The bacterial culture was suspended in 0.1 M $MgSO_4$ buffer, washed twice and re-suspended in distilled water at about 10^8 CFU/ml.

Seeds

Seeds of maize (*Zea mays* L.) and faba bean (*Vicia faba* L.) were purchased from Saaten-Union GmbH.

RP

The RP contained 13.1% P (30% P_2O_5) and came from Kola Peninsula, Russia. The extractability of P from the RP powder was 6.7% in water and 57.3% in ammonium citrate, whereas the pH value was 8.4.

Experimental setup

Four pot experiments were conducted in the greenhouse (Uni Rostock) in 2007, 2008 and 2009 under semi-controlled conditions. The information about the management of experiments during this investigation period is summarised in Table 2.

Table 2 Experimental setup of the four pot experiments under semi-controlled conditions.

Experiment	Sowing date	Harvesting time (days)	Application of fertiliser before seed sowing
2007	11 June	75 after sowing (24 August)	RP*
2008/1	15 June	55 after sowing (8 August)	RP*
2008/2	25 Aug	45 after sowing (8 October)	RP*
2009/1	15 June	55 after sowing (8 August)	RP*+ NH_4NO_3 (1.4 g/pot)

RP=rock phosphate. *= the control did not receive any addition from RP.

The experiments were carried out in complete randomised design with four replicates for each treatment using Mitscherlich pots. Each individual pot contained 6 kg air-dried and sieved (10 mm) soil. Eight seedlings were sown in each pot and thinned to four plants after germination.

Four different treatments, in addition to the control, were performed for each crop in the four experiments including C, RP, RP+PF, RP+AMF, and RP+PF+AMF. Three additional treatments were conducted in the fourth experiment consist of PF, AMF, and PF+AMF. The control in each experiment did not receive any inoculum or fertiliser except the control of the fourth one which received 1.4 g NH_4NO_3.

Before sowing, 2 g of RP powder and 25 ml of mycorrhizal inoculum were added to the respective treatments at a depth of 10 cm. After germination, when the second leaf of the plant appeared, 20 ml (20 x 10^8 CFU) of PF was applied. Irrigation was done with distilled water.

Measurements and analyses

The dry matter (DM) yield was determined as dry shoot biomass. The plant stems were cut above ground and dried in an oven at 60°C for 72 h, weighed and ground for further analyses. Before sowing and at the harvest, soil samples were taken and dried at room temperature and then sieved using a 2 mm sieve for chemical analyses (the data are shown in Table 1).

The investigation of mineral nutrients **(Ca, K, Mg and P) in plant biomass** was performed on ground, dried plants. Samples were prepared by filling porcelain dishes with ground plants and drying to ash in a muffle furnace at 550°C for 5 h before cooling to room temperature. The ashes were hydrated with distilled water in volumetric flasks and then 20 ml HCl was added. The samples were boiled for 15 min and subsequently cooled. The solutions were filtered and then made to 100 ml with distilled water. The values for Ca, K and Mg were determined by flame photometry. The P content was evaluated in 35 ml of the solution by adding 15 ml of vanadomolybdate. Samples were measured in a

spectrophotometer at 430 nm (Page *et al*., 1982). Plant P uptake was calculated by multiplying P content of the shoots with the shoot biomass yield.

To determine the N level in plants, nearly 1 g of ground dried plant material was placed into digestion tubes, along with 20 ml of concentrated H_2SO_4 and one digestion tablet consisting of K_2SO_4, $CuSO_4$ and TiO_2. The tubes were heated at 200°C for 30 min, then cooled and cautiously diluted with 70 ml of distilled water.

The protein content was obtained by measuring N concentrations in diluted solutions using a Kjeldahl apparatus according to Jones *et al*. (1991).

The P content in the soil was determined by the double lactate (Pdl) method. For this procedure 12 g of air-dried soil was shaken with 150 ml double lactate solution with pH 3.6 for 90 min and then filtered. Next, 15 ml of vanadomolybdate was added to 25 ml filtered soil solution and the volume completed to 50 ml with double lactate solution. The P content was measured using a spectrophotometer at 430 nm. Soil-filtrated suspension was also used for K and Mg determination: K was measured by flame photometer and Mg was measured by spectrometer.

Soil pH was measured in 0.01 M $CaCl_2$ using a 1:2.5 soil to solution ratio according to Blume *et al*. (2000).

Soil organic matter was determined by drying fine soil in a crucible at 105°C for 4 hrs and by weighing the crucible with the soil (w1). The samples afterwards were put into a muffle furnace at 550°C for 4 hrs and weighed again (w2). Soil organic matter (OM) was calculated as:

OM % = (w1 – w2)/w2 ×100.

The height of plants was measured with a ruler only in 2009.

The number of flowers and pods (in 2009) were determined by counting.

2.2 Determination of organic acid production by PF and AMF colonised by maize plant

Soil

The soil that was utilised in this experiment had the same characteristics as the 2009/1 experiment soil conducted in a greenhouse (Table 1).

Microorganisms, RP and seeds

The AMF inoculums, PF inoculum, RP and maize seeds were the same as those used in the greenhouse experiments.

Experimental setup

Experiment was carried out at 22-25°C. Each clay pot contained 500 g air-dried and sieved (10 mm) soil. Two different types of soil were used (sterilised vs. non-sterilised). Twenty pots were filled with non-sterilised soil and another 20 pots were filled with sterilised soil. Sterilisation was performed using an autoclave at 121°C under 1.1 atmo pressures for one hour.

Prior to sowing, 0.25 g of RP and 7 ml of AMF (10^5 spores per litre) were added at a depth of 10 cm. Two sterilised maize seeds were sown in each pot. After the appearance of the second leaf, the soil was inoculated with 5 ml (5×10^8 CFU) of PF in liquid medium. Plants were irrigated with distilled water when required. Four replicates were used for each treatment. The following treatments were applied in sterilised and non-sterilised soil:

Control (soil without any application), PF, AMF, RP+PF, RP+AMF.

At the end of week eight plants were uprooted and the loosely adhering soil was gently shaken off. This soil, in addition to soil from the pots, was considered as the rhizosphere soil sample. Root particles left in the rhizosphere soil were removed by sieving. All of the soil in each pot was taken and air dried. Then, 10 g of each soil sample was used for preparing the organic acid extraction.

Organic acids were determined in 1:4 (w/v) soil: 10 mM NaH_2PO_4 extract incubated for 4 hrs at 20°C before centrifuging at 15000 rpm for 30 min. The resulting supernatant from each sample was filtered through 0.45 µm nylon filters. Detection and quantification of organic acids were done by using high performance liquid chromatography (HPLC),

supplied with a UV detector at 210 nm. The mobile phase was H_2SO_4 as carrier solution within a column of aminex 87-H (250 x 4.6 mm).

2.3 Determination of organic acid production by PF in vitro

The tryptic soy broth (TSB) used as the growth medium for the microorganisms contains (per 1 litre distilled water) 17 g enzymatic digest of casein, 3 g enzymatic digest of soybean meal, 5 g sodium chloride, 2.5 g dipotassium phosphate and 2.5 g dextrose. Prior to sterilisation, 400 ml TSB with pH 7.3 was divided into 4 portions. The first portion was considered as a control without RP and PF. The second portion had 0.25 g RP added. The third portion was inoculated with PF and supplemented with 0.25 g RP. The fourth was inoculated with only PF (5 x 10^8 CFU); four replicates were done for each treatment. PF was allowed to grow in TSB for seven days at 22-25°C in a rotary shaker at 180 rpm. The cultures were then centrifuged at 15000 rpm for 30 min. The pH changes in TSB cultures were determined daily during the seven days of incubation. The supernatant of each treatment was filtered and the clear solution was collected in flasks, from which 2 ml of each solution was taken to prepare the extract and determine the organic acids as was described above. The volume of each volumetric flask was made up to 100 ml with distilled water.

Solubilised P in solution was extracted with ammonium bicarbonate diethylene triamine penta-acetic acid (AB-DTPA) (Soltanpour and Workman, 1979). The supernatant was decanted and 5 ml of supernatant was added to 20 ml of AB-DTPA extracting solution. The mixture was then shaken on a reciprocating shaker for 15 min at 180 rpm in open flasks. The extract was stored in plastic bottles. Broth P was determined by the ascorbic acid method (Watanabe & Olsen, 1965). One ml of each broth sample extract was placed into 50 ml conical flasks and 9 ml distilled water plus 2.5 ml freshly prepared colour reagent (12 g ammonium molybdate plus 250 ml distilled water and 0.29 mg antimony potassium tartrate in 1000 ml of 5N H_2SO_4) were added. Both solutions were mixed and the volume was raised to 2 litres. Next, 140 ml of this mixture was added to 0.74 g ascorbic

acid and stirred gently. The optical density of the blue colour that had developed after 15 min was measured at 880 nm in a spectrophotometer and the available P was calculated.

For determination of **immobilised P** from the PF biomass in TSB, supernatants were collected after centrifugation. The supernatants were digested using the wet ash method (Chapmann and Pratt, 1961) by adding 10 ml mixed acids (perchloric and nitric acid) to the supernatants in 50 ml volumetric flasks. The flasks were heated on a hot plate until the solutions (about 2 ml) became colourless at which point they were cooled. Each solution was made to 50 ml with distilled water. Immobilised P was estimated by the vanadomolybdophosphoric acid colour method (Jackson, 1958). To each 5 ml solution digest 5 ml colour reagent was added. The colour reagent comprised of 50 g/l ammonium molybdate + 2.5 g/l ammonium vanadate + 20 ml conc. HNO_3 + 250 ml/l HNO_3. After 30 min the optical density of the yellow colour was measured with a spectrophotometer at 430 nm and immobilised P was calculated.

2.4 Determination of the effect of specific organic acids on solubilisation of RP

This experiment was established to study the effect of the organic acids that were produced by PF in the *in vitro* experiment on P availability from RP.

The three organic acids selected—gluconic acid (Glu-A), lactic acid (Lac-A) and succinic acid (Suc-A)—were used on the fact that they were the main organic acids produced by PF *in vitro* experiment.

A weight of 0.25 g of RP was added to 100 ml distilled water in volumetric flasks and then different concentrations (1.42, 2.42 and 3.42 mg/ml) from the three organic acids were individually added to the volumetric flasks. Each acid was used in three treatments. The first treatment consisted of 1.42 mg/ml Glu-A + 0.25 g RP + 100 ml distilled water; the second treatment consisted of 2.42 mg/ml Glu-A + 0.25 g RP + 100 ml distilled water; the third treatment consisted of 3.42 mg/ml Glu-A + 0.25 g RP + 100 ml distilled water. The same preparation was carried out for Lac-A and Suc-A. In the last treatment the three organic acids were applied together. A concentration of 2.42 mg/ml for each acid was used in the last treatment. The concentration was chosen based on observations from

the highest concentration of organic acid detected in the previous experiments conducted *in vitro*. Thus, the total concentration in solution of combined organic acids was 7.26 mg/ml.

Flasks were sterilised at 121°C for 20 min and then shaken on a rotary shaker at 100 rpm at 22-25°C for seven days before the solutions were finally filtered. The control was prepared using the same procedures, but only consisted of distilled water and RP without any organic acids.

Solubilised P content was estimated using the ascorbic acid method (Watanabe and Olsen, 1965).

2.5 Statistical analyses

Data were subjected to the General Linear Model procedure of SPSS version 15. Treatment effects were tested by one-way analysis of variance followed by Duncan's multiple range test used for multiple comparisons. Mean differences were considered significantly different at $p < 0.05$.

The single and interaction effect of RP and microorganisms was tested by univariate analysis of variance. The significance level was identified as follows: * ($p \leq 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$).

Correlation analyses were performed to determine the relationship between the major parameters by using Pearson's correlation coefficient (r).

To make the four experiments comparable, it was applied the liner transformation ratio. The estimated parameters are then factors with which the results of the control are multiplied to estimate the effects of RP, PF and AMF.

In each experiment the relative value to the control was done then the four experiment were analysed together to study the general single effects of the RP, AMF, and PF on DM yield and P uptake with analysis of covariance with the following model:

$$\mathbf{Y = constant\ term + b_1 * RP^{(0-1)} + b_2 * PF^{(0-1)} + b_3 * AMF^{(0-1)}}$$

Y=Dependent variable (DM yield or P uptake). Constant term indicates the increase in DM yield or P uptake without any addition from RP or microbial inoculums. RP, PF and AMF were considered as covariate factors. The b_1, b_2 and b_3 are regression coefficient values

and indicate the percentage contribution of each of RP, PF and AMF in increasing the DM yield or P uptake. To know the increase in the dependent variable (DM yield or P uptake) under the effect of each addition, two prospects were used (0, 1). The number zero indicates no addition and the number one indicates a single addition of the fertiliser or microorganisms. The increases in the constant term were considered significant at $p < 0.05$. The single effect of RP, PF and AMF in increasing the DM yield or P uptake in each experiment alone was also estimated.

3. Results

3.1 Effects of microorganisms inoculation and RP application in soil with medium P content in greenhouse

The aim of this experiment was to study the effect of single and dual inoculations with PF and AMF along with RP for improving the DM yield of maize and faba bean in soil with medium P content.

Effects on plant yield

The DM yield of maize and faba bean increased significantly in all applied treatments compared to the control (Figure 1). Although the soil P content was medium, addition of RP enhanced the yield of both crops about 10% in comparison to the control (Figure 1). Inoculation with either PF or AMF along with RP achieved a higher yield than singly applied RP. Compared to the control, the highest yield was obtained from the dual inoculation of maize and faba bean up to 50% and 19% respectively. Generally, the effect of applied treatments on maize yield was higher than for faba bean yield.

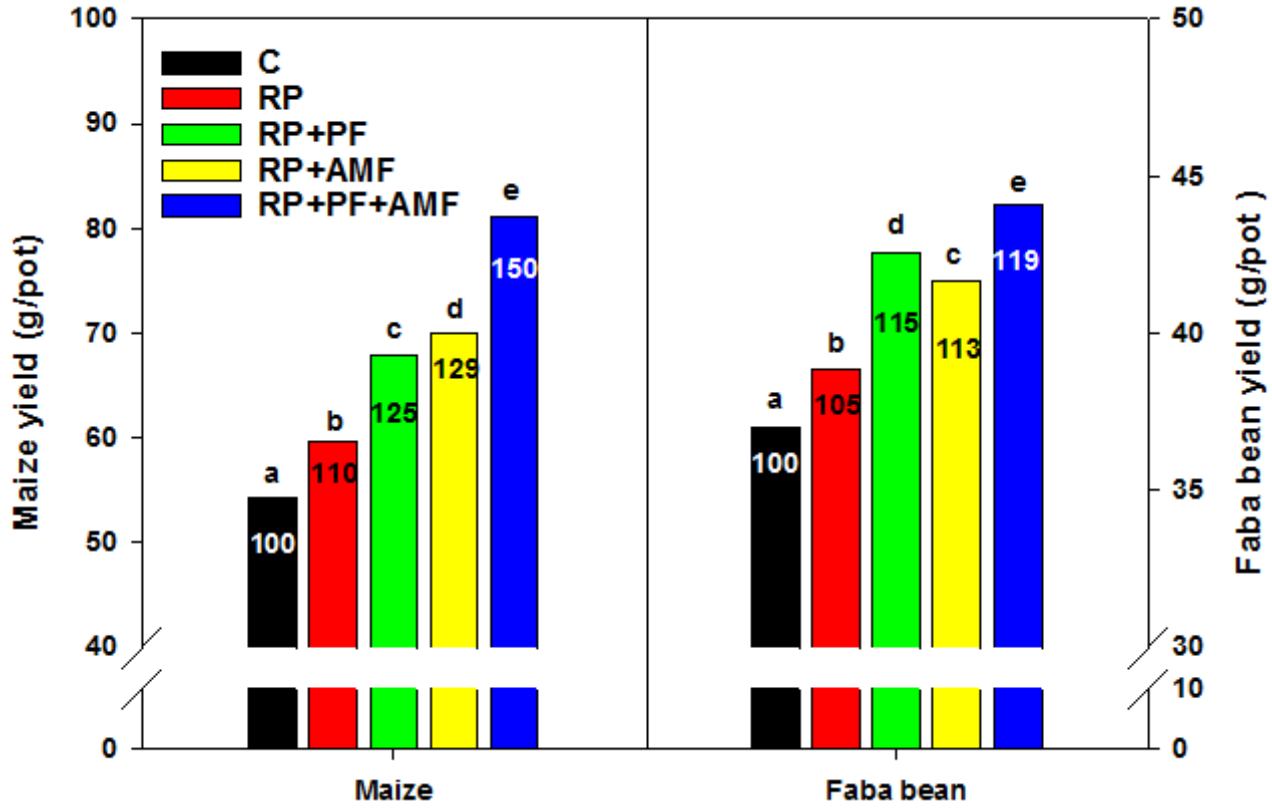

Figure 1 Yield (g per pot and % as relative value to the control) of maize (lift) and faba bean (right) cultivated in soil with medium P content (2007) as affected by RP and microorganisms inoculation.

