Arbuscular mycorrhizal fungus influence maize root growth and architecture in rock phosphate amended tropical soil

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Resumen

Influencia del hongo arbuscular micorrícico en el crecimiento y arquitectura radicular en suelo tropical modificado con fosforita

Hemos ensayado la influencia del homgo micorrícizo arbuscular (AM) *Scutellospora calospora* en la estructura, crecimiento, asimilación de nutrientes, actividad fosfatasa y dependencia micorrizal de raíces de maíz por adicción de 0-5% de fosforita (RP) en suelos deficientes de fósforo (P). La adicción de RP aumentó significativamente la longitud total de la raíz, el número de raíces a diferentes niveles y el diámetro de los pelos radiculares de las plantas AM. El hongo AM influyó positivamente el crecimiento del maíz y la asimilación de nutrientes. Las actividades fosfatasa ácida y alcalina fueron mayores en las plantas AM en suelos mejorados. Al aumentar las concentraciones RP se redujeron no linealmente el porcentaje de colonización del hongo AM. Entonces, la inoculación de hongos AM junto a la mejora de fósfoso proveniente de RP podría sustituir fertilizantes químicos y hacer disponible el P proveniente de RP.

Palabras clave: Hongo AM, Dependencia micorrizal, Nutrientes, Fósforo, Fosfatasa, Pelos radiculares.

Abstract

We evaluated the influence of arbuscular mycorrhizal (AM) fungus *Scutellospora calospora* on root architecture, growth, nutrient uptake, root phosphatase activity and mycorrhizal dependency of maize in 0-5% rock phosphate (RP) amended phosphorus (P) deficient soil. RP amendment significantly increased total root length, number of roots in different orders, and root hair diameter of AM plants. The AM fungus positively influenced maize growth and nutrient uptake. Acid and alkaline phosphatase activities were higher for AM plants in RP amended soils. In contrast, increasing concentrations of RP reduced the percentage of AM fungus colonization non-linearly. Thus, AM fungus inoculation along with RP amendment could substitute chemical fertilizers and make available the P in RP to the plants.

Key words: AM fungi, Mycorrhizal dependency, Nutrients, Phosphorus, Phosphatase, Root hairs

Introduction

Phosphorus (P) is one of the most essential, but least available macronutrient for plant growth in many tropical soils. Phosphate, the available form of P though modulate the fundamental trait of plants, it is neither soluble in the soil solution, nor readily transported by mass flow (Bagyaraj *et al.* 2015). Many soils contain more organic forms of phosphate (Po) compared to inorganic forms. Therefore, frequent application of inorganic P (Pi) in the form of synthetic fertilizers becomes obligatory in crop production systems. But, the use of inorganic synthetic fertilizers on a regular basis is not only expensive but also environmentally undesirable (Arcand & Schneider 2006). Therefore, the current tendency is either to avoid or trim down the use of synthetic fertilizers and to increase the use of natural materials like rock phosphate (RP) in crop production (Arcand & Schneider 2006).

The microorganisms involved in solubilizing minerals and organic phosphates are rife in soil (Barea & Richardson 2015). In nutrient deficient soils, these microorganisms utilize the energy derived from the breakdown of fresh carbon compounds to release Pi from organic sources (Arcand & Schneider 2006). Nevertheless, this effect might be even more important in the presence of the obligate endosymbiotic arbuscular mycorrhizal (AM) fungi which aid in the uptake of the released Pi from the soil. The AM fungi belonging to the phylum Glomeromycota are ubiquitous in most plant communities and interconnect the intraradical with the extraradical environment (Smith & Read 2008). Although, the capacity of AM fungi to solubilize P is controversial, its capacity to enhance the uptake of P from the soil is well proven (Smith & Read 2008).

Plant species exhibit different strategies to acquire nutrients from nutrient-deficient soils. These include changes in root morphology, exudation of enzymes and organic acids into the rhizosphere that dissolves and release nutrients from organic compounds (Arcand & Schneider 2006). Although roots play an important role in the acquisition of nutrients from the soil by plants, its architecture is a highly plastic trait. Root architecture differs with plant species and is strongly controlled by plant growth regulators, inherent genetic factors, and soil conditions (Niu *et al.*

2012). The availability of P in the soil also induces changes in root architecture like primary root length, branching, number and length of lateral roots and root hair development (Niu *et al.* 2012). However, the AM fungal colonization depends on the anatomical features of the root system, and also it could alter the host root morphology (Dreyer *et al.* 2014). Further, it is generally believed that mycorrhizal plants have a coarse root system with no or few short root hairs and exhibit increased growth response to AM fungal colonization. Nevertheless, this view has been recently disputed by Maherali (2014). As AM fungi can induce changes both in plant hormone and nutrient contents (Smith & Read 2008), it would be interesting to assess the role of AM fungi in plant root architecture.

