# ARBUSCULAR MYCORRHIZA FUNGI PROMOTES GROWTH OF TOMATO SEEDLINGS IN THE ABSENCE OF PHOSPHATE IN NUTRIENT SOLUTION

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## ABSTRACT

*Greenhouse experiments were conducted to investigate the effect of commercial arbuscular mycorrhiza fungi (AMF) inoculation on growth of tomato (Solanum lycopersicum) plants grown in cocopeatsin which phosphate ($KH_2PO4$) was either added or omitted.Non inoculated control plants as well as those inoculated with Glomusintraradices, Glomusmossea or a mixture of both AMF species were grown with and without phosphate. Seven weeks after planting, the plants were analysed for mycorrhiza colonization of the roots, growth enhancement, and phosphate uptake.The percentage root colonization of the inoculated plants ranged from 42.67-76.67% and the highestroot colonization were found in plants that did not receive phosphate. The highest shoot and root dry weights were obtained in plants supplied with phosphates and inoculated with either G. intraradices(5.2 g and 4.7 g respectively) or G. mossea (5.7 g and 4.3 g respectively). Co-inoculationof both AMF resulted in a lower shoot (3.5 g) and root dry weight (2.4 g) when phosphate was omitted, but higher shoots (5.2 g) and root dry weights (3.6 g) when phosphate was added. G. intraradices and G. mossea inoculated plants without phosphate addition produced significantly higher shoot dry weight (5.1 g and 5.0 g respectively) compared to non-inoculated treatments with added phosphate (4.1 g). Analysis of phosphorus concentration in the leaves showed that G. intraradices and G. mossea inoculated plants had the highest phosphorus concentrations (0.69 and 0.63 % respectively), while the non-mycorrhizal plants (0.54 %) and plants that were coinoculatedwith both AMFs (0.54 %)hadthe lowest phosphorus concentration, when phosphate was omitted from the nutrient solution.The results suggest that it may be possible to reduce phosphate application while increasing the growth of tomato plants by inoculating plants with AMFs*

**Keywords**: Glomus intraradices; Glomus mossea; Solanum lycopersicum

## INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are an important component of the plant root rhizosphere, since they form a symbiotic association with roots of over 90% of land plants (Smith and Read, 2008). They are beneficial in plant ecosystem through improvement of plant establishment, growth, and protection against environmental stresses such as salinity (He et al., 2007), drought (Augé, 2004) and metal toxicity (Reuscher et al., 2013). AMFs have been reported to improve the growth of plants under phosphate-limiting conditions (Elbon and Whalen, 2014). The symbiosis of plants with AMF often results in increased nutrient uptake (Bücking et al., 2012) suggesting that plant growth promoting activity by AMF under low phosphate is as a result of enhancement of phosphate uptake. The enhanced nutrient supply to plants in the presence of AMFs, results from the increase in the absorption surface area of the root by the fungal hyphae and the high affinity of hyphal surface area for phosphate (Johri et al., 2015).



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Tomato (*Solanum lycopersicum*) is the second most important vegetable crop globally after potato. Global production has been on a steady rise and was estimated at 170 million tonnes per year with a total production area of about 5.0 million ha (FAO, 2014). Phosphate availability has a direct effect on yield and quality of crops such as tomato (Di Candilo and Silvestri, 1995). In soils with low available phosphorus, it is a common practice to apply phosphate fertilizers to achieve increased tomato fruit yields. Phosphorus is an essential macronutrient for crop growth and yield. It is required for nucleic acid synthesis, membrane build-up and stability and energy metabolism (Lambers et al., 2015). Phosphorus limitation is a major constraint to crop production. Despite its abundance in soil, phosphate has poor bioavailability particularly in acidic soils, due to its extremely low rate of diffusion and substantial fixation by soil minerals (Shen et al., 2011). As a result, there is an overuse of phosphorus fertilizers to enhance crop production leading to underground water contamination or eutrophication (Guo, 2007) and accelerated depletion of phosphorus mineral reserves (Elser and Bennett, 2011). One approach towards achieving improved phosphate use efficiency is through the use of AM fungal symbioses for efficient phosphorus mining and uptake.