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi, DM=dry matter of shoot biomass. Means labelled with the same letter are not significantly different at $p < 0.05$, according to Duncan test within each crop. Numbers within the columns indicate values relative to the control.

No correlation was found between the yield of either crop and soil parameters (Table a 1, Table a 2).

Effects on nutrient uptake

As for the yield, the direct application of RP to maize and faba bean increased the P uptake significantly compared to the control in spite of the medium P content in the soil. Similarly, the single inoculation with either PF or AMF caused a significant increase in P uptake in both crops compared to the control (Table 3). Inoculation with PF along with RP

was better in enhancing P uptake than the addition of RP alone. Dual microorganisms inoculation together with RP showed the most distinctive outcome since maize and faba bean P uptakes increased by 112% and 83%, respectively, compared to the control (Table 3).

N uptake increased only significantly in the inoculated treatments in combination with RP fertiliser in both crops compared to the control (Table 3). N uptake of maize plants under the effect of any treatment compared to the control was lower than that of faba bean plants. However, the relative values to the control of maize were higher than in faba bean under the effect of applied treatments (Table 3).

Dual inoculation resulted in the highest outcome regarding K, Mg and Ca uptake by both crops in comparison with the control (Table 5).

Table 3 P, N, K, Ca and Mg uptakes (mg per pot and % as relative values to the control) of maize and faba bean cultivated in soil with medium P content, as affected by RP and microorganisms inoculation.

Treatment	Maize			Faba bean		
	P uptake					
C	134.2	a	100.0	96.6	a	100.0
RP	198.0	b	147.6	109.1	b	112.9
RP+ PF	202.0	c	150.6	118.7	c	122.8
RP+ AMF	203.6	c	151.7	124.9	c	129.2
RP+PF+AMF	284.3	d	211.9	177.1	d	183.3
Mean	**204.4**			**125.3**		
Treatment	N uptake					
C	309.7	a	100.0	1035.3	a	100.0
RP	332.4	a	107.3	1059.8	a	102.4
RP+ PF	410.2	b	132.4	1142.0	b	110.3
RP+ AMF	412.5	b	133.2	1183.6	bc	114.3
RP+PF+AMF	456.9	c	147.5	1228.7	c	118.7
Mean	**384.4**			**1129.9**		
Treatment	K uptake					
C	856.5	a	100.0	616.2	a	100.0
RP	915.6	a	106.9	688.8	bc	111.8
RP+ PF	886.9	a	103.5	619.1	a	100.5
RP+ AMF	1017.0	b	118.7	692.3	c	112.3
RP+PF+AMF	1108.0	b	129.3	717.4	c	116.4
Mean	**981.8**			**666.8**		
Treatment	Mg uptake					
C	122.7	a	100.0	90.2	a	100.0
RP	145.1	b	118.3	99.7	a	110.6
RP+ PF	170.7	c	139.1	112.0	b	124.5
RP+ AMF	176.6	c	143.9	113.0	b	125.3
RP+PF+AMF	201.5	d	164.2	115.0	b	127.3
Mean	**163.3**			**110.0**		
Treatment	Ca uptake					
C	135.7	a	100.0	219.3	a	100.0
RP	160.1	b	118.0	238.0	a	108.5
RP+ PF	165.4	bc	121.8	258.6	b	117.9
RP+ AMF	173.1	c	127.6	268.2	b	122.3
RP+PF+AMF	206.8	d	152.4	284.6	b	129.7
Mean	**168.2**			**262.4**		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Means followed by same letter within columns are not significantly different ($p < 0.05$) according to Duncan test in each element. Numbers after the letters indicate relative values to the control.

Effects on soil parameters

When the soil is medium content with P the soil values revealed a lower response to the applied treatments compared to the plant parameters. However, no significant was found in Pdl values compared to the control with RP amendment and also for treatments with RP+PF, RP+AMF and RP+PF+AMF (**Table 4**). With respect to soil cultivated with faba bean, soil properties showed only small alterations in response to treatments, confirming results seen with maize.

Mgdl in the soil of both crops decreased in tendency for the applied treatments compared to the control. However, no negative correlation was found between Mgdl and Mg uptake despite the uptake increasing and the available Mg in both crops decreasing.

Regarding Kdl, it was noticed that all values of Kdl for both crops decreased a lot compared to the initial value before planting. Moreover, Kdl in maize soil was lower than Kdl in faba bean soil under the same treatments.

The soil pH and the organic matter (OM) cultivated with maize and faba bean did not reveal any significant alterations of the values in all treatments compared to the control.

Table 4 Plant-available P, Mg and K contents in soil with medium P content **(**mg per kg and % as relative value to the control) and changes in soil pH and OM after cultivation of maize and faba bean , as affected by RP and microorganisms inoculation.

Treatment	Maize			Faba bean		
			Pdl			
C	57.3	a	100.0	62.8	a	100.0
RP	61.2	a	106.8	67.2	a	107.0
RP+ PF	59.5	a	103.8	66.6	a	106.1
RP+ AMF	59.8	a	104.4	62.5	a	-99.5
RP+PF+AMF	62.1	a	108.4	68.8	a	109.6
Mean	**60.0**			**65.6**		
			Mgdl			
C	154.1	a	100.0	162.8	a	100.0
RP	150.3	a	-97.5	161.9	a	-99.4
RP+ PF	146.5	a	-95.1	155.1	a	-95.3
RP+ AMF	148.4	a	-96.3	150.1	a	-92.2
RP+PF+AMF	146.2	a	-94.9	155.6	a	-95.6
Mean	**149.1**			**157.1**		
			Kdl			
C	26.2	b	100.0	52.1	ab	100.0
RP	23.2	ab	-88.5	57.6	b	110.0
RP+ PF	22.2	ab	-84.7	48.1	ab	-92.3
RP+ AMF	21.2	a	-80.9	46.9	a	-90.0
RP+PF+AMF	24.3	ab	-92.7	52.1	ab	100.0
Mean	**23.4**			**51.4**		
			pH			
C	5.8	a	100.0	6.0	b	100.0
RP	5.8	a	-99.3	5.8	ab	-98.2
RP+ PF	5.7	a	-98.4	5.7	a	-96.5
RP+ AMF	5.7	a	-98.8	5.8	ab	-98.2
RP+PF+AMF	5.7	a	-98.6	5.8	ab	-97.8
Mean	**5.7**			**5.8**		
			OM %			
C	2.3	a	100.0	2.3	a	100.0
RP	2.3	a	100.0	2.3	a	100.4
RP+ PF	2.3	a	101.3	2.4	a	103.5
RP+ AMF	2.3	a	100.4	2.3	a	101.3
RP+PF+AMF	2.4	a	102.2	2.4	a	102.2
Mean	**2.3**			**2.3**		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Means followed by same letter within columns are not significantly different ($p < 0.05$) according to Duncan test in each element. Numbers after the letters indicate the relative values to the control. Pd, Mgdl and Kdl double lactate soluble P, =Mg, K. OM=organic matter in the soil

3.2 Effects of microorganisms inoculation and RP application in P poor soil in greenhouse

The aim of these two experiments was to study the effects of single and dual inoculations with PF and AMF in combination with RP on growth and nutrient uptake in P poor soil. These studies were conducted with crops sown in June 2008 and August 2008.

Effects on plant yield

In P poor soil, the yield of both cultivated crops in both experiments increased significantly (14-16%) compared to the control when the soil was inoculated with (PF, AMF) along with RP except maize plants in August experiment (Figure 2, Figure 3). Single inoculation with either PF or AMF plus RP resulted in a higher yield only for faba bean sown in the June experiment.

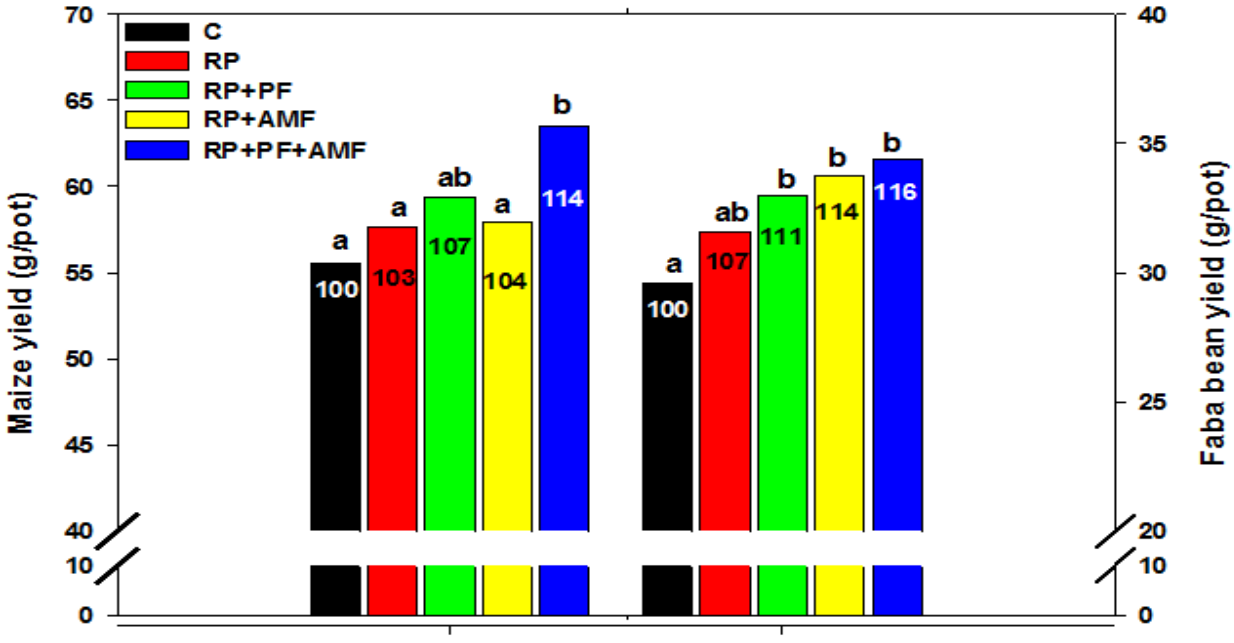

Figure 2: The yield (g per pot and % as relative value to the control) of maize (left) and faba bean (right) sown in June 2008 in P poor soil, as affected by RP and microorganisms inoculation.

(C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi). Means labelled with the same letter are not significantly different in each crop ($p < 0.05$) according to Duncan test. Numbers onto the columns indicate the relative value to the control.

Generally, the yield of maize and faba bean in the June experiment was higher than the yield of both crops in the August experiment (Figure 2, Figure 3).

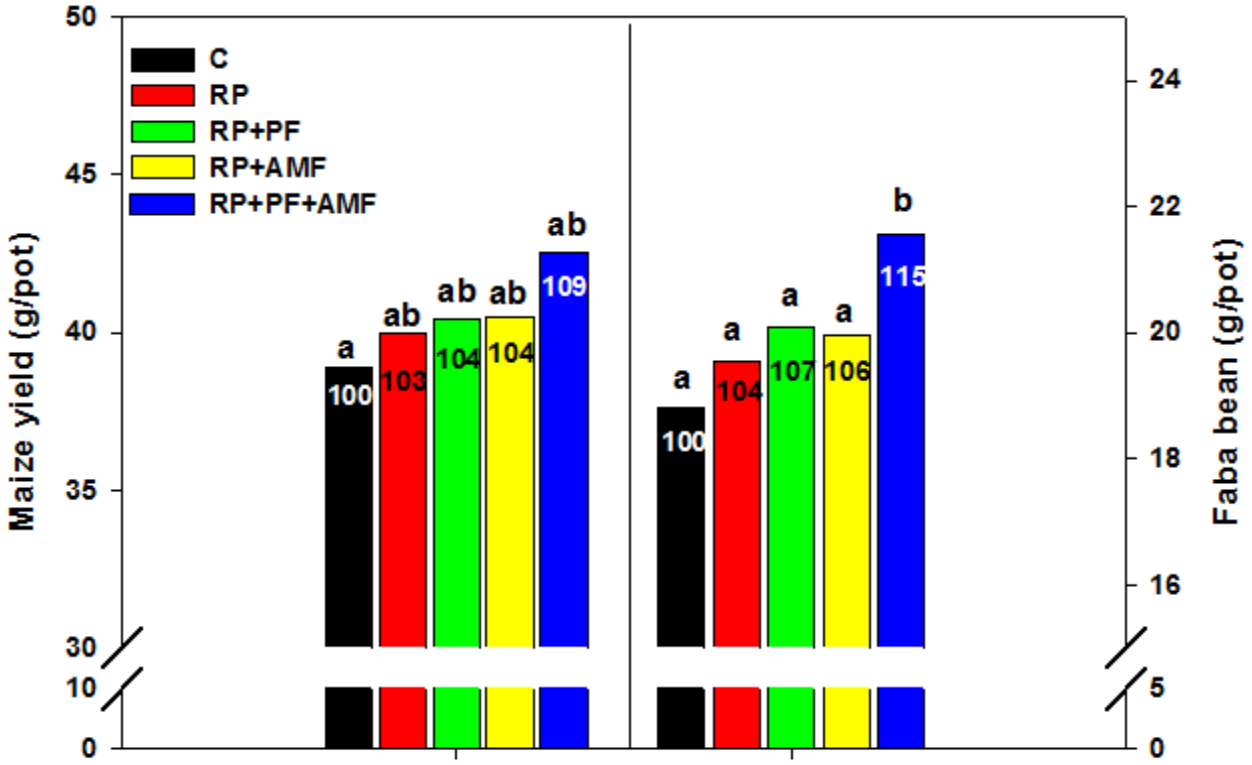

Figure 3: The yield (g per pot and % as relative value to the control) of maize (left) and faba bean (right) sown in August 2008 in P poor soil, as affected by RP and microorganisms inoculation.

(C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi, DM=dry matter of shoot biomass). Means labelled with the same letter are not significantly different in each crop ($p < 0.05$) according to Duncan test. Numbers onto the columns indicate the relative value to the control.

A high significant correlation was found between the DM yield from faba bean and Pdl in the June experiment (Table a 4). Low negative correlations were found in the same experiment between DM yield and pH for faba bean (r = -0.55, $p < 0.05$) and maize (r = -0.50, $p < 0.05$) (Table a 3, Table a 4).

Effects on nutrient uptake

When the soil was low with P, all inoculated treatments caused an increase in P uptake by faba bean in both experiments of 2008 compared to the control (Table 5). In the June experiment, higher P uptake by faba bean was measured in combined treatment (RP+PF+AMF), as well as with single inoculations of microorganisms (RP+PF and RP+AMF). In the August experiment all RP treatments by the faba bean showed higher P uptakes compared to their control. Faba bean plants responded to treatments more than maize plants in both 2008 experiments compared to the control, indicating that faba bean was better able to use P from RP than maize (Table 5).

Compared to control conditions, N uptake by maize plants in the June experiment increased significantly under the effect of any inoculated treatment (Table 5). Faba bean plants also responded to all inoculated treatments in June experiment, since significant increases were observed in N uptake of the inoculated treatments compared to the control. Dual inoculation caused the highest increase in N uptake in both crops in both experiments (Table 5).

Table 5 P and N uptake (mg per pot and % as relative value to the control) by maize and faba bean cultivated in P poor soil, as affected by RP and microorganisms inoculation.

Treatment	Maize						Faba bean					
	June			August			June			August		
	P uptake											
C	72.4	a	100.0	85.7	a	100.0	38.8	a	100.0	36.6	a	100.0
RP	77.7	a	107.3	93.9	a	109.6	42.5	ab	109.5	42.2	b	115.3
RP+PF	81.3	a	112.3	94.9	a	110.7	48.3	bc	124.5	45.1	b	123.2
RP+AMF	78.5	a	108.4	95.5	a	111.4	49.7	bc	128.1	42.4	b	115.8
RP+PF+AMF	81.3	a	112.3	87.7	a	102.3	50.5	c	130.2	44.5	b	121.6
Mean	**78.2**			**91.5**			**46.0**			**42.2**		
	N uptake											
C	293.6	a	100.0	371.4	a	100.0	668.2	a	100.0	658.8	a	100.0
RP	299.4	ab	102.0	376.9	a	101.5	673.5	a	100.8	671.8	ab	102.0
RP+PF	339.3	bc	115.6	447.5	a	120.5	793.4	b	118.7	689.7	ab	104.7
RP+AMF	339.3	bc	115.6	459.8	a	123.8	761.1	b	113.9	684.7	ab	103.9
RP+PF+AMF	362.2	c	123.4	443.9	a	119.5	815.3	b	122.0	738.4	b	112.1
Mean	**326.8**			**419.9**			**742.3**			**688.7**		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Means followed by same letter within columns are not significantly different ($p < 0.05$) according to Duncan test in each element. Numbers after the letters indicate relative values to the control.