Like other soil fungi, AM fungi also exhibit phosphatase activity both in alkaline and acid pH ranges. In addition, AM fungi can also enhance plant's uptake of Pi from P-deficient soils through their extraradical hyphae that scavenge soil volumes beyond the reach of plant roots (Smith & Read 2008). In mycorrhizal plants, phosphatase enzyme appears during the initial stages of mycorrhization and peaks just prior to or during mycorrhizal establishment, thus playing a key role in the P assimilation (Khade *et al.* 2010). Both, intracellular and extracellular phosphatase produced by AM fungi has been shown to liberate Pi from Po (Khade *et al.* 2010). Acid phosphatase localized in mature arbuscules, intraradical hyphae and at the fungal entry points hydrolyse vacuolar polyphosphate to Pi and release them into the plant-fungal interface (Abdel-Fattah 2001). In contrast, alkaline phosphatases that are mainly present in vacuoles in addition to arbuscules and intraradical hyphae are also involved in the transfer of P from the fungus to the host (Kojima *et al.* 1998). Although many studies have examined the solubilization of RP by phosphate solubilizing microorganisms and its influence on plant growth (Arcand & Schneider 2006 and references there in), studies on the effect of RP on AM formation and function are limited.

Maize (*Zea mays* L.), the major food source for humans and livestock in many parts of the world has a unique root system which is highly efficient not only in anchoring the plant to the soil but also in acquiring nutrients and water from the soil (Hochholdinger & Tuberosa 2009). In addi-

tion, the root system of maize also possesses several morphological and metabolic traits that are essential for increased efficiency like adventitious roots, long and dense root hairs, basal-root shallowness, root etiolation and cortical aerenchyma (Hochholdinger & Tuberosa 2009). Several studies reported the mycorrhizal status and colonization patterns like *Arum-*, *Paris-* and intermediate-type in maize (Muthukumar & Prakash 2009; Muthukumar & Tamilselvi 2010; Chandra Gandhi *et al.* 2017). Further, maize genotypes exhibit variation in their responsiveness to mycorrhizal colonization (Kaeppler *et al.* 2000). Recently, Wang *et al.* (2017) in a long term experiment showed that increasing P fertilization in spite of reducing root colonization and community structure of AM fungi can still contribute substantially to P nutrition of maize plants. Nevertheless, this AM fungal benefit depends on the growth stage of maize plants and the soil layer in which roots are located. Though previous studies have examined the influence of RP on AM fungal colonization of roots or on root architecture, there is no information on the simultaneous application of RP and AM fungi on root architecture. So, the main objective of the present study was to assess the influence of AM fungal inoculation and RP amendment on root growth and architecture, and its related changes in plant growth, nutrient uptake, root phosphatase enzyme activities and mycorrhizal dependency of maize in a P-deficient tropical soil.

Materials and methods

Experimental site and soil preparation

The experiment was conducted in the shade house of the Botany Department, Bharathiar University (11º 01' N, 96º 93' E, altitude 410 MASL), Coimbatore, India. The physicochemical characteristics of the soil as determined by standard methods (Jackson 1971) were as follows: pH 6.1, electrical conductivity 0.11 dSm^{-1}, 45 mg/kg of total N, 8.5 mg/kg of available P and 500 mg/kg of exchangeable K. The micronutrients iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were 4.75, 2.64, 1.06 and 1.50 mg/kg as determined by the DTPA method (Lindsay & Norvell 1978). The soil was heat sterilized at 121 ºC at 15 psi for 3 h thrice with 24 h intervals between subsequent sterilization and shade dried for 15 days for refixation of liberated nutrients.

Plant material and AM fungus inoculum

Maize (*cv* CO 6) seeds procured from Tamil Nadu Agricultural University, Coimbatore, India was used for this study. The AM fungus *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders used in this study originated from the semi-arid grassland of Maruthamalai Hills, Coimbatore. The fungus was maintained as a pure culture in sterile soil with *Plectranthus scutellarioides* (L.) R.Br., as the host plant. The fungal inoculum consisted of spores, hyphae, and mycorrhizal roots. The number of AM fungal propagules per gram of the inoculum was assessed using the most probable number (MPN) technique (Feldmann & Idczak 1992). Results were expressed as the number of AM infective propagules per gram of soil.

Experimental design

A two-factorial experiment [concentrations of RP (containing 10.23% P$_2$O$_5$) and AM inoculation] was conducted in a completely randomized block design with ten replicates. Inoculation of AM fungus was assigned to the main plot and RP concentrations to the subplots. The RP treatment consisted of six different concentrations of RP (0%, 1%, 2%, 3%, 4% and 5%, v/w) mixed thoroughly with the sterilized soil. A 225 g of the soil mix was filled in each of the 120 (6×2×10) plastic pots (9×7.5 cm, height ×width). Half of the total number of pots in each treatment was inoculated with three grams of the AM fungal inoculum consisting of 220 infective propagules per gram of inoculum. The AM fungal inoculum was layered 4 cm below the soil surface and each pot was seeded with two maize seeds. Twenty milliliters of microbial wash prepared from AM fungal inoculum as per Muthukumar *et al.* (2001) was added to pots not inoculated with the AM fungus to equalize the background microflora except the AM fungus across treatments. The pots were rearranged every week to expose all the plants to uniform conditions. Plants were watered as necessary with tap water and no nutrients were added. The destructive harvest of the plants was carried out after 58 days of growth.

Harvest and measurements

At harvest, half of the plants from each treatment were used for phosphatase assay and for AM colo-

nization determination. The roots of these plants were washed thoroughly free of soil and a portion of the roots was removed and preserved in FAA solution for the assessment of AM colonization. The remaining portion was used for assessing acid and alkaline phosphatase activity. The other half of the replicates was used for determination of growth, root characteristics and nutrient parameters. Shoots and roots were separated and dried at 40 ºC for 72 hrs for determination of dry mass. Plant growth was assessed using standard parameters like shoot and total root length, and dry weights of shoot and root. In addition, other morphological parameters like root diameter, root hair number, length and diameter, and lateral root numbers in differed root orders were also measured. Total root lengths of plants in different treatments were measured according to Newman (1966). Root diameter and root hair characteristics were measured at ten 1-cm root bits floated in water using a calibrated ocular micrometer. The 1st-and 2nd- order roots in each root system were determined manually after spreading the entire plant root system in a 40×30 cm (l×b) plastic tray containing water. The roots were divided into two orders, primary roots directly arising from the crown roots, 1st - order arising from the primary root and roots of 2nd - order arising from the 1st - order roots.