There is increasing interest in the cultivation of tomato with AMF. They have been shown to improve tomato seedling growth, fruit yield and nutrient uptake under low levels of fertilization (Ortas et al., 2013) and under environmental stress (Al-Karaki et al., 2004; Al-Karaki and Hammad, 2001). It is now common nursery practice to apply AM fungal inoculum to seedling's growing medium (Azcona-Auailar and Barea, 1997) or into planting hole at the time of transplanting, as modern cultivation techniques such as nursery substrate sterilization have resulted in reduced AM fungal diversity and frequency in agricultural soil (Nouri et al., 2014). Pre-inoculation in the nursery leads to superior growth of seedlings, reduces mortality and improves performance of matured plants in the field (Sorensen et al., 2008; Waterer and Coltman, 1988).

However, the possibility of using AMF in soilless media such as cocopeat is an open question. Studies on mycorrhiza in soilless media have reported varying results ranging from enhanced growth (Dasgan et al., 2008) to no effects (Mueller et al., 2009) on growth, fruits yield or nutrient uptake. These discrepancies are probably caused by the phosphate supply in easily absorbable forms in these media, which in turn affects the degree of colonization of the plant roots by AMF (Kowalska et al., 2015). As AMF increases nutrient uptake, it is possible that low level of phosphate in the nutrient solution would be beneficial to the AMF themselves, while still supplying enough for the plant.

The objectives of this study, therefore, were to assess root colonization, growth and leaf phosphorus concentration of tomato plants inoculated with AMF with and without the addition of phosphate to the nutrient solution.

## MATERIALS AND METHODS

### Experimental Design

Four AMF inoculation treatments (A. Non-inoculated control plants; B. *G. intraradices* inoculated plants; C. *G. mossea* inoculated plants; and D. Mixed *G. intraradices* and *G. mossea* inoculated plants) and two phosphate treatments (i. added phosphate; and ii. no added phosphate) The experiment was laid out in a factorial design.

### Growth Medium and Biological Materials

Tomato seeds (var ANNA F1) and AMF inocula (*G. intraradices* and *G. mossea*) were obtained from Amiran Ltd (Kenya) and Dudutech Division of Finlays Horticulture, respectively. Thirty grams of the crude inoculum was added to each planting hole prior to direct seeding of the pots with surface-sterilized tomato seeds. Cocopeat, which is low in nutrient (particularly phosphate) and free from AMFs, was used as planting medium.

### Growth Conditions

The greenhouse pot experiment (using 5 L cylindrical plastic pots) was carried out at Jomo Kenyatta University of Agriculture and Technology (JKUAT) from June to December, 2016. All plants were irrigated daily and fertilised weekly with Hoagland nutrient solution with or without phosphate addition (Hoagland and Arnon, 1950).

### Root colonization by AMF

Estimation of root colonization was done by detecting the presence or absence of AMF hyphae, arbuscules, vesicles and internal spores. After washing with tap water, root samples were cleared in 10% (w/v) KOH solution and stained in ink/vinegar solution (Vierheilig et al., 1998). Quantification of root colonization was done by counting the number of root segments colonized and expressed as a percentage of total root segments examined (Giovannetti and Mosse, 1980).

$$\%\ \text{colonization} = \frac{\text{number of colonized segments}}{\text{total number of segments examined}} \times 100$$

### Biomass

The root and shoot of seven weeks old plants were oven dried for 48 hrs at 70 ºC. Five plants per treatment were randomly sampled for dry weight analysis per replicate. The experiment was replicated three times.

### Phosphorus concentration

Total Phosphorus concentration in leaves was determined by colorimetric method using the ammonium-molybdate-vanadate method (Anderson and Ingram, 1989) and a spectrophotometer at 400 nm wavelength. The method involves drying, homogenization, digestion of the leaf samples (in sulphuric acid, salicylic acid, selenium powder and hydrogen peroxide). The samples were then filtered and pH of the filtrate was adjusted (using p-nitrophenol, 6N $NH_3$, 1N $HNO_3$) and yellow colour was developed by adding Ammonium molybdate/ammonium vanadate mixed reagent. The absorbance of the solution was measured using a colorimeter at 400 nm wavelength. The amount of phosphorus present was extrapolated from a calibration curve of standard phosphorus.

### Statistical Analysis

Data on percentage colonization were subjected to angular transformation, and this with the srest of the data, were subjected to analysis of variance (ANOVA) with inoculation treatment and phosphate as sources of variation and the means were separated using Tukey's test ($P < 0.05$) using SPSS statistical software. Means were compared at 5% level of significance.