K, Mg and Ca uptake in maize plants were promoted when microorganisms were added together to the soil along with RP in both experiments. The K and Ca uptake by faba bean were especially enhanced by microorganisms application in June experiment. The effects of treatment on enhancing K, Mg and Ca uptake were more pronounced in maize than in faba bean plants in both experiments (Table 6). The increase in nutrient uptake was partially higher than the increase in yields.

Table 6 K, Mg and Ca uptakes (mg per pot and % as relative value to the control) by maize and faba bean cultivated in P poor soil, as affected by RP and microorganisms inoculation.

Treatment	Maize						Faba bean					
	June			August			June			August		
	K uptake											
C	656.0	a	100.0	956.8	a	100.0	229.5	a	100.0	280.8	a	100.0
RP	700.8	ab	106.8	1003.6	ab	104.9	246.4	ab	107.4	285.7	a	101.7
RP+PF	759.4	b	115.8	1149.6	b	120.2	273.2	b	119.0	295.3	a	105.2
RP+AMF	724.4	ab	110.4	1140.9	b	119.2	279.2	bc	121.7	295.0	a	105.1
RP+PF+AMF	752.6	b	114.7	1460.1	c	152.6	309.8	c	135.0	320.7	a	114.2
Mean	**718.6**			**1142.2**			**267.6**			**295.5**		
	Mg uptake											
C	153.6	a	100.0	84.1	a	100.0	125.6	a	100.0	81.8	a	100.0
RP	155.3	ab	101.1	85.7	a	101.9	133.0	a	105.9	84.7	a	103.5
RP+PF	166.8	ab	108.6	93.6	ab	111.3	138.2	a	110.0	87.6	ab	107.1
RP+AMF	164.3	ab	107.0	93.4	ab	111.1	141.2	a	112.4	87.8	ab	107.3
RP+PF+AMF	177.2	b	115.4	108.4	b	128.9	141.0	a	112.2	99.0	b	121.0
Mean	**163.4**			**93**			**135.8**			**88.2**		
	Ca uptake											
C	186.4	a	100.0	146.0	a	100.0	409.6	a	100.0	343.7	a	100.0
RP	203.4	ab	109.1	187.1	a	128.2	427.8	a	104.4	364.6	a	106.1
RP+PF	207.4	ab	111.3	262.6	ab	179.9	563.8	b	137.6	390.8	a	113.7
RP+AMF	215.3	ab	115.5	343.2	bc	235.1	580.8	b	141.8	378.5	a	110.1
RP+PF+AMF	274.0	b	147.0	397.6	c	272.3	608.5	b	148.6	414.2	a	120.5
Mean	**217.3**			**267.3**			**518.1**			**378.4**		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test in each element. Numbers after the letters indicate relative values to the control.

Effects on soil parameters

Positive effects of PF and AMF were observed regarding soil values. The dual inoculation with microorganisms in combined with RP recorded the maximum Pdl values despite the high P uptake by plants with this treatment. The addition of RP together with only one inoculant resulted in higher soil P values mainly in the June experiment that had the longer experimental time in contrast to the August experiment with the shorter time. The Pdl in soil cultivated with faba bean exceeded those in maize soil at both times (Table 7).

Table 7 P, Mg and K available in soil (mg per kg and % as relative value to the control) after cultivation of maize and faba bean in P poor soil, as affected by RP and microorganisms.

Treatment	Maize						Faba bean					
	June			August			June			August		
	Pdl											
C	42.8	a	100.0	47.0	a	100.0	46.1	a	100.0	48.6	a	100.0
RP	45.9	ab	107.2	50.2	a	106.8	51.8	b	112.4	50.8	ab	104.5
RP+ PF	49.9	bc	116.6	53.4	a	113.6	53.7	b	116.5	53.8	ab	110.7
RP+ AMF	52.8	c	123.4	51.5	a	109.6	56.4	b	122.3	54.5	ab	112.1
RP+PF+AMF	51.6	c	120.6	53.5	a	113.8	55.3	b	120.0	55.6	b	114.4
Mean	**48.6**			**51.1**			**52.7**			**52.7**		
	Mgdl											
C	260.1	b	100.0	266.3	b	100.0	252.7	a	100.0	259.2	a	100.0
RP	240.5	a	-92.5	262.5	ab	-98.6	258.3	a	102.2	264.4	a	102.0
RP+ PF	257.3	b	-98.9	249.8	a	-93.8	260.6	a	103.2	258.3	a	-99.6
RP+ AMF	255.1	b	-98.1	251.8	a	-94.6	263.4	a	104.3	255.9	a	-98.7
RP+PF+AMF	243.3	a	-93.5	256.1	ab	-96.2	264.4	a	104.6	263.4	a	101.6
Mean	**251.3**			**257.3**			**259.9**			**260.3**		
	Kdl											
C	30.5	ab	100.0	28.7	a	100.0	37.8	a	100.0	31.3	a	100.0
RP	28.7	a	-94.1	40.6	c	141.4	39.2	ab	103.8	33.1	ab	106.0
RP+ PF	32.0	b	104.9	38.8	bc	135.4	36.5	a	-96.6	31.0	a	-99.2
RP+ AMF	31.0	b	101.7	33.4	ab	116.4	42.9	b	113.5	39.1	b	125.2
RP+PF+AMF	31.1	b	102.0	37.3	bc	130.0	40.9	ab	108.4	39.2	b	125.4
Mean	**30.7**			**35.8**			**39.4**			**34.7**		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Pdl=double lactate soluble P, Mgdl= double lactate soluble Mg, Kdl= double lactate soluble K. Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test in each element. Numbers after the letters indicate relative values to the control. Minus sign before the relative values indicates decreased values compared to the control.

Significant decreases in soil pH were recorded for combined treatment compared to the control in each experiment in 2008 in both crops (Table 8). In general, all OM values after planting were increased over OM values before planting.

Table 8 Soil pH, OM after cultivation of maize and faba bean in P poor soil, as affected by RP and microorganisms inoculation.

Treatment	Maize						Faba bean					
	June			August			June			August		
	pH											
C	6.93	c	100.0	6.94	b	100.0	6.93	c	100.0	6.93	b	100.0
RP	6.82	bc	-98.4	6.93	b	-99.9	6.93	c	100.0	6.90	b	-99.6
RP+ PF	6.08	a	-87.7	6.56	b	-94.5	6.87	c	-99.1	6.68	b	-96.4
RP+ AMF	6.56	b	-94.7	6.49	ab	-93.5	6.57	b	-94.8	6.54	ab	-94.4
RP+PF+AMF	5.98	a	-86.3	6.08	a	-87.6	6.06	a	-87.4	6.19	a	-89.3
Mean	**6.47**			**6.60**			**6.67**			**6.65**		
	OM %											
C	2.97	c	100.0	2.85	a	100.0	2.99	b	100.0	2.86	ab	100.0
RP	2.94	bc	-99.0	2.85	a	100.0	2.85	a	-95.3	2.83	a	-99.2
RP+ PF	2.86	ab	-96.3	2.94	b	103.3	2.92	ab	-97.7	2.94	cd	103.0
RP+ AMF	2.82	a	-94.9	3.01	bc	105.8	2.97	b	-99.3	2.99	d	104.7
RP+PF+AMF	2.87	ab	-96.6	2.90	ab	101.6	2.85	a	-95.3	2.91	bc	101.9
Mean	**2.89**			**2.91**			**2.92**			**2.91**		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi, OM=organic matter in the soil. Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test. Numbers after the letters indicate relative values to the control. Minus sign before the relative values indicates a decrease in the value compared to the control.

3.3 Effects of microorganisms inoculation and RP application in soil fertilised with additional N in greenhouse

This study investigated effects of additional N supply during plant growth on the role of PF and AMF in combination with RP regarding yield and nutrient uptake of maize and faba bean

Effects on plant growth parameters

With additional N soil the combined treatment (RP+AMF+PF) caused the greatest increases in terms of plant height of maize and faba bean, maize flower number and faba bean pod number compared to control plants (Table 9, Table 10). The highest protein content in both crops compared to the control was recorded when RP was added along with AMF (Table 10).

Table 9 Plant height (cm and % as relative to the control) of maize and faba bean, as affected by RP and microorganisms inoculation in the experiment with additional N.

Treatment	Maize			Faba bean		
	Plant height					
C	113.8	a	100.0	69.3	a	100.0
RP	126.1	b	110.9	75.6	b	109.2
PF	123.1	b	108.2	79.9	b	115.4
RP+PF	130.1	bc	114.4	106.8	c	154.2
AMF	121.8	ab	107.0	77.9	b	112.4
RP+AMF	134.5	e	118.2	79.2	b	114.3
AMF+PF	139.3	de	122.4	104.3	c	150.5
RP+PF+AMF	156.0	f	137.1	124.1	d	179.1
Mean	**130.6**			**89.6**		
Source of variation						
(RP)	*			ns		
microorganisms	***			***		
(RP)×microorganisms	ns			**		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test in each element. Numbers after the letters indicate relative values to the control (%). ***** Significant at $p \leq 0.05$, ****** Significant at $p \leq 0.01$**,** ******* Significant at $p \leq 0.001$, ns= not significant.

Table 10 Protein content (%), flower number per pot of maize and pod number per pot of faba bean as affected by RP and microorganisms inoculation in the experiment with additional N.

Treatment	Maize			Faba bean		
	Protein					
C	3.9	a	100.0	16.0	ab	100.0
RP	4.2	a	107.8	14.6	a	91.4
PF	4.7	ab	122.5	15.3	a	95.3
RP+PF	7.4	d	190.2	20.4	d	127.2
AMF	6.0	bc	153.7	18.9	bcd	118.3
RP+AMF	4.9	ab	125.3	16.2	abc	101.2
AMF+PF	6.2	cd	159.7	17.8	abcd	111.2
RP+PF+AMF	6.8	cd	176.2	19.4	cd	120.9
Mean	5.5			17.3		
Source of variation						
(RP)	**			**		
microorganism	***			***		
(RP)× microorganism	ns			***		
Treatment	**Flower number**			**Pod number**		
	Maize			**Faba bean**		
C	1.0	a	100.0	5.0	a	100.0
RP	1.0	a	100.0	9.3	a	185.0
PF	1.3	ab	125.0	10.0	ab	200.0
RP+PF	1.8	bc	175.0	12.3	bc	245.0
AMF	1.3	ab	125.0	11.5	ab	230.0
RP+AMF	2.3	c	225.0	14.0	cd	280.0
AMF+PF	3.3	d	325.0	15.5	d	310.0
RP+PF+AMF	4.0	e	400.0	19.3	e	385.0
Mean	2.0			12.1		
(RP)	**			**		
microorganism	***			***		
(RP)× microorganism	ns			***		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test. Numbers after the letters indicate values relative values to the control (%). ****** Significant at $p \leq 0.01$, ******* Significant at $p \leq 0.001$, ns=not significant.

The results showed that the DM yields of maize and faba bean plants increased significantly under all inoculated treatments compared to the control.

The highest increment in maize and faba bean yield was obtained when the soil was inoculated with PF and AMF along with RP; 17% for maize and 70% for faba bean (Figure 4).

It was found that faba bean plants responded more than maize plants to all inoculations with and without RP compared to controls. All relative values of the control for DM yield of faba bean plants were higher than those for maize plants, except for the RP treatment. DM yield of both crops correlated negatively with soil pH (Table a7, Table a8).

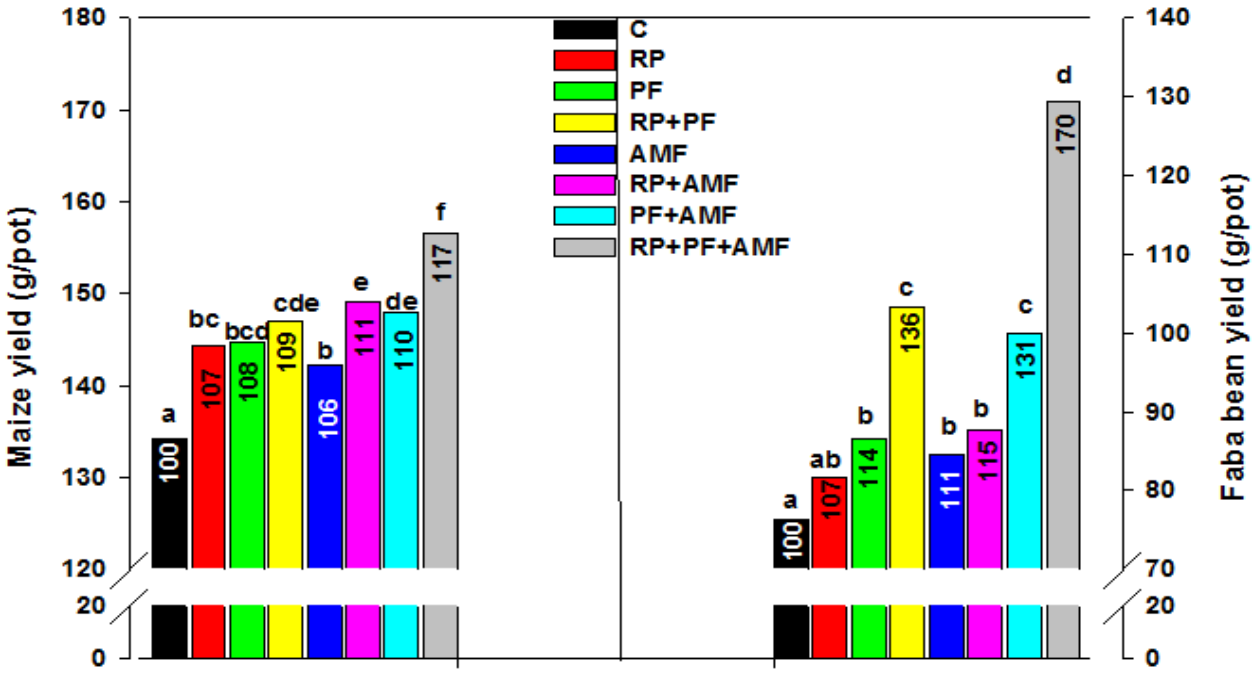

Figure 4 DM yields of maize (right) and faba bean (left) plants (g per pot and % relative value to the control) in the experiment with additional N as affected by RP and microorganisms inoculation.

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Numbers onto columns of the same crop indicate values relative to the control. Different letters above the columns of the same crop indicate significant differences between the treatments ($p < 0.05$) according to Duncan test.

The interactive effect between RP and microorganisms was clearly more noticeable in faba bean plants than in maize.

Table 11 Single and combined effects of microorganisms and RP on yield of maize and faba bean in the experiment with additional N.

Source of variation	DM yield	
	Maize	Faba bean
(RP)	***	***
microorganisms	***	***
(RP)× microorganisms	*	***

***** Significant at $p \leq 0.05$ ****** Significant at $p \leq 0.01$ ******* Significant at $p \leq 0.001$

Effects on nutrient uptake

When RP was added, P and N uptake increased in tendency to the control in maize and faba bean plants (**Table 12**). Nevertheless, addition of RP together with single and dual inoculations with PF and AMF increased P and N uptake significantly compared to the control. The combined treatment (RP+PF+AMF) demonstrated the highest increase in P and N uptake in both crops compared to the control. The effect of the combined treatment (RP+PF+AMF) on P and N uptake was more pronounced in faba bean than in maize crop.

Table 12 P and N uptake (mg per pot and % as relative value to the control) by maize and faba bean as affected by RP and microorganisms inoculation in the experiment with additional N.