Phosphatase assay

One gram of fresh root tissue was homogenized in 10 ml of ice-cold 50 mM of citrate buffer (pH-5.3) for acid phosphatase or 50 mM glycine-NaOH buffer (pH-10.4) for alkaline phosphatase in a pre-chilled pestle and mortar. The homogenate was filtered and centrifuged at 10,000 g for 10 min. The supernatant was used as the enzyme source. Phosphatase activity was measured at 405 nm in a spectrophotometer (UV-1601 visible spectrophotometer) and expressed in terms of m moles of p-nitrophenol released per gram of fresh tissue per min at 37 ºC (Sadasivam & Manickam 1992).

Estimation of macronutrients in plant tissues

In the shoot, N was determined by the micro-Kjeldahl digestion method using concentrated H_2SO_4 digest and potassium sulphate - copper sulphate in 5:1 ratio as a catalyst. After wet-ashing the plant samples in a nitric-sulphuric-perchloric acid mixture, the P was determined by the Vanado molybdate method (Jackson 1971) using a spectrophotometer at 470 nm and the K was estimated by flame photometry (Jackson 1971).

Preparation of roots and assessment of AM fungal colonization

Fixed roots were washed free of FAA, cut into 1-cm bits, cleared in 2.5 % KOH at 90 ºC, acidified with 5N HCl and stained with trypan blue (0.05% in lactoglycerol) overnight. The stained roots were examined with a compound microscope (400 ×) for AM fungal structures and the percentage of root length colonization was estimated according to a magnified intersection method (McGonigle *et al.* 1990).

Mycorrhizal dependency

Mycorrhizal dependency was determined by using the biomass of AM and non-AM plants as per Plenchette *et al.* (1983).

Statistical analysis

Analysis of variance (ANOVA) was performed on all data to compare treatment effects and influence of AM and RP application on maize plant growth and phosphatase activity. Means were separated using Duncan's Multiple Range Test (DMRT). Pearson's correlation and regression analysis were used to assess the relationship between different variables. Percentage data on mycorrhizal colonization were arcsine square root transformed prior to analysis (SPSS, windows Version 9).

Results

Plant growth

Maize plants raised in various concentrations of RP showed significant variations in growth parameter both under mycorrhizal and non-mycorrhizal conditions (Table 1). Non-mycorrhizal plants grown in soils amended with 2%RP were the tallest and those grown in 1%RP amended soils were the shortest. A decline in plant height was observed in plants raised in 2% to 5%RP amended soils. In contrast, increasing concentrations of RP increased plant height under mycorrhizal condition.

    The RP amendment had no significant influence on shoot and root dry weights of maize plants (Table 1). AM fungal inoculation signifi

Parameters		Rock phosphate concentrations (%)						Mean	F statistics		
		0	1	2	3	4	5		RP (A$_{5,108}$)	AM (B$_{1,108}$)	A × B$_{(5,108)}$
Plant height (cm)	-AM	51.93a	42.90a	55.12a	52.51a	47.87a	45.32a	49.27	<1ns	19.37***	<1ns
	+AM	56.79a	57.90a	57.28a	62.08a	63.46a	60.52a	59.67***			
Dry weight (mg)											
Shoot	-AM	610ab	500ab	770a	690ab	390b	520ab	580	<1ns	2.64ns	2.18ns
	+AM	620a	640a	630a	620a	710a	760a	663.33ns			
Root	-AM	40b	40b	80a	60ab	40b	40b	50	1.52ns	3.98*	1.18ns
	+AM	60a	70a	70a	60a	50a	80a	65*			
Root shoot ratio	-AM	0.06b	0.08ab	0.10a	0.09ab	0.12a	0.10a	0.09	<1ns	<1ns	2.37*
	+AM	0.10a	0.10a	0.09a	0.09a	0.07a	0.10a	0.09ns			
Total root length (m plant^{-1})	-AM	1.23b	1.12b	4.10a	3.29ab	1.20b	1.21b	2.03	1.826ns	19.146***	2.870*
	+AM	4.03ab	3.58ab	4.14ab	3.10b	3.25b	6.21a	4.16***			
Root characters											
Root numbers (plant^{-1})											
Primary	-AM	5.80a	3.80a	7.40a	8.20a	5.80a	4.00a	5.83	<1ns	10.62**	2.79*
	+AM	7.80ab	11.20a	8.00ab	6.20b	8.80ab	8.40ab	8.40**			
1st order	-AM	73.13a	65.47a	77.73a	86.27a	52.20a	54.67a	68.25	<1ns	22.15***	1.30ns
	+AM	107.40a	111.33a	98.07a	89.93a	86.00a	114.67a	101.23***			
2nd order	-AM	12.13a	9.93a	21.60a	21.47a	5.00a	8.00a	13.02	2.00ns	22.07***	4.66**
	+AM	28.53bc	52.80a	23.67bc	12.40c	21.80bc	39.87ab	29.85***			
Primary root diameter (µm)	-AM	734.47ab	576.91b	662.96ab	778.71a	712.66ab	762.95a	704.78	<1ns	<1ns	1.73ns
	+AM	739.32a	724.17a	712.66a	650.84a	647.21a	714.47a	698.11ns			
Root hair characters											
Number (mm^{-1})	-AM	12.03a	11.66a	15.25a	12.69a	12.91a	10.71a	12.54	<1ns	12.06**	1.30ns
	+AM	15.69a	17.31a	15.11a	14.96a	15.84a	19.73a	16.44***			
Diameter (µm)	-AM	6.70a	6.36a	8.79a	7.42a	6.97a	6.82a	7.18	1.12ns	12.88***	1.82ns
	+AM	12.12a	9.64ab	8.79ab	8.48ab	10.91ab	7.27b	9.54***			
Length (µm)	-AM	521.16a	452.68a	486.62a	481.77a	462.98a	477.53a	480.46	<1ns	<1ns	<1ns
	+AM	459.35a	510.86a	468.44a	409.66a	487.22a	469.04a	467.43ns			