## RESULTS

Both *G. intraradices* and *G. mossea* were able to colonize the root of the tomato plants and colonization was significantly enhanced by the elimination of phosphate from the nutrient solution. Additionally, there was a significant effect of inoculation with *G. intraradices* and



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*G. mossea* on growth of tomato plants with and without the addition of phosphate to the nutrient solution.

## Root Colonization

The effect of mycorrhiza inoculation was dependent on phosphate addition in the media for root colonization, as indicated by significant interaction (AMF*Phasphate) ($F(3, 32) = 39.779$, $P= 0.000$. Root colonization was significantly higher in all the three AMF treatments where phosphate was absent in the nutrient solution (Table 1).

**Table 1: Root colonization (represented as % of total root fragments) by *G. intraradices* and *G. mossea* with and without phosphate in the nutrient solution**

| Mycorrhiza treatments | Root Colonization (%) | |
|---|---|---|
| | +P | -P |
| Control | $0^{a}$ | $0^{a}$ |
| *G. intraradices* | $47.33^{b}$ | $63.33^{d}$ |
| *G. mossea* | $56.0^{c}$ | $68.67^{d}$ |
| Mixed | $42.67^{b}$ | $76.67^{e}$ |

+P indicates addition of 0.5mM $KH_2P0_4$ per pot, -P indicates no $KH_2PO_4$ addition. Values with different letters indicate significant difference at $P \leq 0.05$.

The highest percentage colonization (76.67%) was obtained in the mixed AMF inoculated plants without phosphate addition, and the lowest percentage colonization (42.67%) was obtained in the mixed AMF treated plants with phosphate addition to the nutrient solution. Although, colonization by *G. mossea* was significantly higher when phosphate was added to the nutrient solution, there was no significant difference between colonization by either AMF species when phosphate was absent in the nutrient solution.

## Effect of AMF inoculation on Growth

To determine the effect of AMF inoculation on growth, the plants were grown with AMF inoculation and all required mineral nutrients were supplied including phosphate at the first instance of fertilization, i.e. one week after plant germination. Subsequently, phosphate was omitted from the nutrient solution and plant biomass was determined after 5 weeks of cultivation.

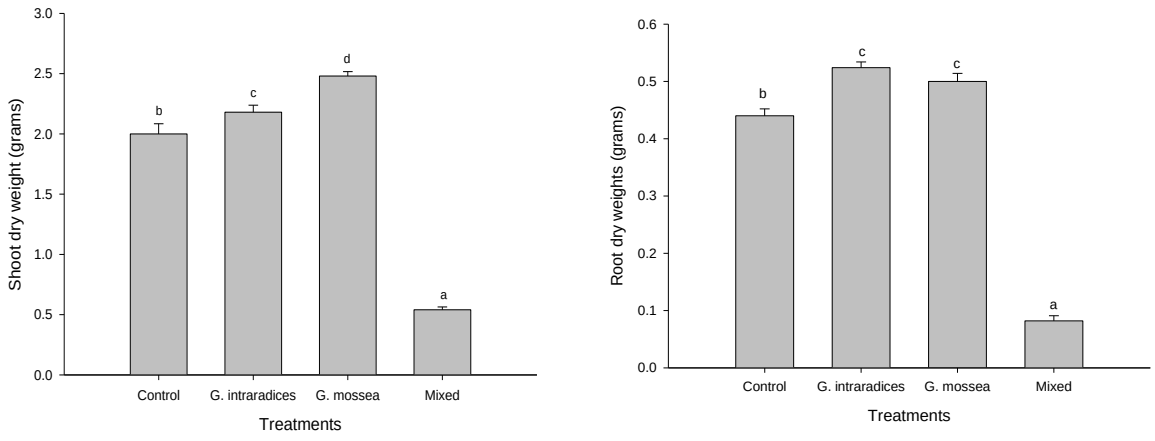


Figure 1: Effects of inoculation with AMF on tomato shoot and root biomass. (A) Effect of

inoculation with AMF on shoot dry weight. (B) Effect of inoculation with AMF on root dry weight. Error bars represent S.E. Different letters above bars represent significant differences at P≤0.05

Inoculation with either *G. intraradices* or *G. mossea* significantly increased shoot and root dry weights. However, co-inoculation with both AMF resulted in a significantly reduced shoot and root dry weight (Figure 1).