Treatment	Maize			Faba bean		
	P uptake					
C	128.5	a	100.0	102.0	a	100.0
RP	131.1	a	102.0	118.4	abc	116.0
PF	151.1	b	117.6	111.7	ab	109.5
RP+PF	173.5	c	135.0	141.2	cd	138.4
AMF	137.9	ab	107.3	118.7	abc	116.4
RP+AMF	152.2	b	118.4	151.1	d	148.1
AMF+PF	174.9	c	136.1	134.2	bcd	131.5
RP+PF+AMF	216.0	d	168.1	194.5	e	190.6
Mean	**158.2**			**134.0**		
Source of variation						
(RP)	*			***		
microorganisms	***			***		
(RP)×microorganisms	*			**		
Treatment	**Maize**			**Faba bean**		
	N uptake					
C	832.5	a	100.0	1911.1	a	100.0
RP	962.7	a	115.6	1958.9	a	102.5
PF	1352.8	bc	162.5	2559.1	bc	133.9
RP+PF	1755.6	d	210.9	2855.8	c	149.4
AMF	1095.3	ab	131.6	2115.6	ab	110.7
RP+AMF	1139.6	b	136.9	2674.5	c	139.9
AMF+PF	1462.9	cd	175.7	2849.6	c	149.1
RP+PF+AMF	1701.6	d	204.4	4163.6	d	217.9
Mean	**1287.9**			**2636.0**		
(RP)	ns			**		
microorganisms	***			***		
(RP)×microorganisms	ns			***		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Numbers after the letters indicate relative values to the control (%). Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test in each element. ***** Significant at $p \leq 0.05$. ****** Significant at $p \leq 0.01$**, ***** Significant at $p \leq 0.001$, ns= not significant.

Maize plants recorded the highest increases of K, Mg and Ca uptake in the RP+PF and RP+PF+AMF treatments. The highest increases in element uptake in faba bean plants were observed with dual inoculation along with RP treatment (**Table 13**).

Table 13 K, Mg and Ca uptake (mg per pot and % as relative value of the control) by maize and faba bean, as affected by RP and microorganisms inoculation in the experiment with additional N

Treatment	Maize			Faba bean		
			K uptake			
C	795.4	a	100.0	639.0	a	100.0
RP	832.2	ab	104.6	692.7	a	108.4
PF	979.7	abc	123.2	1168.6	b	182.9
RP+PF	1290.3	e	162.2	1691.9	c	264.8
AMF	879.8	abc	110.6	703.9	a	110.2
RP+AMF	1022.3	bcd	128.5	923.3	b	144.5
AMF+PF	1069.2	cd	134.4	1158.0	b	181.2
RP+PF+AMF	1205.6	de	151.6	2363.9	d	370.0
Mean	**1009.3**			**1167.7**		
Source of variation						
(RP)	**			**		
microorganisms	***			***		
(RP)×microorganisms	ns			***		
			Mg uptake			
C	315.9	ab	100.0	270.6	a	100.0
RP	335.7	abc	106.3	294.1	ab	108.7
PF	353.2	bcd	111.8	300.4	ab	111.0
RP+PF	380.4	d	120.4	336.5	bc	124.3
AMF	294.8	a	93.3	312.2	ab	115.3
RP+AMF	345.2	abcd	109.3	378.0	c	139.7
AMF+PF	345.2	abcd	109.3	358.7	c	132.5
RP+PF+AMF	361.5	cd	114.4	475.6	d	175.7
Mean	**341.5**			**340.8**		
(RP)	ns			**		
microorganisms	**			***		
(RP)×microorganisms	ns			**		
			Ca uptake			
C	337.2	a	100.0	1323.7	a	100.0
RP	367.0	a	108.8	1463.1	a	110.5
PF	412.5	ab	122.3	1536.3	a	116.1
RP+PF	496.2	c	147.1	1608.8	ab	121.5
AMF	365.3	a	108.3	1523.8	a	115.1
RP+AMF	400.8	ab	118.9	1885.6	b	142.4
AMF+PF	444.5	bc	131.8	1849.3	b	139.7
RP+PF+AMF	493.6	c	146.4	2328.3	c	175.9
Mean	**414.6**			**1689.9**		
(RP)	*			ns		
microorganism	***			***		
(RP)× microorganism	ns			**		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Numbers after the letters indicate values relative to the control (%). Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test in each element. ***** Significant at $p \leq 0.05$. ****** Significant at $p \leq 0.01$, ******* Significant at $p \leq 0.001$. ns=not significant.

Generally it was observed that all inoculation treatments in single or dual form, along with RP, trend to exceed all corresponding inoculation treatments without RP by enhancing not only P uptake, but also all other nutrient uptakes, yield and all plant growth parameters.

Effects on soil parameters

In soil cultivated with maize or faba bean plants, Pdl was increased significantly in all inoculated treatments along with RP compared to the control. Pdl in soil of faba bean plants was higher than Pdl in soil cultivated with maize plants which can be explained with the higher P uptake of maize (**Table 14**).

Table 14 Plant-available P content in the soil **(**mg per kg and % as relative value to the control) of maize and faba bean as affected by RP and microorganisms inoculation in the experiment with additional N

Treatment	Maize			Faba bean		
	Pdl					
C	42.2	a	100.0	45.9	a	100.0
RP	48.9	b	115.9	54.6	cd	119.0
PF	43.1	a	102.1	50.3	bc	109.6
RP+PF	49.7	b	117.8	59.3	e	129.2
AMF	42.2	a	100.0	51.1	bc	111.3
RP+AMF	48.5	b	114.9	60.1	e	130.9
AMF+PF	42.2	a	100.0	49.7	ab	108.3
RP+PF+AMF	48.3	b	114.5	58.1	de	126.6
Mean	**45.6**			**53.6**		
Source of variation						
(RP)	***			***		
microorganism	ns			**		
(RP)× microorganism	ns			ns		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Pdl=double lactate soluble P, Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test, ****** Significant at $p \leq 0.01$**, ***** Significant at $p \leq 0.001$, ns= not significant. Numbers after the letters indicate values relative to the control (%).

No significant differences between the treatments were found for the available Mg contents in soil cultivated with faba bean (**Table 15**).

Table 15 Plant-available Mg and K content in the soil (mg per kg and % as relative value of the control) of maize and faba bean as affected by RP and microorganisms inoculation in the experiment with additional N

Treatment	Maize			Faba bean		
	Mgdl					
C	179.4	ab	100.0	208.1	a	100.0
RP	183.8	ab	102.4	196.9	a	-94.6
PF	177.5	ab	-99.0	203.8	a	-97.9
RP+PF	187.5	b	104.5	205.0	a	-98.5
AMF	178.8	ab	-99.7	208.8	a	100.3
RP+AMF	181.9	ab	101.4	199.4	a	-95.8
AMF+PF	180.0	ab	100.3	206.9	a	-99.4
RP+PF+AMF	175.0	a	-97.6	202.5	a	-97.3
Mean	**180.5**			**203.9**		
Source of variation						
(RP)	ns			ns		
microorganism	ns			ns		
(RP)× microorganism	ns			ns		
Treatment	**Maize**			**Faba bean**		
	Kdl					
C	13.7	a	100.0	53.2	bc	100.0
RP	22.6	b	164.8	52.0	abc	-97.7
PF	14.1	a	102.7	42.0	ab	-78.9
RP+PF	15.6	a	113.3	45.9	abc	-86.3
AMF	15.9	a	115.8	66.9	d	125.8
RP+AMF	13.9	a	101.1	42.9	ab	-80.7
AMF+PF	15.6	a	113.7	56.7	cd	106.5
RP+PF+AMF	14.6	a	106.0	40.8	a	-76.6
Mean	**15.7**			**50.0**		
(RP)	ns			ns		
microorganism	*			***		
(RP)× microorganism	ns			ns		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. Mgdl= double lactate soluble Mg, Kdl= double lactate soluble K. Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test. ***** Significant at $p \leq 0.05$. ******* Significant at $p \leq 0.01$. ns= not significant. Numbers after the letters indicate values relative to the control (%). Minus sign before the relative values indicate a decrease in the value compared to the control.

No significant increase was found in soil OM content in any of the applied treatments in maize plants compared to the control (**Table 16**). Soil pH dropped consistently with all inoculated treatments in both crops compared to the control and to the pH before planting.

The lowest pH value was noticed under the combined treatment consisting of AMF, PF and RP in both crops (**Table 16**).

Table 16 Soil OM and pH values after cultivation of maize and faba bean as affected by RP and microorganisms inoculation in the experiment with additional N

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Treatment	OM %					
	Maize			Faba bean		
C	2.46	a	100.0	2.47	ab	100.0
RP	2.59	a	104.9	2.79	b	113.0
PF	2.47	a	100.2	1.79	a	-72.5
RP+PF	2.53	a	102.7	2.13	ab	-86.0
AMF	2.55	a	103.5	2.50	ab	101.2
RP+AMF	2.52	a	102.4	1.94	a	-78.5
AMF+PF	2.64	a	107.2	2.90	b	117.2
RP+PF+AMF	2.55	a	103.4	2.14	ab	-86.6
Mean	**2.5**			**2.3**		
Source of variation						
(RP)	ns			ns		
microorganisms	ns			*		
(RP)× microorganisms	ns			ns		
Treatment	**pH**					
	Maize			**Faba bean**		
C	6.35	e	100.0	6.31	e	100.0
RP	6.24	de	-98.3	6.27	de	-99.4
PF	6.15	cd	-96.9	6.15	cd	-97.5
RP+PF	6.12	bcd	-96.4	6.10	bc	-96.7
AMF	6.09	bc	-95.9	6.18	cd	-97.5
RP+AMF	6.05	bc	-95.3	6.13	bcd	-97.1
AMF+PF	5.99	b	-94.3	5.99	a	-94.9
RP+PF+AMF	5.71	a	-89.9	5.83	a	-92.4
Mean	**6.1**			**6.1**		
(RP)	*			ns		
microorganisms	***			***		
(RP)× microorganisms	*			ns		

C=control, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi, OM=organic matter in the soil. Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test. Numbers after the letters indicate values relative to the control (%). ***** Significant at $p \leq 0.05$. ******* Significant at $p \leq 0.01$. ns=not significant. Minus sign before the relative values indicates a decrease of the value compared to the control.

3.4 Factors affecting DM yield and P uptake of crops in greenhouse experiments

It is known that the four experiments have different conditions, so it is difficult to estimate the average effects each parameter on the yield and P uptake. In this part of the study, it can make the four experiment comparable (see section 2.5- Statistical analyses). The average effects of each single factor (RP, PF, and AMF) on DM yield and P uptake in the four greenhouse experiments together were estimated. The single effect of each factor on DM yield and P uptake in each experiment with their special conditions were also estimated.

Parameters affecting DM yield

Generally, the inoculation with RP, PF and AMF contributed to enhance maize DM yield by 7 % for each factor with percentage of the total variability (Partial eta-squared) 10%, 12% and 13% respectively. The DM yield of faba bean was enhanced significantly higher than maize under effect each addition from PF and AMF (**Table 17**).

Table 17 Parameters affecting DM yield (g/pot) of maize and faba bean plants during the four experiments.

Parameter estimates*							
Dependent variable: DM yield of **maize** (four experiments)							
Parameter	Regression coefficient B	Standard error	T	Sig.	95%-confidence interval of B		Partial eta-squared
					Lower bound	Upper bound	
Constant term	0.993	0.019	51.127	0.000	0.954	1.031	0.967
RP	0.067	0.022	3.041	0.003	0.023	0.111	0.095
PF	0.070	0.020	3.450	0.001	0.030	0.110	0.119
AMF	0.074	0.020	3.665	0.000	0.034	0.114	0.132
Dependent variable: DM yield of **faba bean** (four experiments)							
Constant term	1.005	0.025	40.404	0.000	0.956	1.055	0.949
RP	0.027	0.028	0.949	0.345	-0.029	0.083	0.010
PF	0.153	0.026	5.919	0.000	0.102	0.205	0.285
AMF	0.105	0.026	4.070	0.000	0.054	0.157	0.158

RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. (n=60, Significant at $p < 0.05$). Constant term indicates the increase in DM yield without any treatment additions (the control). Parameter tested by analysis of covariance. * All data were analysed as standard values to the control.

When comparing results from the three years, the 2007 experiment (the longer experimental time) showed significant increases in DM yields of maize and faba bean plants with any addition of fertiliser or inoculum (Table a 9, Table a 10). In the 2009 experiment when the plants were fertilised with N, again the yield of both crops increased significantly with any addition of fertiliser or inoculum (Table a 9, Table a 10). However, in the 2008 experiments (the shorter experimental time compared with the other experiments) no significant contribution to DM yield of either maize or faba bean was noted for any fertiliser or inoculum addition.

Factors affecting P uptake

From Table 18, it can be seen that the supply with each RP, PF and AMF contributed to increase significantly P uptake by maize and faba bean. However, P uptake of faba bean increased clearly than maize by the single effect of each factor.

Table 18 Parameters affecting P uptake (mg/pot) of maize and faba bean plants during the four experiments.

Parameter estimates*							
Dependent variable: P uptake of maize plants (four experiments)							
Parameter	Regression coefficient B	Standard error	T	Sig.	95%-confidence interval of B		Partial eta-squared
					Lower bound	Upper bound	
Constant term	0.994	0.050	19.744	0.000	0.894	1.094	0.816
RP	0.131	0.057	2.280	0.025	0.017	0.245	0.056
PF	0.138	0.052	2.623	0.010	0.033	0.242	0.072
AMF	0.188	0.052	3.576	0.001	0.083	0.292	0.127
Dependent variable: P uptake of faba bean plants (four experiments)							
Constant term	0.971	0.037	26.182	0.000	0.897	1.045	0.886
RP	0.153	0.042	3.632	0.000	0.069	0.237	0.130
PF	0.175	0.039	4.536	0.000	0.098	0.252	0.189
AMF	0.213	0.039	5.525	0.000	0.137	0.290	0.258

RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. (n=60, significant at $p < 0.05$). Constant term indicates the increase in DM yield without any treatment additions (the control). Parameter tested by analysis of covariance. * All data were analysed as standard values.

All factors (RP, PF and AMF) contributed toward significantly increasing P uptake by maize in the experiment with the longest vegetation interval, as well as the additional N experiment (Table a 11). Microorganisms (PF, AMF) caused a significant increase in faba bean P uptake in both previous experiments (Table a 12).

3.5 Production of organic acids by PF and AMF colonised with maize plants

Quantities of organic acids in non-sterilised soil exceeded those in sterilised soil in all treatments (Table 19). The addition of RP with PF in both types of soil resulted in production of the same organic acids that were found in PF treatment but without RP. The same organic acids were detected in AMF treatments regardless of the presence or absence of RP in both soil types. However, the addition of RP to the single inoculation with PF or AMF caused slight increases in the quantity of organic acids compared to those in the absence of RP (Table 19).

Table 19 Effects of RP addition on quantity and type of organic acids (mM/l) produced by PF and AMF colonised with maize plants in greenhouse (2009/2)**.**

Type of soil	Treatment	Organic acid							
		Glu-A	Lac-A	Suc-A	Cit-A	For-A	Mal-A	Oxa-A	αKG-A
Non-sterilised	Control	NF	NF	NF	0.06	NF	0.07	0.12	0.07
	PF	10.75	1.20	0.68	0.06	NF	0.06	0.11	0.08
	PF+RP	10.86	1.48	0.78	0.07	NF	0.09	0.13	0.09
	AMF	NF	3.92	NF	4.32	1.61	0.06	0.12	0.08
	AMF+RP	NF	4.65	NF	4.55	1.78	0.07	0.14	0.09
Sterilised	Control	NF	NF	NF	NF	NF	NF	NF	NF
	PF	9.13	1.06	0.68	NF	NF	NF	NF	NF
	PF+RP	10.76	1.42	0.75	NF	NF	NF	NF	NF
	AMF	NF	3.82	NF	4.26	1.54	NF	NF	NF
	AMF+RP	NF	4.54	NF	4.51	1.73	NF	NF	NF

NF=not found, RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi, Glu-A=gluconic acid, Lac-A=lactic acid, Suc-A=succinic acid, Cit-A=citric acid, For-A=formic acid, Mal-A=malic acid, Oxa-A=oxalic acid, αKG-A=α-Ketoglutaric acid.

The non-sterilised soil showed a small amount of citric acid (Cit-A), malic acid (Mal-A), oxalic acid (Oxa-A) and α-ketoglutaric acid (αKG-A) with PF and PF+RP treatments

compared to the other organic acids under investigation. These organic acids were not detected in sterilised soil.

Mal-A, Oxa-A and αKG-A were also found in quantities lower than the other organic acids under investigation when AMF was added in single form or in combination with RP to non-sterilised soil. Unlike the other organic acids under investigation, Mal-A, Oxa-A and αKG-A were absent from sterilised soil with the same treatments (Table 19).

Regardless of the presence or absence of RP, Glu-A was the predominant organic acid under the effect of inoculation with PF in both soil types, while Cit-A had the highest quantity among the other organic acids when the soils were inoculated with AMF (Table 19).

3.6 Effects of organic acids production by PF in vitro on P solubilisation and P immobilisation from RP

In liquid growth medium, the organic acids produced by PF were estimated. As well immobilised P in PF biomass and when RP was added was determined. Solubilised P from RP under effects of PF was also measured.