Means in a row followed by a same letter(s) are not significantly (P>0.05) different according to Duncan's Multiple Range Test.
*, **, ***, ns: significant at P<0.05, P<0.01, P<0.001 and non significant respectively.

Tabla 1. Crecimiento de planta de maíz y caracteres de la raíz por influencia de la aplicación de diferentes concentraciones de fosforita (RP) e inoculación de hongo micorrícico arbuscular (AM). -AM/+AM incidan la ausencia o preencia de la inoculación fúngica.

Table 1. Maize plant growth and root characters as influenced by different concentrations of rock phosphate (RP) application and arbuscular mycorrhizal (AM) fungus inoculation. -AM, +AM indicate the absence or presence of AM fungus inoculation.

cantly affected root dry weight, but not the shoot dry weight in maize. Non-mycorrhizal plants raised in 2% and 4%RP amended soils had the maximum and minimum shoot dry weights respectively. In contrast, the maximum shoot dry weight of mycorrhizal plants occurred in 5%RP, and minimum in 0% and 3%RP amended soils respectively. Like shoot dry weight, maximum and minimum root dry weights of non-mycorrhizal maize plants occurred in 2%RP and other concentrations of RP except 3% at RP amendment. Among AM plants, maximum root dry weight was recorded in 5%RP and minimum root dry weight was recorded in 4%RP respectively.

The interaction of different concentrations of RP application and AM inoculation on R/S ratios of maize plant was evident (Table 1). Nevertheless, neither AM inoculation nor the various concentrations of RP amendment had any influence on maize R/S ratios. Generally, the R/S ratios of AM plants were almost similar or lower compared to their non-AM conspecifics (except at 1%RP). Lowest R/S was recorded for plants raised in the 0%RP amendment under non-AM and in 4%RP amendment under AM conditions.

Influence of RP on root characters

Different concentrations of RP had no significant influence on total root length in maize. Nevertheless, AM inoculation significantly enhanced the total root length of maize both under RP amended and non-amended conditions (Table 1). Nonmycorrhizal plants raised in the highest concentration of RP (5%) had the minimum root length, while those raised on 2%RP had the maximum root length. In contrast, AM maize plants raised in 5% and 1%RP amended soils had the maximum and minimum root lengths (Table 1).

Application of RP at different rates had no significant influence on lateral root numbers, whereas, AM fungus inoculation significantly influenced lateral root production (Table 1). Generally, AM-plants had significantly higher number of lateral roots except for plants in 3%RP where the primary and 1st - order root numbers of AM plants was 24% and 42% lower than non-AM plants. The primary root production was 8% to 198% higher for AM-plants compared to non-AM plants.

Mycorrhizal inoculation significantly influenced the 1st - and 2nd - order root numbers. The 1st - order root numbers of AM plants were 4% to

110% higher compared to non-AM plants. The 2nd - order root numbers of AM plants were 0.1% to 432% higher than non-AM plants (Table 1). In contrast to root numbers, the primary root diameter was not influenced by RP concentrations, AM fungus inoculation and their interactions (Table 1).

Root hair characteristics

Rock phosphate application had no significant effect on root hair numbers (Table 1). Nevertheless, AM-plants had 18 to 84% more root hairs compared to non-AM plants (except in plants in 2%RP). Similarly, root hairs of AM plants were 6 to 81% thicker than non-AM plants (Table 1). Contrarily, neither AM inoculation nor RP application rates had any significant influence on root hair length (Table 1).

Plant tissue nutrients

Application of RP and AM inoculation significantly affected tissue N, P and K concentrations in maize shoots (Fig. 1). There was a sequential increase in the shoot N, P and K contents of AM and non-AM maize with increasing concentration of RP (Fig. 2). The maximum and minimum shoot nutrient concentrations in AM and non-AM maize plants occurred in 5% and 0%RP application rates. Average N, P, and K concentrations were 15%, 20%, and 22% higher of AM plants compared to non-AM plants.

Phosphatase enzyme activity

Both inoculation of AM fungus and RP application rates significantly influenced acid and alkaline phosphatase activities of maize roots. Nevertheless, the interactions between these factors were only significant for alkaline phosphatase activity (Table 2). Acid phosphatase activity of AM roots was only 0.2% to 6% higher than non-AM roots, whereas alkaline phosphate activity of AM roots was 1.4% to 26.9% higher than non-AM roots.

Extent of AM fungus colonization

No AM fungal structures were observed in the roots of uninoculated plants. The percentage of total root length colonized by *S. calospora* (%RLTC) varied significantly ($F_{5,89}=18.83$; $P<0.001$) with concentrations of RP and ranged from 22.20% (2%RP) to 46.78% (0%RP). The %RLTC decreased with increasing concentrations
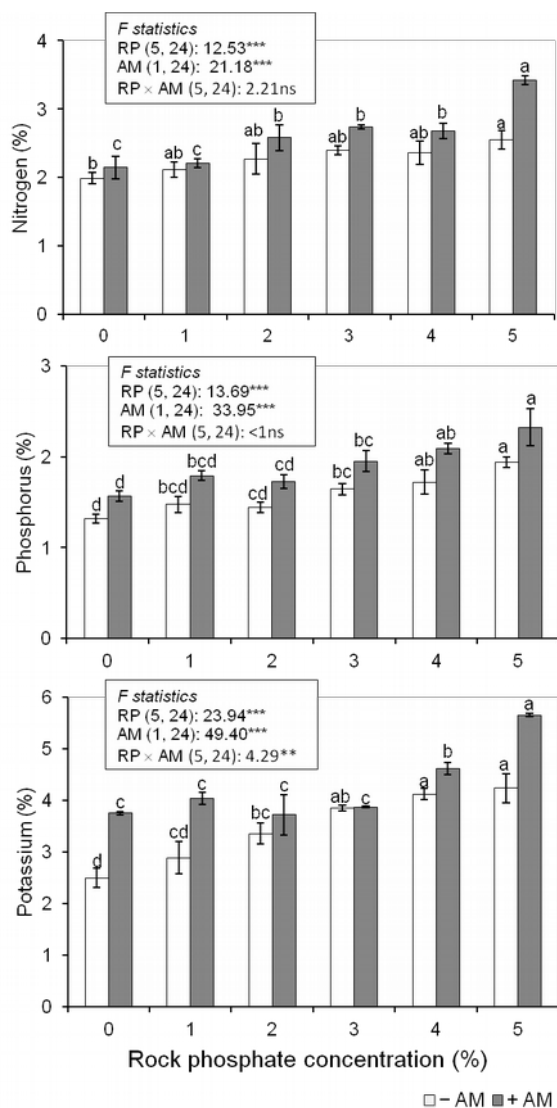
Figura 1. Concentraciones de nitrógeno, fósforo, potasio en tejidos de brote de plantas de maíz cultivadas a distintas concentraciones de fosforita (RP) e inoculadas (+AM) o no (-AM) por el hongo micorrícico arbuscular *Scutellospora calospora*. Estadísticos: Las diferencias entre el numerador y el denominador se representa entre paréntesis. **, **, ns: significantivo a P<0,01, P<0,001 o no significativo, respectivamente. Las barras de error indican ± el error estándar. Las barras para +AM o -AM que muestran la misma(s) letra(s) no son significativamente diferentes (P>0,05) de acuerdo con el DMRT.

Figure 1. Shoot tissue nitrogen, phosphorus, potassium concentrations of maize plants raised on different concentrations of rock phosphate (RP) and inoculated (+AM) or uninoculated (-AM) with the arbuscular mycorrhizal (AM) fungus *Scutellospora calospora*. F-statistics: Numerator and denominator differences are presented in parenthesis. **, ***, ns: significant at P<0.01, P<0.001 and non significant respectively. Error bars indicate ± standard errors. Bars for +AM or -AM bearing same letter(s) are not significantly different (P>0.05) according to DMRT.

Treatments[a]	Enzyme activity (mM/g FW/min)			
	Acid phosphatase		Alkaline phosphatase	
	-AM	+AM	-AM	+AM
0%RP	0.498a	0.506ab	0.386a	0.413b
1%RP	0.472ab	0.496bc	0.368a	0.373c
2%RP	0.482a	0.483bc	0.378a	0.386bc
3%RP	0.490a	0.501abc	0.376a	0.424ab
4%RP	0.450b	0.477c	0.349a	0.408bc
5%RP	0.498a	0.525a	0.357a	0.453a
Mean	0.482	0.498**	0.369	0.410***
RP concentr. ($A_{5,48}$)	7.16***		2.72*	
AM ($B_{1,48}$)	9.60**		33.51***	
A × B($_{5,48}$)	<1 ns		4.10**	

Means in a column followed by a same letter(s) are not significantly (P>0.05) different according to Duncan's Multiple Range Test.
*, **, ***, ns: significant at P<0.05, P<0.01, P<0.001 and non significant respectively.

Tabla 2. Actividad fosfatasa ácida y alcalina en raíces de maíz en diferentes concentraciones de fosforita e inoculadas (+AM) o no (-AM) por hongo micorrícico arbuscular.