Organic acids excretion by PF *in vitro* (Table 20) were similar to in greenhouse (2009/2) in sterilised soil (Table 19). Similarly, Glu-A was the major organic acid detected under the effect of inoculation with PF in single form along with RP under *in vitro*.

The highest production of the three organic acids *in vitro* (Glu-A, Lac-A and Suc-A) was recorded for PF combined with RP compared to the single inoculation with PF.

Table 20 Effect of RP addition on quantity and type of organic acids (mM/l) produced by PF *in vitro.*

Treatment *in vitro*	Organic acid		
	Glu-A	**Lac-A**	**Suc-A**
Control (TSB)	NF	NF	NF
RP	NF	NF	NF
PF	9.18	1.09	0.69
PF+RP	12.34	1.57	0.79

NF=not found, RP=rock phosphate, PF=*Pseudomonas fluorescens*, Glu-A=gluconic acid, Lac-A=lactic acid, Suc-A=succinic acid, TSB=tryptic soy broth.

It was shown in Table 21 that incubation of PF in medium amended with RP resulted in higher organic acids excretion into the growth medium and a higher percentage immobilised P compared to the addition of PF alone. In addition, RP was highly solubilised when it was added together with PF compared to the single application. The pH decreased clearly in PF treatment and recorded the lowest value in PF+RP treatment.

Table 21 Effect of PF inoculation on solubilisation and immobilisation of P (from 0.25 g RP) and quantity of all organic acids (Glu-A, Lac-A and Suc-A) production *in vitro.*

Treatment *in vitro*	Organic acid (mM/l)	Solubilised P %	Immobilised P %	pH
Control (TSB)	ND	ND	ND	7.3 d
RP	ND	6.32 a	ND	6.8 c
PF	10.96 a	ND	0.06 a	3.7 b
RP+PF	14.69 b	19.59 b	0.43 b	3.1 a

Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test. RP=rock phosphate, PF=*Pseudomonas fluorescens*, TSB=tryptic soy broth, ND=not detected

The pH of PF treatments over the seven days of incubation is shown in Figure 5. The pH of PF treatment during solubilisation of RP dropped from 7.3 to 3.1 compared to the control which remained constant at 7.3 (**Figure 5**). Also, PF alone caused a decrease in pH from 7.3 to 3.7 over the seven days of incubation.

The addition of RP alone did not cause a clear change in pH during the seven days. It can be noticed that pH of the PF media during solubilisation of RP was lower than the pH with PF alone.

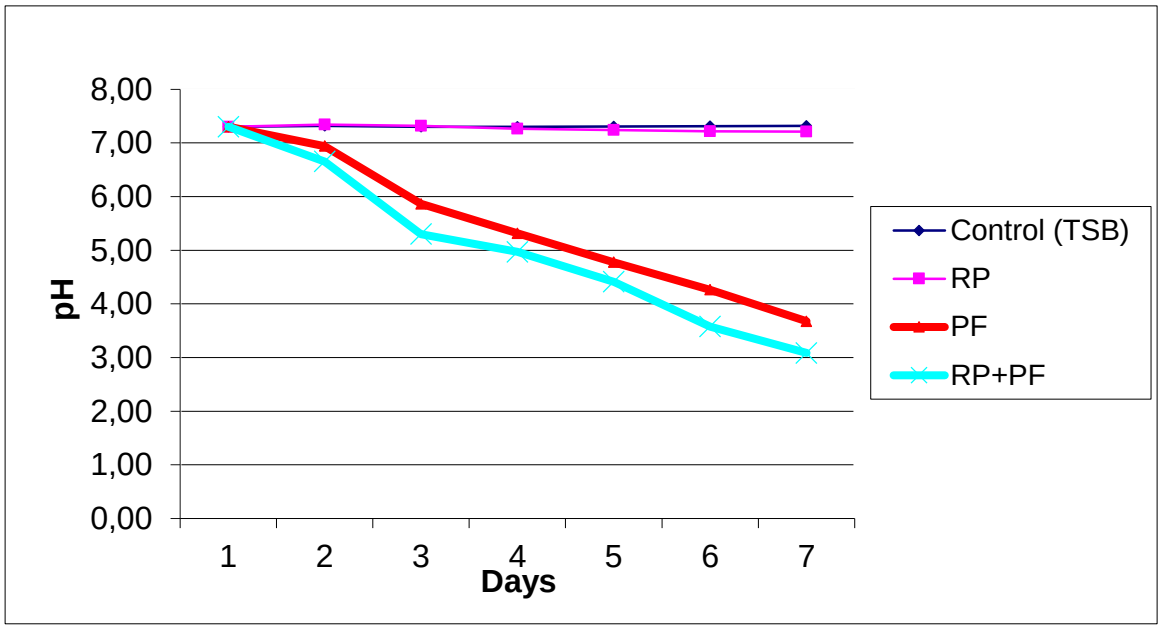

Figure 5 Effects of PF incubation under *in vitro* conditions on pH values during 7 days.

3.7 Effect of specific organic acids on solubilisation of RP

Three organic acids, specifically Glu-A, Lac-A, and Suc-A were used in different concentrations in this experiment. These organic acids were the main acids produced by PF *in vitro* in the abovementioned experiment.

Under the effect of the three different concentrations of the three particular organic acids applied, solubilisation of RP increased significantly compared to the control and to the single addition of RP (.

Table 22).

The higher the concentration of the specific organic acid applied, the more P was solubilised and the lower the pH became. The same concentrations of the three organic acids did not show the same rates of P solubilisation or the same pH. The highest concentration of Suc-A recorded the highest solubilised P compared to the other organic acids.

When the organic acids were combined in the same solution for a final concentration of 7.26 mg/ml, there was superior RP solubilisation compared to the control or with the individual organic acids in different concentrations.

Table 22 Effect of the different concentrations of Glu-A, Lac-A and Suc-A on solubilisation of RP and pH values in the laboratory experiment.

Treatment	Acid concentration		Solubilised P (from 0.25 g RP)		pH	
	(mg/ml)	mM/l	%			
Control (DW)			0.00	a	7.5	l
RP			6.69	b	6.8	k
Glu-A+RP	**1.42**	**7.24**	7.43	c	3.8	j
	2.42	**12.34**	7.49	c	3.6	i
	3.42	**17.43**	8.38	d	3.5	h
Lac-A+RP	**1.42**	15.76	8.88	e	3.4	g
	2.42	26.87	9.90	f	3.1	f
	3.42	37.97	14.21	h	1.7	c
Suc-A+RP	**1.42**	12.02	9.13	e	2.8	e
	2.42	20.49	10.81	g	2.1	d
	3.42	28.96	14.86	j	1.6	b
Glu-A+Lac-A+Suc-A+RP	**7.26** (**2.42*3**)	**59.74**	15.33	k	1.4	a

Means followed by the same letter within columns are not significantly different ($p < 0.05$) according to Duncan test. Glu-A=Gluconic acid, Lac-A=Lactic acid, Suc-A=Succinic acid, DW=distilled water.

In **Figure 6** it can be observed that the trend line between solubilised P and pH values under different organic acid treatments had a significant negative correlation (r=-0.80, $p < 0.01$). Solubilised P increased whenever pH decreased.

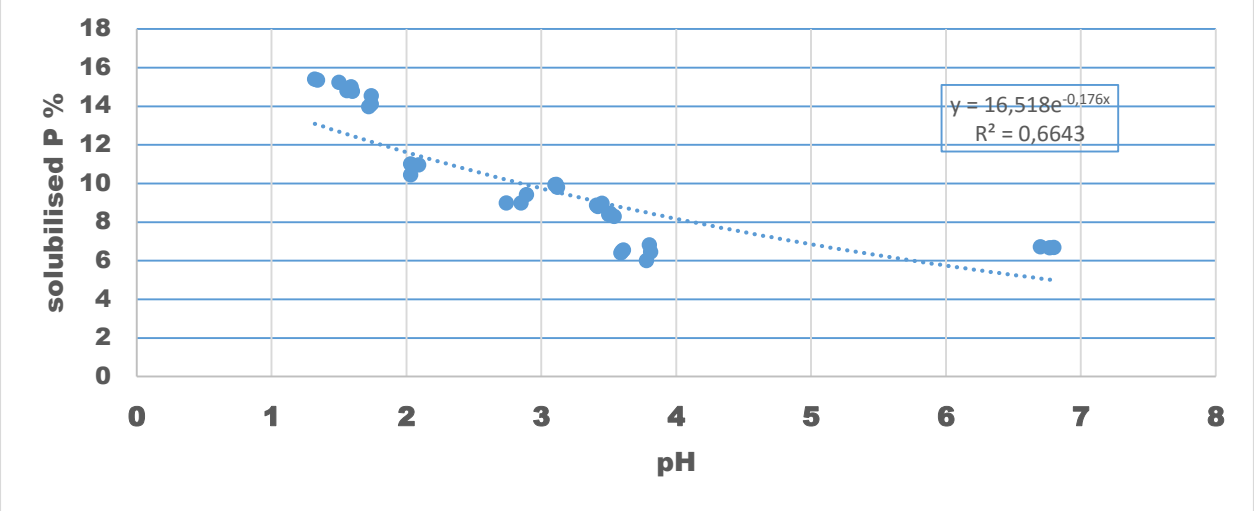

Figure 6 Relationship between pH and solubilised P (%) under different concentrations of gluconic acid, lactic acid and succinic acid.

4. Discussion

4.1 Effect of RP application on plant growth and nutrient uptake

The response of plants to low P soluble fertiliser as RP depends on many factors as soil pH and time. Although the medium P content of the soil in the first experiment, P uptake and plants growth clearly increased in both maize and faba bean crops due to addition of RP. While no increase was noticed in the other experiments with low P in soil in the present study. This may be due to many causes. The first cause is the differences in length of vegetation interval between experiments, which may have influenced the content of P uptake by plants, as well as the concentration of P available to the plants. It may be that the longer the vegetation interval of the first experiment, the higher the yield and P uptake. This suggests that crops cannot utilise P from RP during short vegetation intervals as in 2008 and 2009 experiments. It is possible that RP as a fertiliser dissolves slowly and needs a long time to release its P, which only then becomes available for plants. Truong *et al.* (1978) studied changes of the estimated relative agronomic effectiveness (RAE) coefficients for many types of rock phosphates (RPs) with time. The RAE coefficients based on P uptake changed considerably between 1 and 4 months for most RPs. The Gafsa and Tilemsi RPs solubilised quickly and their effects increased slightly after 4

months, while less-reactive RPs required more time to transmit their potential effectiveness. As Akande *et al*. (1998) reported, Sokoto RP was more effective in supplying P for maize growth in the second year of the experiment than in the first year. The second possible cause is the lower soil pH in the first experiment than the other experiments which may be played a role to more availability of P from RP.

Under P-deficient soil experiments, P uptake and DM yield did not increase significantly when RP was added alone. Although the initial soil P content was suboptimal according to the German soil classification in P-deficient soil. However, the shorter vegetation interval may be not sufficient for the plants to take P from RP in both experiments. As well, no significant increase in DM yield and P uptake of both crops in the experiment with additional N. since the short time may be did not allow to appear a positive changes in the plants.

4.2 Effect of PF on plant growth and nutrient uptake

Solubilisation of less soluble P forms is mainly caused by decreased pH values. This feature is often associated with *Pseudomonas* spp. Stevenson (2005) reported that PF is considered a phosphate solubilising microorganism as it possesses the ability to convert insoluble soil phosphates into soluble forms by many mechanisms that lead to low soil pH. Maliha et al. (2004) found that the pH of broth with bacteria dropped significantly (5.96 to 2.75) compared to broth without microorganisms. Richardson (2001) reported that soil microorganisms directly affect the ability of plants to use P from soil through a many of process or structural interposed mechanisms. These findings agree with the present results in that soil pH dropped between 1.6 and 12.3% in all experiments under the effect of RP+PF treatment when compared to the control.

Maize plants in P medium soil content experiment achieved the greatest increases in DM yield and P uptake and the lowest increase in pdl under the effects of PF and RP compared to the other experiments. Soil pH in this experiment under the effect of RP+PF treatment was lower than in the other experiments; pH was 5.71, 6.08, 6.56, and 6.12 in the experiments from 2007, 2008/1, 2008/2, and 2009, respectively. The lowest pH in the

experiment in 2007 (beside the longest experimental time – see previous chapter) may have been responsible for the significant effects of RP on soil and plant characteristics, although the P content in soil was higher than in the other experiments

In accordance with other studies confirming the high potential of P solubilisation by legumes (see the introduction), obviously the faba bean was better able to use P from RP than maize in P deficient soil.

Some of the effects that were anticipated but not observed with applied PF on maize yield in P-deficient soil experiments are in accordance with a previous pot experiment by Krey *et al*. (2011). In both experiments it was found higher levels of available P in soil after application of PF, but the yield and P uptake did not increase. Although the initial soil P levels were suboptimal according to the German soil classification, it was expected that stronger yields following PF application might have occurred in compliance with even lower soil P levels. This supposition was also supported by strong promotion of plant growth after inoculation of maize with *Pseudomonas alcaligenes*, *Bacillus polymyxa*, and *Mycobacterium phlei* to a nutrient deficient Calcisol in a greenhouse pot experiment by Egamberdiyeva (2007).

N fertilisers may have some effect on PF growth and their role in solubilising RP. This statement is supported by both crops demonstrating enhanced DM yield, as well as P and N uptakes over their control in experiment with additional N when compared to crops in the first P deficient soil experiments under similar soil conditions and the same vegetation interval (55 days). This hypothesis is supported by the findings from Balamurugan *et al*. (2010) who studied the utilisation of different N sources by strains of phosphate-solubilising bacteria. They reported that N compounds in the form of NH_4NO_3 support growth of these bacterial strains and also support perfectly their activity in phosphate solubilisation.

The N fertilisation effect was also clear in K uptake when PF and RP were applied together. The increase in nutrient uptake are essentially associated with the increase of the yield from the additional N fertiliser that encourages vegetation growth. Furthermore PF behaves as plant growth-promoting bacteria through their role in solubilising RP and making P available, which is important for growing plants, both PF and RP may have

helped enhance plant growth and, in turn, have increased the need for K. Sarathchandra *et al*. (1993) reported that application of fertilisers to soil affects the activities of diverse soil microorganisms, indirectly through greater carbon availability from root secretions resulting from increased plant growth, and directly by supplying nutrients that are important for plant growth.

The increase in protein content in maize and faba bean plants in the experiment with additional N might be due to the higher N supply which resulted in higher N and protein contents in plants. Moreover, Rokhzadi et al. (2008) reported that *Pseudomonas* inoculants were superior to other inoculants with respect to protein yield of chickpea grain.

4.3 Effect of AMF on plant growth and nutrient uptake

Generally, colonisation of plant roots with AMF increase absorption surface of the roots, which helps to enhance the acquisition of important nutrients for plant growth.

However, colonisation of plant roots with AMF related to P concentrations in soil. In the experiments with P deficient in soil, a very low Pdl in soil due to the addition of AMF and RP together may be caused high percentages of root colonization that achieved the high P uptake of faba bean (Graham and Abbott, 2000). As well, Chen et al. (2006) reported that in P deficient soil, the beneficial effect of mycorrhiza on P uptake of plants is high.

The mycorrhiza effect increases usually with running experimental time (Marschner and Baumann. 2003). This finding agrees with the results of both P poor soil experiments, where Pdl values was higher in June experiment with the longer vegetation interval than in the August experiment.

Douds and Nagahashi (2000) reported that the branching and growth of hypha increase plant compounds exudation that controls hyphal branching intensity in low P concentrations in the soil. However, this result did not concur with the first experiment with the medium P content in the soil. Since higher DM yield and P uptake of both crops were observed although the medium P content in the soil. The longest vegetation interval compared to the other experiments may have increased the colonisation of AMF by roots

of both crops over the time. This caused a larger surface area on the roots to facilitate absorption of P. The work of Wu et al. (2011) showed that the total hyphal and vesicle colonisation by AMF in AMF-inoculated Bermuda grass roots increased significantly over the time. In another study conducted by Marschner and Baumann (2003), it was declared that mycorrhizal colonisation of maize roots increased from 15–34% after 3 weeks to 78–87% after 6 weeks. El-Ghandour et al. (1996) reported that application of AMF increased dry weight of faba bean plants in the presence of an additional P source in a pot experiment.

Liu et al. (2002) reported that AMF can improve absorption of K, Mg and Ca. this confirms the findings of enhanced K, Mg and Ca uptake in both crops under AMF treatment in most experiments of the present study. Farzaneh et al. (2011) found that the inoculation of chickpea with AMF increased the P, K, Mg and Ca uptake and promoted the shoots growth.

The highest increase in faba bean DM yield and nutrient uptake in the experiment with additional N was in the AMF+RP treatment. The synergy between many factors (faba bean as legume, AMF and both fertlisers RP and NH_4NO_3) may be caused the increase in yield and nutrient uptake. This meets with the study of Norris *et al* (1994) that proved the nutritional relationship between faba bean roots and mycorrhiza, in the presence of high nitrogen requirement of the chitin walled AMF and high P requirement for the N fixing root nodule.

Phosphorus is considered as an energy source for legumes. When each molecule of N2 is converted to NH3, 16 molecules of adenosine triphosphate (ATP) are transformed to adenosine diphosphate (ADP).The ATP is formed during photosynthesis process, when light energy is transformed and stored in the form of ATP for later use by the plant (Sultenfuss and Doyle, 1999). In the present study, AMF may be could supply their host plant the faba bean with P from RP added. Medina et al. (2006; 2007) reported that plants colonised with AMF respond to the addition of insoluble forms of phosphate, such as RP, which are not readily available to non-mycorrhizal roots. Many researchers explained some mechanisms of AMF when insoluble forms of phosphate are added. Bago *et al.*

(1996) cited that AMF are able to acidify the environment, which facilitates inorganic P solubilisation. George *et al*. (1995) reported that the exudation of organic substances by roots and the production of fungal hyphae might be important to make P more available. Shibata and Yano (2003) concluded that AMF could expedite P acquisition by the plants, from insoluble sources in soil through interaction between the roots and hyphae.

The N fertiliser added as NH_4NO_3 maybe also have contributed in stimulating the AMF growth. Many previous studies confirmed the present result. They have found an increase in the plant growth response to mycorrhizal infection in the presence of NO_3^- (Barea *et al*., 1989; Azco´n *et al*., 1991, 1992). Other works have shown that mycorrhizal plants prefer NH4+ as N source (Cuenca and Azco´n, 1994). Nevertheless, the absorbing hyphae of AMF are able to take up and transport both NH_4^+ and NO_3^- (Johansen et al., 1993; Hawkins and George, 2001). Cornejo *et al*. (2007) investigated the interaction of NO_3^- or NH_4^+ as the N source with an inoculated AMF in an Andisol from southern Chile. They found that mycorrhizal root length was greater with NO_3^- than with NH_4^+. The NO_3^- source also improved AMF mycelium density and spore number in the rhizosphere soil. Different N sources change the pH around the AMF roots in different ways. An increase in pH with NO_3^- uptake and a decrease in the pH of the hyphosphere with NH4+ uptake (Gianinazzi-Pearson and Smith, 1993). The pH changes may influence the microbial activity in the rhizosphere and the solubility and mobilisation of nutrients. Rhizosphere changes, caused by the N sources can convert the extent of AMF propagule formation, symbiosis functionality and root colonization (Ortas *et al.,* 1996; Kabir *et al*., 1997b).

4.4 Effect of the dual inoculation with AMF and PF on plant growth and nutrient uptake

As in the previous treatments the increase in DM yield and nutrient uptake due the dual inoculation along with RP in the experiment with the medium P content in the soil was not expected. Since many studies showed that the fungal and microbial activity increases in P deficient soil (see previous chapters). However, the longer vegetation interval in this

experiment may be caused a cumulative effect of microorganisms to enhance plant growth and nutrition of both crops. As it was explained previously that the single effects of AMF and PF on plant growth and nutrient uptake increase over the time. Moreover, RP is a fertiliser solubilised slowly and needs a time to become available to the plants. Accordingly, the combined application of microorganisms along with RP could achieve the higher yield and nutrient uptake by plants than single addition of each one over the time. Moreover, PF considers as mycorrhiza helper bacteria (Garbaye, 1994). Boer *et al. (*2005) reported that in the presence of mycorrhiza-helping bacteria, AMF root colonisation is enhanced, which promotes plant growth and nutrient uptake. They found that mycorrhizal colonisation achieved with *G. intraradices* alone was about 20%, while dual inoculation with AMF and PF enhanced fungal colonisation up to 40%. Founoune *et al*. (2002) demonstrated that the mycorrhizosphere activity exerts a significant stimulating effect on the populations of fluorescent pseudomonads.

In both poor P soil experiments the yields significantly increased. The positive influences of AMF and PF together with RP indicate the increased effectiveness of combined inoculation regarding the nutrient availability and plant growth in P deficient soil, which was also shown in a study of Boer et al. (2005). However, the short vegetation interval of both experiments may be did not help the plants to utilise P from RP (see previous chapters). This in turn negatively affected P uptake in the combined treatment.

The increase in plant growth and nutrient uptake in the experiment with additional N fertiliser could be also due to the combined effect of microorganisms and both fertilisers together. Afzal and Bano (2008) reported that all inoculations with *Rhizobium leguminismarum* and *Pseudomonas* spp., along with P fertiliser and dual inoculations without P had significantly greater plant heights than other treatments. Roesti (2005) reported that the plant height increased in all treatments compared to the control, especially when PGPR and AMF were co-inoculated. These findings agree with the present results when dual inoculations with PF and AMF achieved a significant increase in plant height compared to the control in the additional N experiment. The pod number of faba bean plants in the experiment with the additional N significantly increased compared to the control under the effect of dual inoculations with AMF and PF. Both P and N

fertilisers in addition to microorganisms may be caused root development which enhanced supply water and the other nutrients to the growing parts of the plants, resulting in an increased photosynthetic and then more dry matter accumulation and seed formation (Prihar and Tripathi, 1989). Hossain *et al*. (2007) reported that additions of P and N fertilisers significantly influenced the number of mature pods per plant of groundnut. Barbar (1984) found that addition of N fertilizer increases root-shoot ratio, and pod yield of groundnut (Patra *et al*., 1995).

It is important to note that the increased nutrient uptake, and especially K uptake, was partially higher than the increase in yields in all experiments. This can be explained by the higher availability of nutrients in the soil and therefore higher nutrient concentrations in plant tissue. Similarly, other studies also found higher responses in plant nutrient uptake than plant yield (Eichler *et al*., 2004).

4.5 Production of organic acids by PF and by AMF colonised by maize plants

Organic acids in the control with non-sterilised soil could have been produced by other microorganisms that were not identified in current experiments. The absence of organic acids in the control with sterilised soil confirmed that the organic acids that were found in the inoculated treatments were only produced by the added microorganisms. Accordingly, it can be said that the source of organic acids in the sterilised soil is only from the added microorganisms. It is important to point out that maize plants could produce organic acids, but in very low concentrations, under the selected values on HPLC for determining organic acids in the soil solution. For that, no organic acids were determined in sterilised soil.

The presence of Glu-A, Lac-A and Suc-A in PF treatments (i.e., PF, PF+RP) in both soil types in convergent concentrations confirmed that these organic acids were produced mainly by the PF strain. However, four organic acids (Cit-A, Mal-A, Oxa-A and αKG-A) were detected in non-sterilised soil in PF treatments, as well as in the control and in both AMF treatments (i.e., AMF, AMF+RP), albeit in very small quantities. The finding of Vyas

and Gulati (2009) confirmed the current results. They studied the production of organic acids during inorganic phosphate solubilisation and the influence on plant growth as a function of phosphate solubilisation by fluorescent *Pseudomonas*. They found that *P. fluorescens* strain secreted Oxa-A and αKG-A in very low concentrations (12.7 ± 1.0, 67.0 ± 2.6 µg/ml) respectively and did not produce Cit-A and Mal-A.

The existence of organic acids in non-sterilised soil in quantities higher than in sterilised soil was likely due to the presence of other microorganisms. The presence of Lac-A, Cit-A and For-A in all AMF treatments in both soil types confirmed that AMF-maize plants produced these organic acids. Klugh and Cumming *et al*. (2007) reported that Lac-A, Cit-A and For-A were detected in the soil solution collected from the roots and hyphae compartments of *Liriodendron tulipifera* plants inoculated with the AM fungi *G. claroideum*.

The higher organic acids secretion in PF+RP treatment than in PF treatment indicates that the presence of RP as P source for growth and development of microorganisms, could promoted PF to produce a higher quantity of organic acids. Maliha *et al*., (2004) cited that P is essential for microbial cell synthesis. Illmer and Schinner (1995) noticed a high significant correlation between P mobilisation and the production of microbial biomass. The work of Jurinak *et al*. (1986) showed that solubilisation mechanisms of inorganic phosphate depend on the activation of microbial biomass to produce organic acids.

The difference in the amount of solubilised P under the same concentration of different organic acids is attributed to the fact that each organic acid has a specific solubilisation ability. The three organic acids do not have the same molar mass and the same acidity. For instance, the single addition of Glu-A, Lac-A and Suc-A at 2.42 % concentration resulted in different amounts of solubilised P from 0.25 g RP (7.49 %, 9.9 % and 10.81%) respectively. Moreover, the higher concentration of Suc-A (3.42%) recorded the highest value of solubilised P between the single additions of each organic acid. This superiority might be due to the higher acidity of Suc-A compared to the other used acids, since Suc-

A has two carboxylic acid groups while Glu-A and Lac-A have only one carboxylic acid group.

In the laboratory experiment, Glu-A at 2.42 % concentration solubilised 7.49 % P from 0.25 g RP. In vitro experiment, PF solubilised 19.59 % P from 0.25 g RP and immobilised 0.43 % P from 0.25 g RP. This means that the whole solubilised P from 0.25 g RP was 20.02 % from 0.25 g RP. Production of Glu-A *in vitro* was in such a high concentration (2.42%). On the other hand, production of Lac-A and Suc-A by PF *in vitro* were in very low concentrations, so it is reasonable to presume from these results that Glu-A produced by PF *in vitro* has the potential of playing the major role in solubilisation of RP, while both Lac-A and Suc-A have only a slight effect on RP solubilisation. Illmer and Schiner (1992) and *Di* Simine et al., (1998) reported that Glu-A was the major organic acid produced during phosphate solubilisation by *Pseudomonas* sp and *P. fluorescens.* Accordingly. By the comparison between the effect of Glu-A on solubilisation of RP in the laboratory experiment and the effect of Glu-A produced by PF *in vitro* experiment at the same concentration (2.42%) under relatively similar conditions of the both experiments, it was found that Glu-A as a major organic acid produced by PF during RP solubilisation *in vitro* solubilised 20.02 % from 0.25 g RP while Glu-A used in the laboratory experiment solubilised 7.49 % P from 0.25 g RP at the same concentration (2.42%). As well, the effect of PF in vitro was better than the effect of combined treatments with the three types of organic acids (Glu-A, Lac-A and Suc-A) at a total concentration of 7.26 mg/ml. This may indicate that the RP solubilisation mechanism of PF does not depend only on the production of organic acids, but that additional mechanisms could contribute to RP solubilisation. One mechanism could be the production of CO_2 by bacteria that play a role in forming carbonic acid and thus solubilising P even more. Illmer and Schinner (1992) suggested that microbial respiration may be involved in the drop in pH. Nevertheless, negative correlation between solubilised P and pH ($r=-0.538$, $p < 0.05$) in the present study in the laboratory experiment agreed with findings reported by Sheshadri et al. (2004). These authors mention that inoculating the media with *Aspergillus* caused release of P from RP, which in turn depended on the low pH.

5. Conclusions and suggestions

1. The use of microbial inoculants (PF, AMF) in single and combined forms to maize and faba bean crops showed a positive effectiveness in the various conditions of the present study for plant nutrition and growth especially in the longest experimental interval and when the soil was supplied with additional N fertiliser.
2. The present results proved that the combined application of PF and mycorrhizal inoculum of the genus Glomus can improve the P availability from RP more effectively than single inoculations in all experiments.
3. The single inoculation with PF or AMF showed the same effects for growth and plant nutrition.
4. In P deficient soil, higher Pdl and lower pH values in AMF treatments compared to the control produce the direct P mobilisation by AM fungi.
5. With running of the experimental time, the effects of RP and microbial inoculants become more pronounced, since plants had the higher P uptake from RP and the higher yield.
6. The use of additional NH_4NO_3 in the present investigations as N fertiliser enhanced the role of PF and AMF in the soil to increase the solubility and mobilisation of nutrients. This result suggests addition of N fertiliser to increase the effects of soil microorganisms. Since high N requirement is important for the chitin walled AMF and to support bacterial growth.
7. The higher yield and P uptake of faba bean due to the addition of RP, PF and NH_4NO_3 together suggest the multiple potentials for reducing the fixation process of P in the soil.

8. The use of PF liquid culture along with RP could be considered as a good means for bio activation of RP for practical application in field.
9. The use of PF-DR54 inoculum along with RP achieved higher solubilised P than the use of the same organic acids produced by the same bacteria strain, when they applied at higher concentrations to increase solubilisation of RP. This finding can confirm that their mechanisms in RP dissolving did not depend only on production of organic acids. Therefore, more studies are needed to understand the mechanism of PF-DR54 to solubilise RP.
10. According to the present study, the use of RP and NH_4NO_3 along with both microorganism inoculums might make it the best choice to obtain a safe P bio-fertiliser and suitable technologies for reinstituting soil fertility, which will be required for sustainable agriculture in a time when intensively produced fertilisers, with undesirable environmental consequences, are likely to become increasingly expensive.
11. In order to obtain a more accurate and practical conclusions, future studies will focus on the interactions between the host plant, AMF and soil bacteria mainly under three different conditions—namely *in vitro*, greenhouse and field conditions.
12. For a better understanding of the efficiency of the microbial inoculants on RP solubilisation and mineral nutrient availability by the time, it is recommended to collect plant and soil samples from experiments in different stages of growth. Since it was demonstrated from the present study that the higher yield and nutrient uptakes were by the longer vegetation interval.

6. Summary

Conservation of soil fertility is one of the most important necessities for sustainable agriculture. This is not possible unless the nutrients taken up by plants are replenished. The direct application of rock phosphate (RP) as a fertiliser is an easy and somewhat cheap way to add P to soils. However, the solubility of phosphorus (P) from RP is low. Many researchers reported that the amount of P released from directly applied RP may be very low to provide sufficient P for crop needs. The use of soil microorganisms in combined with RP may consider comparatively a good way to enhance utilisation of P from RP in agro-ecosystems. For this purpose the effects of single and dual inoculation with *Pseudomonas fluorescens* DR-54 (PF) and arbuscular mycorrhizal fungi (AMF) on solubilisation of RPwere investigated in different conditions.

The growth and nutrient uptake of maize (*Zea mays*) and faba bean (*Vicia faba*), as well as soil properties, were estimated. Organic acid exudates from PF and AMF colonised with maize plants in greenhouse and from PF *in vitro* were determined. The effects of specific organic acids on solubilisation of RP were then studied.

Four pot experiments were carried out in the greenhouse for investigation the effect of PF and AMF on solubilisation of RP over three consecutive years (2007, 2008/1, 2008/2, 2009/1) and differed in their soil mineral contents, vegetation length (75, 55, 45, 55 days) and N supply (NH_4NO_3 in 2009/1 for all treatments). In 2007, 2008/1, and 2008/2 experiments, five treatments were arranged for each crop including the control (no addition from fertiliser or microorganisms), RP, RP+PF, RP+AMF and RP+PF+AMF. In addition, three additional treatments without RP supply were applied with single inoculations of either PF or AMF and dual inoculations with PF+AMF in the 2009/1 experiment. The four experiments were carried out in complete randomized design with four replicates for each treatment in Mitscherlich pots. After plants were harvested the nutrient concentration in plant tissue (P, N, Mg, K), levels of plant-available P, K and Mg (Pdl, Kdl, Mgdl) and pH values of the soil were analysed.

In sterilised and non-sterilised soil, pots experiment to determine the organic acids produced by PF-DR54 strain and AMF colonised by maize roots, was carried out on maize plants with the following treatments for both soils (4 replicates for each treatment): the control consisting of soil without any applications, RP+AMF, AMF, RP+PF, PF.

In the experiment of determination of organic acids produced by PF-DR54 strain *in vitro*, four treatments were applied: the single incubation of PF with and without RP in tryptic soy broth (TSB), in addition to RP treatment and the control (TSB). This *in vitro* experiment required rotary shaking for a span of seven days.

The laboratory experiment established to study the effect of the organic acids that were produced by PF in the *in vitro* experiment on P availability from RP. Three organic acids gluconic acid (Glu-A), lactic acid (Lac-A) and succinic acid (Suc-A) were used in three concentrations along with RP. The three organic acids were applied individually at 2.42% and collectively (7.26% owing to 2.42% of each acid) along with RP. Two other concentrations of the organic acids were applied also at 1.42% and 3.42%. The experiment lasted 7 days and included 4 replicates in each treatment.

In the experiment with the longer vegetation interval and P medium content soil, the results showed that the yield and P uptake of maize and faba bean increased significantly when the soil was fertilised with RP alone and combined with single and dual inoculation with PF and AMF.