Table 2. Acid and alkaline phosphatase activity in maize plant roots raised on different concentrations of rock phosphate and inoculated (+AM) or uninoculated (-AM) with arbuscular mycorrhizal fungus.
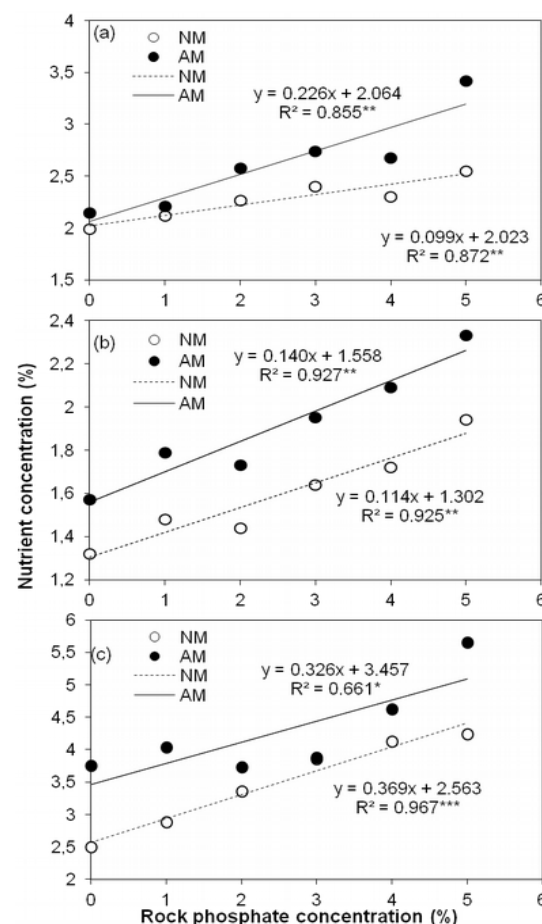

Figura 2. Relación de las concentraciones de nitrógeno (a), fósforo (b) y potasio (c) con diferentes proporciones de adición de fosforita con micorrización (AM) no (NM). *, **, **: significativo P<0,05; P<0,01 y P<0,001 respectivamente.

Figure 2. Relationship of shoot tissue nitrogen (a), phosphorus (b) and potassium (c) concentrations to different rates of rock phosphate amendment under non mycorrhizal (NM) or mycorrhizal (AM) conditions in maize. *, **, *** significant at P<0.05; P<0.01 and P<0.001 respectively.
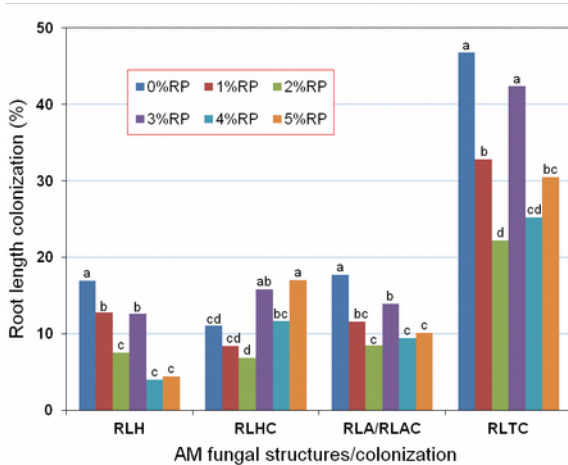
Figura 3. Colonización micorrícica arbuscular (AM) en raíces de maíz cultivadas en diferentes concentraciones (0-5%) de fosforita. RLH, RLHC, RLA/RLAC y RLTC indican el porcentaje de longitud de raíz conteniendo hifas, ovillos, arbúsculos/ovillos arbusculares y el total de colonización total AM. La barras que presentan letra(s) similar(s) no son significativamente diferentes (P>0,05) de acuerdo con el DMRT.

Figure 3. Arbuscular mycorrhizal (AM) colonization in maize roots raised in different concentrations (0-5%) of rock phosphate. RLH, RLHC, RLA/RLAC and RLTC indicate percentage root length containing hyphae, hyphal coils, arbuscules/arbusculate coils and AM total colonization. Bars for an AM structure/colonization bearing same low case letter(s) are not significantly (P>0.05) different according to DMRT.
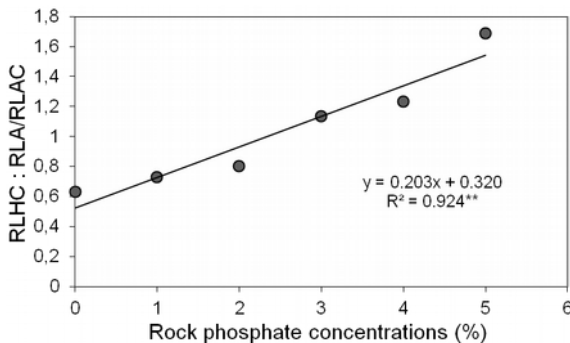

Figura 4. Relación entre la proporción de longitud de raíz conteniendo ovillos hifales (RLHC)/ longitud de raíz conteniendo arbúsculos/ovillos arbusculares (RLA/RLAC) y las diferentes concentraciones de fosforita. ** significante a P<0,01.

Figure 4. Relationship between the ratio of percentage root length containing hyphal coils (RLHC) to root length containing arbuscules/arbusculate coils (RLA/RLAC) and different concentrations of rock phosphate. ** significant at P<0.01.