However, N, Mg and Ca uptakes increased significantly only under effect the inoculated treatments.

The soil values revealed a lower response to the applied treatments compared to the plant parameters.

There were no significant increases in Pdl and pH of the soil under effects all applications compared to the control.

In both P deficient soil experiments, the combined treatment RP+PF+AMF achieved the highest plant yields, P, N uptakes and Pdl in the soil compared to the controls for both

crops. The increase in nutrient uptake was partially higher than the increase in yields. As expected, the plant yields of the June experiment with the longer vegetation interval were higher than the yields of the second experiment in August with the shorter vegetation interval.

Higher Pdl and lower pH values compared to the controls after the single AMF inoculation indicate a direct P mobilisation by AMF.

Application of RP together with only one inoculant resulted in higher P available in the soil mainly in the first experiment with the longer experimental interval.

The pH was lower in the treatments with the microorganism inoculation.

Faba bean was more efficient than maize in using P from RP.

In the experiment with additional N in the soil, the DM yields, P, N uptakes and Pdl in the soil cultivated with maize and faba bean plants increased significantly under all inoculated treatments compared to the control.

The highest increase in maize and faba bean yield, P and N was obtained when the soil was inoculated with PF and AMF along with RP.

Faba bean plants responded more than maize plants to all inoculations with and without RP compared to the control.

Generally it was observed that all inoculation treatments in single or dual form, along with RP, trend to exceed all corresponding inoculation treatments without RP by enhancing not only P uptake, but also all other nutrient uptakes, yield and all plant growth parameters.

Soil pH dropped consistently with all inoculated treatments in both crops compared to the control and to the pH before planting. The lowest pH value was noticed under the combined treatment consisting of AMF, PF and RP in both crops

In the 2009/2 experiment (determination the organic acids) and *in vitro*,

The investigated organic acids that were produced by PF in sterilised soil and *in vitro* were Glu-A, Lac-A and Suc-A.

The organic acids produced by AMF colonised by maize plants in sterilised soil were Cit-A, Lac-A and For-A.

Addition of RP together with PF or with AMF resulted in higher organic acid quantities than the inoculated treatments without RP in both 2009/2 and *in vitro* experiments.

PF showed high solubilisation of RP (20.02% of 0.25g RP) and it caused pH levels to drop from 7.3 to 3.09 during RP solubilisation and from 7.3 to 3.7 without RP.

In the laboratory experiment, the three organic acids, each at three concentrations, showed that soil pH was the lowest with the highest organic acid concentration and the amount of dissolved P from RP was the highest.

The highest concentration of Suc-A caused the highest values of solubilised P compared other organic acids.

The lower pH was accompanied by higher solubilised P, immobilised P and organic acid quantities.

Overall, it can conclude that the addition of AMF and PF-DR54 along with RP increased availability of P from RP and improved DM yield, plant growth and nutrient uptakes in the related and described experimental conditions. However, plant responses were varied, depending on the crop and the conditions of the experiment (the length of the experimental interval, soil P content and the additional N supply).

7. Zusammenfasung

Die Erhaltung der Bodenfruchtbarkeit ist eine der wichtigsten Aufgaben für eine nachhaltige Landwirtschaft. Dies ist nur möglich, wenn die Nährstoffe, die von den Pflanzen aufgenommen werden, wieder zurückgeführt werden. Die direkte Anwendung von Rohphosphat (RP) als Düngemittel ist eine einfache und günstige Möglichkeit den Böden etwas Phosphor hinzuzufügen. Jedoch ist die Löslichkeit von Phosphor (P) vom RP gering. Viele Forscher berichten, dass die Menge an P aus direkt angewendetem RP zu gering ist um eine ausreichende P-Versorgung der Pflanzen zugewährleisten. Die Verwendung von Mikroorganismen im Boden in Kombination mit RP könnte ein guter Weg sein, um Nutzung von P aus RP in Agrarökosystemen zu verbessern.

Hierzu wurde die Wirkung einer einfachen oder kombinierten Inokulation von Pseudomonas fluorescens-DR-54 (PF) und arbuskulären Mykorrhiza-Pilzen (AMF) auf die P-Düngewirkung von RP unter verschiedenen Bedingungen untersucht. Das Wachstum und die Nährstoffaufnahme von Mais (Zea mays) und Ackerbohnen (Vicia faba) sowie die Bodeneigenschaften wurden analysiert. Die Ausscheidung organischer Säurer durch PF und AMF, welche an Maispflanzen im Gewächshaus als auch in vitro kolonisiert wurden,wurden ermittelt. Abschließend wurde die Auswirkung bestimmte organischer Säuren auf Lösung von RP untersucht.

Vier Topfversuche wurden im Gewächshaus zur Untersuchung der Wirkung von PF und AMF auf die Lösung von RP in drei aufeinander folgenden Jahren (2007, 2008/1, 2008/2, 2009/1) und in Böden mit unterschiedlichem P-Status, unterschiedlicher , Vegetationslänge (75, 55, 45, 55 Tage) und Stickstoff (N) - Versorgung durchgeführt.

In den Versuchen 2007, 2008/1 und 2008/2 wurden fünf Behandlungen für jede Fruchtart eingerichtet, einschließlich der Kontrolle, die keinen Zusatz von Düngemitteln oder Mikroorganismen hatte: RP, RP + PF, RP + AMF und RP + PF + AMF. Außerdem wurden drei zusätzliche Behandlungen ohne RP-Versorgung aber mit einzelner Inokulationen von entweder PF oder AMF und kombinierter Inokulation von PF + AMF im 2009/1 Versuch angewendet.

Die vier Versuche wurden im vollständigen randomisierten Design mit vier Wiederholungen für jede Behandlung in Mitscherlich Töpfen durchgeführt. Nachdem die Pflanzen geerntet wurden, wurde die Nährstoffkonzentration in Pflanzengewebe

(P, N, Mg, K) und die pflanzenverfügbaren P, K und Mg (PdL Kdl, Mgdl) Gehalte und pH-Werte im Boden analysiert.

In sterilisierten und nicht sterilisierten Böden mit Maispflanzen wurden die durch den Bakterien-Stamm (PF-DR54) und die durch AMF produzierten organischen Säuren bestimmt, mit den folgenden Behandlungen (4 Wiederholungen für jede Behandlung): Kontrolle (keine Zugabe von Dünger oder Mikroorganismen), RP + AMF, AMF, RP + PF, PF untersucht

Im in vitro Versuch wurden die durch den PF-DR54 Stamm produzierten organischen Säuren bei vier Behandlungen untersucht: die alleinige Inkubation von PF mit und ohne RP in tryptischer Sojabrühe (TSB), zusätzlich zu den RP-Behandlung und die Kontrolle (TSB). Dieser in vitro-Versuch erfolgte über Schütteln für eine Zeitspanne von sieben Tagen.

Im Laborversuch wurde die Wirkung der organischen Säuren, die von PF in dem in vitro Versuch produziert wurden hinsichtlich ihrer Wirkung auf die Freisetzung von P aus RP untersucht. Die drei organischen Säuren Gluconsäure (Glu-A), Milchsäure (Lac-A) und Bernsteinsäure (Suc-A) wurden in drei Konzentrationen zusammen mit RP verwendet. Die drei organischen Säuren wurden einzeln auf 2,42% angewandt und kollektiv (7,26% durch 2,42% jeder Säure) mit RP. Zwei andere Konzentrationen der organischen Säuren wurden auch bei 1,42% und 3,42% angewendet. Der Versuch dauerte 7 Tage und beinhaltete 4 Wiederholungen in jeder Behandlung.

Die Ergebnisse zeigen, dass vor allem in dem Experiment mit der längeren Vegetationsperiode und dem mittleren P-Gehalt im Boden , die Ausbeute und die PAufnahme von Mais und Ackerbohnen signifikant erhöht war, wenn der Boden mit RP gedüngt wurde und wenn eine alleinige oder kombinierte Inokulation mit PF und AMF erfolgte. Die N, Mg und Ca Aufnahmen waren aber nur signifikant erhöht, wenn eine Inokulation mit PF und/oder AMF erfolgte.

Es gab keine signifikanten Erhöhungen bezüglich der Pdl-Gehalte und des pH-Wert des Bodens durch die verschiedenen Behandlungen im Vergleich zur Kontrolle.

In beiden Experiment mit den P armen Böden ,führte die kombinierte Behandlung von RP + PF + AMF bei beiden Kulturpflanzen zu den höchsten Erträgen, P, N Aufnahmen und PDL-Gehalten im Boden. Die Erhöhung der Nährstoffaufnahme war teilweise größer als die Ertragssteigerung. Wie erwartet, waren die Pflanzenerträge des Juni

Versuches mit der längeren Vegetationszeit höher als die Erträge des zweiten Experiments im August mit der kürzeren Vegetationszeit.

Höhere Pdl-Gehalte und niedrigere pH-Werte im Boden im Vergleich zu den Kontrollen nach der alleinigen Inokulation mit AMF zeigen eine direkte P Mobilisierung durch AMF.

Die Anwendung von RP zusammen mit der Inokulation von PF oder AMF führte zu höheren P-Gehalten im Boden, hauptsächlich in dem ersten Versuch mit der längeren Versuchszeit.

Der pH-Wert war niedriger bei den Behandlungen mit dem Mikroorganismen- Inokulation.

Die Ackerbohne war effizienter als Mais bei der Verwendung P aus RP.

In dem Experiment mit zusätzlicher N-Gabe in den Boden, waren die TM-Erträge, P, N-Aufnahmen und Pdl-Gehalte im Boden nach dem Anbau von Mais oder Ackerbohnen in den inokuliert Behandlungen signifikant erhöht gegenüber der Kontrolle.

Der höchste Anstieg des Mais und Ackerbohnenertrages, sowie der P- und NAufnahmen wurde erreicht, wenn der Boden wurde mit PF und AMF mit RP inokuliert wurde.

Die Ackerbohnenpflanzen reagierten deutlicher als die Maispflanzen auf die Inokulationen, egal ob mit und ohne RP.

Generell ließ sich beobachten, dass alle inokulierten Behandlungen, in einfacher oder kombinierter Form, zusammen mit RP den Behandlungen ohne RP überlegen waren. Das zeigte sich nicht nur in einer erhöhten P-Aufnahme sondern auch in allen anderen Nährstoffaufnahmen, dem Ertrag und allen weiteren untersuchten Pflanzenparametern.

Der Boden-pH fiel durchweg in allen inokulierten Behandlungen und bei beiden Kulturpflanzen im Vergleich zur Kontrolle ab. Die niedrigsten pH-Werte wurden unter der kombinierten Behandlung aus AMF, PF und RP in beiden festgestellt.

Im Versuch 2009/2 (Bestimmung die organischen Säuren) und in vitro,

Die untersuchten organischen Säuren, die von PF in sterilisiertem Boden und in vitro produziert wurden waren Glu-A, Lac-A und Suc-A. Die organische Säuren die von AMF, kolonisiert mit Maispflanzen oder im sterilisiertem Boden, produziert wurden, waren Cit-A, Lac-A und For-A.

Die Zugabe von RP zusammen mit PF oder AMF führte zu einer höheren Produktion von organischen Säure als in dene inokuliert Behandlungen ohne RP sowohl in den 2009/2 und in den in-vitro-Experimenten.

PF zeigte eine höhere Mobilisierung von P aus RP (20,02% der 0,25 g RP), und verursachte eine Abnahme des pH-Wertes von 7.3 auf 3.09 (mit RP) beziehungsweise von 7.3 auf 3.7 (ohne RP).

Im Laborversuch mit den drei organischen Säuren, die in jeweils drei unterschiedlichen Konzentrationen zugeführt wurden, zeigte sich, dass der Boden-pH mit der höchsten organischen Säurekonzentration am niedrigsten war und der Menge des gelösten P von RP am größten war.

Die höchste Konzentration von Suc-A verursacht die höchsten Gehalte an gelösten P im Vergleich zu anderen organischen Säuren.

Der niedrigere der pH-Wert ging einher mit höheren Mengen gelösten P, immobilisiertem P und organischen Säuremengen .

Insgesamt kann aus den Ergebnissen der vorliegenden Arbeit geschlossen werden, dass die Zugabe von AMF und PF-DR54 zusammen mit RP die Verfügbarkeit von P von RP verbessert und sich dadurch der Ertrag, das Pflanzenwachstum und Nährstoffaufnahmen unter den verwendeten und beschriebenen experimentellen Bedingungen verbessert Allerdings waren die pflanzlichen Reaktionen sehr unterschiedlich, je nach den Bedingungen des Experiments (Versuchsdauerl, Boden P-Gehalt und die zusätzliche N-Versorgung).

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9. Appendix

9.1 Results

Table a 1 Pearson correlation coefficients (r) for soil and maize plants characteristics with higher P content in the soil (2007 experiment) (number of samples: n = 20).

	DM yield	P-up	N-up	K-up	Mg-up	Ca-up	OM	P dl	Mg dl	K dl
P-up	0.94 **									
N-up	0.87 **	0.78 **								
K-up	0.57 **	0.56 *	0.32							
Mg-up	0.92 **	0.86 **	0.93 **	0.32						
Ca-up	0.85 **	0.86 **	0.74 **	0.34	0.88 **					
OM	0.10	0.10	0.10	-0.21	0.11	0.17				
P dl	0.42	0.47 *	0.34	0.26	0.42	0.45 *	0.35			
Mg dl	- 0.32	- 0.31	- 0.49 *	0.01	- 0.41	- 0.24	0.35	0.25		
K dl	- 0.25	- 0.2	- 0.41	- 0.01	- 0.44	- 0.30	0.45	0.17	0.55 *	
PH	- 0.21	- 0.33	- 0.10	- 0.40	- 0.13	- 0.20	- 0.10	0.26	0.18	0.34

***** Significant at $p < 0.05$, ** significant at $p < 0.01$, Pdl, Mgdl, Kdl =double lactate soluble P, Mg and K, DM= dry matter. P-up, N-up, K-up, Mg-up = P, N, K, Mg uptakes. OM= organic matter in the soil

Table a 2 Pearson correlation coefficients (r) for soil and faba bean plants characteristics with higher P content in the soil (2007 experiment) (number of samples: n = 20).

	DM yield	P-up	N-up	K-up	Mg-up	Ca-up	OM	P dl	Mg dl	K dl
P-up	0.81 **									
N-up	0.74 **	0.59 **								
K-up	0.49 *	0.40	0.51 *							
Mg-up	0.80 **	0.58 **	0.73 **	0.30						
Ca-up	0.52 **	0.22	0.46 *	0.08	0.71 **					
OM	- 0.13	- 0.06	0.01	- 0.14	- 0.12	- 0.25				
P dl	0.27	0.29	0.07	0.42	0.080	0.35	- 0.10			
Mg dl	- 0.38	- 0.15	- 0.36	- 0.25	- 0.42	- 0.17	- 0.18	0.12		
K dl	- 0.25	- 0.02	- 0.08	0.52 *	- 0.32	- 0.32	- 0.32	0.34	0.28	
PH	- 0.36	- 0.15	- 0.07	0.27	- 0.28	- 0.28	0.04	0.17	- 0.15	0.49 *

***** Significant at $p < 0.05$, ****** significant at $p < 0.01$, Pdl, Mgdl, Kdl =double lactate soluble P, Mg and K, DM= dry matter. P-up, N-up, K-up, Mg-up = P, N, K, Mg uptakes. OM= organic matter in the soil.

Table a 3 Pearson correlation coefficients (r) for soil and **maize** plants sowing in June 2008 experiment (number of samples: n = 20).

	DM yield	P-up	N-up	K-up	Mg-up	Ca-up	OM	P dl	Mg dl	K dl
P-up	0.59 **									
N-up	0.60 **	0.22								
K-up	0.52 *	0.58 **	0.38							
Mg-up	0.61 **	0.36	0.58 **	0.59 **						
Ca-up	0.20	0.19	0.30	0.78 **	0.45 *					
OM	- 0.12	- 0.20	- 0.36	- 0.50 *	- 0.34	- 0.45 *				
P dl	0.41	0.34	0.54 *	0.15	0.10	0.07	- 0.42			
Mg dl	- 0.11	- 0.21	- 0.10	- 0.19	- 0.21	0.01	- 0.02	0.11		
K dl	0.12	- 0.15	0.23	- 0.15	- 0.09	- 0.18	- 0.32	0.61 **	0.40	
PH	- 0.50 *	- 0.37	- 0.64 **	- 0.66 **	- 0.53 *	- 0.48 *	0.50 *	- 0.43	0.09	- 0.22

***** Significant at $p < 0.05$, ****** significant at $p < 0.01$. Pdl=double lactate soluble P, Mgdl= double lactate soluble Mg, Kdl= double lactate soluble K, DM= dry matter of shoot biomass. P-up, N-up, K-up, Mg-up = P, N, K, Mg uptakes. OM= organic matter in the soil.

Table a 4 Pearson correlation coefficients (r) for soil and **faba bean** plant sowing in June 2008 experiment (number of samples: n = 20).

	DM yield	P-up	N-up	K-up	Mg-up	Ca-up	OM	P dl	Mg dl	K dl
P-up	0.65									
	**									
N-up	0.78	0.66								
	**	**								
K-up	0.7	0.77	0.82							
	**	**	**							
Mg-up	0.89	0.64	0.72	0.68						
	**	**	**	**						
Ca-up	0.76	0.75	0.79	0.79	0.56					
	**	**	**	**	**					
OM	0.01	- 0.03	0.05	- 0.01	- 0.03	0.05				
P dl	0.59	0.49	0.41	0.59	0.57	0.53	- 0.22			
	**	*		**	**	**				
Mg dl	0.75	0.45	0.74	0.46	0.72	0.49	0.06	0.22		
	**	*	**	*	**	*				
K dl	0.36	0.15	0.35	0.34	0.39	0.31	0.2	0.25	**0.25**	
PH	- 0.55	- 0.48	- 0.62	- 0.75	-0.34	- 0.68	-0.29	- 0.49	**- 0.25**	**- 0.34**
	*	*	**	**		**		*		

***** Significant at $p < 0.05$. ****** Significant at $p < 0.01$. Pdl=double lactate soluble P, Mgdl= double lactate soluble Mg, Kdl= double lactate soluble K, DM= dry matter of shoot biomass. P-up, N-up, K-up, Mg-up = P, N, K, Mg uptakes. OM= organic matter in the soil.

Table a 5 Pearson correlation coefficients (r) for soil and **maize** plants sowing in August 2008 experiment (number of samples: n = 20).

	DM yield	P-up	N-up	K-up	Mg-up	Ca-up	OM	P dl	Mg dl	K dl
P-up	0.21									
N-up	0.77 **	- 0.10								
K-up	0.60 **	0.10	0.73 **							
Mg-up	0.83 **	- 0.06	0.76 **	0.51 *						
Ca-up	0.55 *	0.13	0.50 *	0.67 **	0.59 **					
OM	0.53 *	- 0.09	0.67 **	0.75 **	0.52 *	0.55 *				
P dl	0.05	0.35	- 0.09	0.08	- 0.34	- 0.02	0.07			
Mg dl	-0.39	0.09	- 0.32	- 0.47 *	-0.35	- 0.45 *	-0.37	- 0.01		
K dl	0.42	0.13	0.15	- 0.01	0.23	0.05	0.10	0.47 *	- 0.31	
PH	0.31	0.26	0.25	0.13	0.35	0.42	0.05	- 0.01	- 0.50 *	0.40

***** Significant at $p < 0.05$, ****** significant at $p < 0.01$. Pdl=double lactate soluble P, Mgdl= double lactate soluble Mg, Kdl= double lactate soluble K, DM= dry matter of shoot biomass. P-up, N-up, K-up, Mg-up = P, N, K, Mg uptakes. OM= organic matter in the soil.

Table a 6 Pearson correlation coefficients (r) for soil and **faba bean** plant sowing in August 2008 experiment (number of samples: n = 20).

	DM yield	P-up	N-up	K-up	Mg-up	Ca-up	OM	P dl	Mg dl	K dl
P-up	0.66 **									
N-up	0.59 **	0.61 **								
K-up	0.75 **	0.62 **	0.58 **							
Mg-up	0.85 **	0.55 **	0.48 *	0.80 **						
Ca-up	0.67 **	0.62 **	0.71 **	0.53 **	0.46 *					
OM	0.40	0.25	0.25	0.45 *	0.30	0.23				
P dl	- 0.09	- 0.07	- 0.13	- 0.13	- 0.21	0.13	- 0.13			
Mg dl	- 0.15	0.22	- 0.06	- 0.17	- 0.28	- 0.01	- 0.16	0.12		
K dl	0.66 **	0.51 **	0.25	0.32	0.49*	0.38	0.11	- 0.06	0.11	
PH	0.28	0.07	- 0.05	0.20	0.35	0.04	0.45 *	-0.19	- 0.23	-0.01

*Significant at $p < 0.05$, ** significant at $p < 0.01$, Pdl, Mgdl, Kdl =double lactate soluble P, Mg and K, DM= dry matter. P-up, N-up, K-up, Mg-up = P, N, K, Mg uptakes, OM= organic matter in the soil.

Table a 7 Pearson correlation coefficients (r) for soil and maize plants under RP addition and single or dual inoculation with AMF and PF in the additional N (number of samples: n = 32).

	DM yield	P-up	N-up	K-up	Mg-up	Ca-up	OM	P dl	Mg dl	K dl
P-up	0.84									
	**									
N-up	0.67	0.65								
	**	**								
K-up	0.55	0.55	0.92							
	*	*	**							
Mg-up	0.7	0.7	0.89	0.75						
	**	**	**	**						
Ca-up	0.61	0.63	0.94	0.84	0.94					
	**	**	**	**	**					
OM	0.82	0.64	0.58	0.44	0.62	0.5				
	**	**	**		**	*				
P dl	0.47	0.21	0.39	0.43	0.44	0.42	0.45			
	*						*			
Mg dl	0.11	0.04	-0.07	-0.13	-0.03	-0.18	0.26	0.1		
K dl	0.32	0.26	0.52	0.47	0.59	0.52	0.51	0.39	0.21	
			*	*	**	*	*			
PH	-0.89	-0.95	-0.56	-0.4	-0.62	-0.53	-0.7	-0.17	-0.1	-0.16
	**	**	**		**	*	**			

***** Significant at $p < 0.05$. ****** $p < 0.01$.
Mgdl= double lactate soluble Mg, Kdl= double lactate soluble K.
OM= organic matter in the soil

Table a 8 Pearson correlation coefficients (r) for soil and faba bean plants under RP addition and single or dual inoculation with AMF and PF in the additional N experiment (number of samples: n = 32).

	DM yield	P-up	N-up	K-up	Mg-up	Ca-up	OM	P dl	Mg dl	K dl
P-up	0.87									
	**									
N-up	0.88	0.91								
	**	**								
K-up	0.75	0.86	0.94							
	**	**	**							
Mg-up	0.95	0.87	0.93	0.83						
	**	**	**	**						
Ca-up	0.9	0.81	0.89	0.77	0.98					
	**	**	**	**	**					
OM	0.59	0.64	0.68	0.68	0.59	0.53				
	**	**	**	**	**	**				
P dl	0.5	0.5	0.43	0.41	0.48	0.43	0.46			
	**	**			*		*			
Mg dl	0.1	0.27	0.35	0.31	0.11	0.11	0.09	0.18		
K dl	0.22	0.55	0.54	0.66	0.33	0.3	0.36	0.38	0.64	
		**	**	**					**	
PH	-0.82	-0.75	-0.75	-0.78	-0.84	-0.8	-0.45	-0.39	-0.04	-0.31
	**	**	**	**	**	**	*			

***** Significant at $p < 0.05$. ****** $p < 0.01$.
Mgdl= double lactate soluble Mg, Kdl= double lactate soluble K.
OM= organic matter in the soil

Table a 9 Parameters affecting DM yield of maize plants in 2007, 2008 and 2009 experiments

Parameter estimates						
Dependent variable: DM yield of maize (g/pot), 2007						
Parameter	Regression coefficient B	Standard error	T	Sig.	95%-confidence interval of B	
					Lower Bound	Upper Bound
Constant term	54.20	0.43	127.00	0.000	53.30	55.10
RP	4.78	0.56	8.40	0.000	3.59	5.98
PF	9.64	0.43	22.60	0.000	8.73	10.50
AMF	11.70	0.43	27.50	0.000	10.80	12.60
Dependent variable: DM yield of maize (g/pot), 2008						
Constant term	47.18	3.49	13.54	0.000	40.11	54.24
RP	1.22	4.61	0.26	0.794	-8.13	10.57
PF	2.19	3.49	0.63	0.533	-4.87	9.26
AMF	1.68	3.49	0.48	0.632	-5.39	8.75
Dependent variable: DM yield of maize (g/pot), 2009						
Constant term	135.00	0.90	150.00	0.000	133.00	137.00
RP	7.00	0.90	7.77	0.000	5.15	8.85
PF	6.54	0.90	7.26	0.000	4.69	8.38
AMF	6.38	0.90	7.08	0.000	4.53	8.22

RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. (n=20, Significant at $p < 0.05$)

Table a 10 Parameters affecting DM yield of faba bean plants in 2007, 2008, 2009 experiments

Parameter estimates						
Dependent variable: DM yield of faba bean (g/pot), 2007						
Parameter	Regression coefficient B	Standard error	T	Sig.	95%-confidence interval of B	
					Lower Bound	Upper Bound
Constant term	36.90	0.29	129.00	0.000	36.30	37.50
RP	2.18	0.38	5.78	0.000	1.38	2.98
PF	3.09	0.29	10.80	0.000	2.48	3.69
AMF	2.16	0.29	7.58	0.000	1.56	2.77
Dependent variable: DM yield of faba bean (g/pot), 2008						
Constant term	24.20	2.39	10.14	0.000	19.36	29.04
RP	1.32	3.16	0.42	0.677	-5.08	7.73
PF	1.04	2.39	0.43	0.666	-3.80	5.88
AMF	1.36	2.39	0.57	0.572	-3.48	6.20
Dependent variable: DM yield of faba bean (g/pot), 2009						
Constant term	69.00	2.72	25.40	0.000	63.40	74.80
RP	13.60	2.72	5.00	0.000	8.03	19.20
PF	22.30	2.72	8.20	0.000	16.80	27.90
AMF	13.40	2.72	4.92	0.000	7.81	19.00

RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. (n=20, Significant at $p < 0.05$).

Table a 11 Parameters affecting P uptake of maize plants in 2007, 2008, 2009

Parameter estimates						
Dependent variable: P uptake of maize plants (mg/pot), 2007						
Parameter	Regression coefficient B	Standard error	T	Sig.	95%-confidence interval of B	
					Lower Bound	Upper Bound
Constant term	134.00	9.75	13.80	0.000	113.00	155.00
RP	44.60	12.90	3.46	0.003	17.30	72.00
PF	42.40	9.75	4.35	0.000	21.70	63.00
AMF	43.90	9.75	4.51	0.000	23.30	64.60
Dependent variable: P uptake of maize plants(mg/pot), 2008						
Constant term	79.00	3.69	21.40	0.000	71.50	86.50
RP	7.95	4.88	1.63	0.112	-1.95	17.90
PF	-0.12	3.69	-0.03	0.975	-7.61	7.37
AMF	-1.16	3.69	-0.31	0.755	-8.65	6.33
Dependent variable: P uptake of maize plants (mg/pot), 2009						
Constant term	115.00	5.04	22.90	0.000	105.00	126.00
RP	20.10	5.04	3.99	0.000	9.80	30.40
PF	24.20	5.04	4.81	0.000	13.90	34.50
AMF	41.50	5.04	8.23	0.000	31.20	51.80

RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. (n=20, Significant at $p < 0.05$)

Table a 12 Parameters affecting P uptake of faba bean plants in 2007, 2008, and 2009

Parameter estimates						
Dependent variable: P uptake of faba bean plants (mg/pot), 2007						
Parameter	Regression coefficient B	Standard error	T	Sig.	95%-confidence interval of B	
					Lower bound	Upper bound
Constant term	96.60	5.89	16.40	0.000	84.10	109.00
RP	1.80	7.80	0.23	0.820	-14.70	18.30
PF	30.90	5.89	5.25	0.000	18.40	43.40
AMF	37.10	5.89	6.29	0.000	24.60	49.60
Dependent variable: P uptake of faba bean plants (mg/pot), 2008						
Constant term	37.70	1.63	23.11	0.000	34.40	41.00
RP	5.40	2.16	2.50	0.017	1.02	9.78
PF	2.88	1.63	1.77	0.086	-0.43	6.19
AMF	2.22	1.63	1.36	0.182	-1.09	5.53
Dependent variable: P uptake of faba bean plants (mg/pot), 2009						
Constant term	89.60	5.83	15.40	0.000	77.70	102.00
RP	34.60	5.83	5.94	0.000	22.70	46.60
PF	22.80	5.83	3.91	0.001	10.90	34.80
AMF	31.30	5.83	5.36	0.000	19.30	43.20

RP=rock phosphate, PF=*Pseudomonas fluorescens*, AMF=arbuscular mycorrhizal fungi. (n=20, Significant at $p < 0.05$)

Erklärung

Ich erkläre, dass ich die vorliegende Doktorarbeit mit dem Titel:
„ Growth and nutrient uptake in maize and faba bean plants inoculated with arbuscular mycorrhizal fungi and Pseudomonas fluorescens DR54 and fertilised with rock phosphate„ selbständig und ohne Benutzung anderer als der angegebenen Quellen und Hilfsmittel verfasst habe.

Rostock, den 06.01.2015

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Theses

Growth and nutrient uptake in maize and faba bean plants inoculated with arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* DR54 and fertilised with rock phosphate

Presented by

DIPL.-ING. AGR. GHINA AL NAJJAR

Problem and Research Approach

- Sustaining and optimizing crop yields are main objectives in modern agriculture. Nutrient deficiency is one obstruction in the development of an economically successful agriculture in many parts in the world. Phosphorus (P) and nitrogen (N) are essential plant elements which strongly influence the agricultural and natural ecosystems.
- Application of P fertilisers to agricultural land can improve crop production and soil P fertility, although negative impacts on environment also occurred in the past. Rock phosphate (RP) is widespread used as mineral P, however its effect on plant nutrition can be limited due to the low solubility of apatite.
- Arbuscular mycorrhiza fungi (AMF) and plant growth promoting rhizobacteria (PGPR) can be applied as bio-fertilizers in order to improve plant growth and nutrition which can help to reduce the application rates of nutrients.
- The mycorrhiza is a symbiotic relationship between fungi and roots of vascular plants. Plants colonised with AMF usually respond better to applications of insoluble phosphate forms than non-mycorrhizal plants.
- *Pseudomonas fluorescens* (PF, strain DR54) is one of most efficient strain of phosphate solubilising bacteria. Furthermore, it shows the ability to increase the

growth and yield of different agricultural plants through several mechanisms such as phytohormone and organic acid production, stimulation of nutrient uptake and bio-control of deleterious soil bacteria and phyto-pathogenic fungi.

- The key aspect of this work was to evaluate the availability of P in combined application of the bio-fertilisers with RP. To achieve this goal, several experiments were conducted under controlled and semi-controlled conditions.
- In pot experiments, the single and combined application of AMF and PF along with RP on growth and nutrient uptake of maize (*Zea mays*) and faba bean (*Vicia faba*) were investigated. Special attention was given to the vegetation length, N fertilisation and P soil content.

  Furthermore, concentrations of organic acids and their ability to dissolve RP in soil were investigated under controlled conditions in pot and in vitro experiments.

Main results and future Outlook

- Application of RP alone can result in higher yields and nutrient uptakes of maize and faba bean. This effect was more pronounced with running experimental time and additional application of PF and AMF.
- Faba bean was more efficient than maize in using P from RP. This effect can be utilized to improve the P nutrition in mixed cropping systems or in a crop rotation.
- The combined application of PF and AMF with RP usually showed better results regarding the P availability from RP than a single inoculation of either PF or AMF. In addition, plant N uptake and yield were also higher in the combined treatment.
- An additional application of NH_4NO_3 to maize plants during the vegetation time of about eight weeks along with PF and AMF resulted in higher P uptake compared to the control without N fertiliser, which was related to the higher biomass production.
- The main organic acids produced by inoculated maize plants were citric acid (Cit-A), lactic acid (Lac-A) and formic acid (For-A). Higher concentrations of these organic acids and P uptake were found when PR was added.

- Lowering the pH value due to the excretion of organic acids by the PF strain may be considered as one mechanism that enhances P availability from RP in this study.
- The co-inoculation of PGPR and AMF represents a promising approach in the bio-fertilizer research. The bacterial strains also may interact synergistically with other bacterial strains, which should be investigated in studies comprising co-inoculation and single inoculation of bacterias in order to clarify potential benefits on plant development.
- Furthermore, it is necessary to investigate the survival rate of the microorganisms after application under field conditions and to evaluate their effect on the native microbial diversity and activity

Application of beneficial PGPR and AMF in combination with RP could be an opportunity to reduce the fertilizer need and to contribute to a sustainable agriculture. However, economical advantages for the farmers will depend on the prices for fertilizers and bio-fertilizers.