of RP, but the decrease was not linear (r=−0.469; P>0.05) (Fig. 3). The percentage of root length with linear hyphae (%RLH) was significantly influenced by RP application ($F_{5,89}$=13.75; P<0.001) and ranged from 4% (4%RP) to 17% (0%RP) and decreased non-linearly (P>0.05) with increasing concentrations of RP. The percentage of root length with hyphal coils (%RLHC) ranged from 7% (2%RP) to 17% (5%RP) and significant
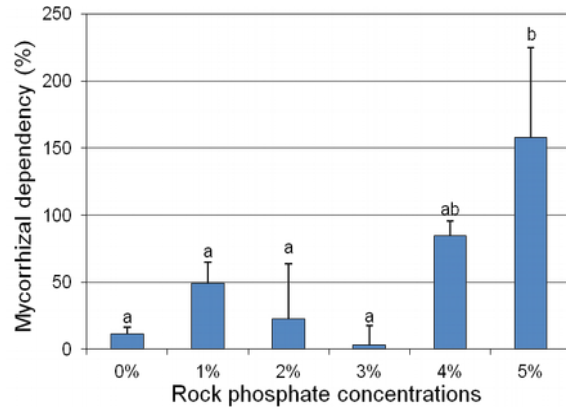

Figura 5. Dependencia de micorrización del maíz cultivado a diferentes concentraciones de fosforita. Las barras con con las mismas letras no son significativamente diferentes (P>0.05) de acuedo con el DMRT. Las barras de error indican ± 1 SD.

Figure 5. Mycorrhizal dependency of maize raised on different concentrations of rock phosphate. Bars bearing same low case letter(s) are not significantly different (P>0.05) according to DMRT. Error bars indicate ± 1 SD.

ly varied ($F_{5,89}$=6.99; P<0.001) with RP application rates. Likewise, percentage root length with arbuscules/arbusculate coils (%RLA/%RLAC) ranged from 8% (2%RP) to 18% (0%RP) and varied significantly ($F_{5,89}$= 8.83; P<0.001) with RP application rates. The ratio of %RLHC to %RLA/ %RLAC linearly increased with increasing concentrations of RP (Fig. 4).

Mycorrhizal dependency

Mycorrhizal dependency was significantly influenced by RP application ($F_{5,54}$=3.022; P<0.018) and ranged from 3.29% (3%RP) to 157.63% (5%RP) under different concentrations of RP amendment (Fig. 5).

Discussion

In this study, increasing concentrations of RP application reduced plant height, and this negative effect of RP on plant growth was ameliorated by AM fungus inoculation. Ramirez *et al.* (2009) also reported a reduction in maize growth in response to RP application and a reversal of the effect by inoculation of the AM fungus *Gigaspora margarita* W.N. Becker & I.R. Hall. Stimulation in plant height in response to dual application of RP and AM fungi has also been reported in several cereal crops (Costa *et al.* 2015; Wahid *et al.* 2016). The increase in plant height in response to AM colonization can be attributed to the efficient uptake and utilization of P solubilized from RP by the AM plants. This increased efficiency is due to

increased root absorbing area brought about by the extraradical mycelial network of AM fungus in the soil (Maherali 2014).

Rock phosphate application had no significant influence on shoot and root dry weights. Nevertheless, the shoot and root dry weights gradually increased with increasing concentrations of RP in AM plants. The higher biomass of AM plants can be attributed to the increased activity of the extraradical mycelial network of AM fungi around the roots as well as increased photosynthesis (Ayoob *et al.* 2011). Generally, the R/S ratios of AM plants in the present study were similar or lower compared to their non-AM conspecifics (except at 1%RP). Khade *et al.* (2010) also recorded higher R/S ratios in mycorrhizal papaya at the end of period of four months of growth, which was attributed to the increased carbon allocation for root production in mycorrhizal plants than non-mycorrhizal plants. Usually, mycorrhizal plants exhibit lower R/S ratios than non-mycorrhizal plants in response to greater increment in shoot mass relative to root mass due to an increase in efficiency of the roots by AM fungi (Maherali 2014).

The increased total root length in response to AM inoculation in maize is similar to those reported by Dickson *et al.* (1999), where *Allium cepa* L. colonized by *S. calospora* and *Glomus* sp. 'City Beach' (*Glomus* Tul. & C. Tul.) had longer roots than their non-AM counterparts. In the present study, inoculation of the AM fungus along with RP application affected the root proliferation in maize. Berta *et al.* (1990) also demonstrated an overall increase in branching of the root system in *Allium porrum* L. when colonized by a *Glomus* sp., in spite of the fact that individual roots were shorter than those of non-AM plants. Similarly, Kaldorf & Müller (2000) also showed an increase in the percentage of fine roots in maize plants colonized by *Rhizophagus irregularis* C. Walker & Schuessler. The increased branching of the root system in AM plants has often been attributed to the influence of tissue P content on root geometry and partitioning of assimilates to lateral root formation (Niu *et al.* 2012). Although not significant, the reduction in the number and length of root hairs observed in maize is expected as the elongation and density of root hairs is dependent on P availability in the soils (Niu *et al.*, 2012). However, inoculation of *S. calospora* influenced all these characteristics except root hair length. This

effect on root hair traits could be attributed to the mycorrhizal mediated changes in plant hormones like auxins that are known to promote root hair formation (Kaldorf & Müller 2000).

There was a sequential increase in the shoot N, P and K concentrations in AM and non-AM maize shoots with increasing concentration of RP application. This is in accordance with studies where RP application has been shown to improve the concentration of P and other nutrients in plant tissues (Barea & Richardson 2015). The increased nutrient content of AM plants as observed in the present and other studies may be due to the extra radical hyphal contribution and probably also from the increased supply of nutrients at the root surface and mass flow resulting in an increased nutrient acquisition by roots (Frey & Schüepp 1993). Although experimental evidence for the possibility of active involvement of AM fungi in accessing P from RP independently of other microorganisms is scarce, such a phenomenon could not be ruled out.

Inoculation of *S. calospora* clearly led to a distinctive increase in the activities of enzymes involved in P dynamics. The acid and alkaline phosphatase activities of AM maize roots were higher when raised at higher concentrations of RP which is in agreement with the observations of Abdel-Fattah (2001) where acid and alkaline phosphatase activities of soybean roots were found to be significantly higher for AM than non-AM roots. Though Abdel-Fattah (2001) suggested that high phosphatase activities were related to the degree of active mycorrhizal colonization, only %RLH in the present study was correlated to alkaline phosphatase activity (r=0.925; P<0.01; n=6). This is similar to the observations of Kojima *et al.* (1998) who showed that only the intraradical mycelium of *G. margarita* colonizing *Allium* cepa responds metabolically to plant P concentration and external P availability. The observations of the present study are supported by another study where root colonization by *R. irregularis* increased the activity of alkaline phosphatase enzyme in *Trifolium alexandrinum* L. (Raisei & Ghollarata 2006). Nevertheless, the activity of alkaline phosphatase in maize roots was significantly higher only at higher concentration (3% to 5%) of RP application. Acid phosphatase activity was significantly higher in the AM than in non-AM roots. In contrast, either a lack of any response or a significant reduction in acid phos-

phatase activity to AM fungi inoculation or increasing levels of P amendment have been reported in different plant species (Baligar *et al.* 2005). However, the acid phosphatase activity was 31% and 21% higher in non-AM and AM maize roots than the respective alkaline phosphatase activities. This is in accordance with Khade *et al.* (2010) who also found a consistently higher acid root phosphatase activity than alkaline root phosphatase activity in four papaya varieties. These increased acid phosphatases activities than alkaline phosphatase activity could be due to the fact that the enzyme acid phosphatase is produced by both plants and microorganisms whereas alkaline phosphatases are exclusively produced by microorganisms (Khade *et al.* 2010). Further, the presence of a significant positive correlation between acid and alkaline phosphatase activities (r=0.714; P<0.009; n=12) suggests the involvement of common factors in the induction of these enzymes.

Even as the application of P to soils deficient in P is known to stimulate AM fungal formation (Sorenson *et al.* 2005), the %RLTC as well as %RLA/%RLAC, decreased with different concentrations of RP application. Takács *et al.* (2006) also showed that arbuscules were more sensitive to the supply of soil P than other AM structures. The negative effects of P application on AM fungi within roots often result from the reduction in the mycorrhizal dependence in response to high P in plant tissues (Takács *et al.* 2006). In contrast to the present study, the mycorrhizal formation was favored by high soil P in *Allium porrum* (Sorenson *et al.* 2005). An interesting observation made in the present study was that the proportion of %RLHC to %RLA/%RLAC increased linearly with increasing concentrations of RP. A similar observation was made by Cavagnaro *et al.* (2003) to increasing application of P in *Asphodelus fistulosus* L. It has been suggested that the long lifespan of the hyphal coils than arbuscules and their large surface area can be the structural adaptation of the fungus to increasing concentrations of P in the soil and plant tissues (Cavagnaro *et al.* 2003). In addition to the transfer of nutrients to the plants, hyphal coils could also store P and carbon which could benefit both the fungus and the plants during unfavourable periods (Cavagnaro *et al.* 2003).

Mycorrhizal dependency defined as the degree of change in plant growth associated with AM

colonization (Plenchette *et al.* 1983) was significantly affected by RP applications. The average mycorrhizal dependency value (54.81%) for the weakly mycorrhizal-dependent maize in the present study falls well within the range (-30% to 83%) reported for maize in other studies (Khalil *et al.* 1994; Kaeppler *et al.* 2000). The concentrations of P in the soil and the ability of the plant roots to acquire P from the soil play a very important role in determining the mycorrhizal dependency of a plant species. Application of different concentrations of RP increased the mycorrhizal dependency of maize plants nonlinearly as against the decreases reported in previous studies (Tawaraya 2003; Takács *et al.* 2006). This is in accordance with an observation where different concentrations of soil P had little influence on the mycorrhizal dependency of *Hancornia speciosa* Gomes plantlets (Cardoso-Filho *et al.* 2008). The low mycorrhizal dependency values obtained for maize plants in certain concentrations of RP application suggests that AM association may not be fully functional under these conditions as noted by Bethlenfalvay *et al.* (1983). Mycorrhizal dependency has been shown to be influenced by host and fungal species, the activity of soil microorganisms, and soil type in addition to the concentrations of nutrients in the soil (Cardoso-Filho *et al.* 2008; Oliveira-Júnior *et al.* 2017; Zangaro *et al.* 2007). Application of RP might have increased the soil microbial populations and the competition for P which might have elevated the mycorrhizal dependency of the maize plants in response to RP application (Nakhro & Dkhar 2010; Ndungu-Magiroi *et al.* 2015).

Conclusions

The AM fungal inoculation along with different concentrations of rock phosphate application had varied response in terms of root architecture, plant growth, nutrient content, enzyme (acid and alkaline phosphatase) activities and AM fungal colonization in maize plants. The performance of maize plants was better when RP application was combined with AM inoculation. This suggests that AM fungi could enhance the availability of P in RP to plants. Hence, AM fungal inoculation has proven to be a better alternative to the chemical fertilizers, especially P to increase and maintain the productivity of crops. Long-term fertility trials with efficient strains of mycorrhizal inoculums

and RP application are needed to test the performance of the maize under field conditions.

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