A second experiment was carried out to determine the effect of AMF inoculation and phosphate application on plant growth (Figure 3). A two-way ANOVA was conducted to examine the effect of mycorrhiza inoculation and phosphate addition on growth enhancement in terms of root and shoot dry weights. There was a statistically significant interaction between the effect of mycorrhiza inoculation and phosphate addition on shoot, (F (3, 112) = 34.555, P = 0.000) and root, (F (3, 112) = 10.433, P= 0.000) dry weights.

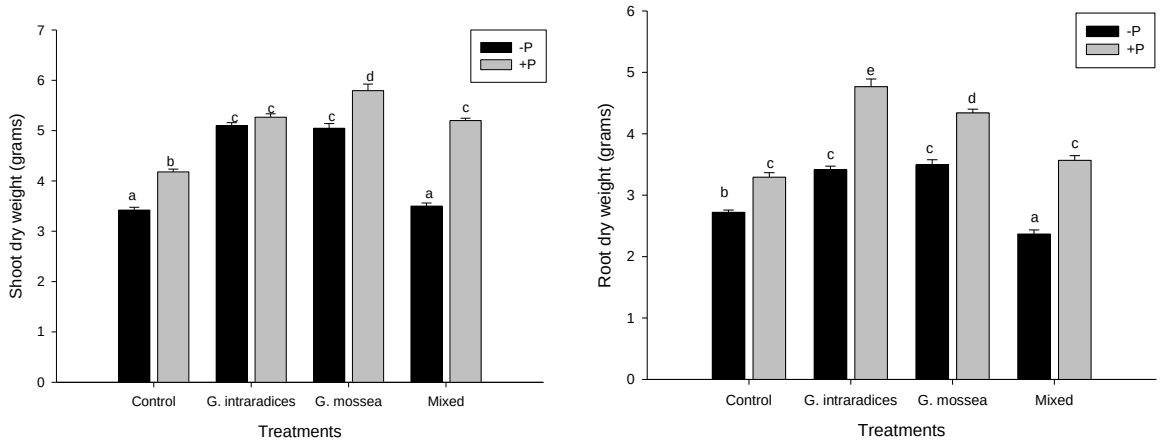


Figure 2: Effects of inoculation with AMF and Phosphate addition on tomato shoot and root biomass. (A) Effect of inoculation with AMF and phosphate addition on shoot dry weight. (B) Effect of inoculation with AMF and phosphate addition on root dry weight. Error bars represent S.E. Different letters above bars represent significant differences at P≤0.05

Regardless of phosphate addition, inoculation with either *G. intraradices* or *G. mossea* increased plant tissue dry weights. The highest dry weights were obtained when inoculation with either of the AMFs was combined with addition of phosphate. Interestingly, inoculation with AMF without phosphate resulted in higher shoot and root dry weights than non-inoculated plants with phosphate addition. Co-inoculation with both AMFs resulted in significantly lower shoot and root dry weights compared to the control and inoculation with either AMFs in the absence of phosphate. With the addition of phosphate however, co-inoculation resulted in higher dry weights (Figure 2).

## Phosphorus Concentration

Four representatives of each treatment combinations above were randomly selected for determination of phosphorus concentration in the leaves. The phosphorus concentration in the leaves showed that *G. intraradices* and *G. mossea* inoculated plants had the highest phosphorus concentrations (0.69 and 0.63 % respectively), while the non-mycorrhizal plants (0.54 %) and plants that were co-inoculated with both AMFs (0.54 %) had the lowest phosphorus concentration, when phosphate was omitted from the nutrient solution. (Table 2).



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**Table 2: Effect of Inoculation with AMF and Phosphate addition on Phosphorus concentration (%) in the leaves of Tomatoes**

| Mycorrhiza treatments | P concentration (%) | |
|---|---|---|
| | +P | -P |
| Control | $0.64^{b}$ | $0.54^{a}$ |
| *G. intraradices* | $0.84^{bc}$ | $0.69^{b}$ |
| *G. mossea* | $0.66^{b}$ | $0.63^{c}$ |
| Mixed | $0.87^{c}$ | $0.52^{a}$ |

+P indicates addition of 0.5mM $KH_2P0_4$ per pot, -P indicates no $KH_2PO_4$ addition. Values with different letters indicate significant difference at P≤0.05

## DISCUSSIONS

The results of this study showed that inoculation of tomato with *G. intraradices* or *G. mossea* enhanced root and shoot growth. Although, the most effective treatment was to add phosphate and AMF, application of AMF without phosphate gave similar or even higher plant dry weights than non-AMF inoculated plants with phosphate addition

AMF inoculation has been observed to increase growth in many plants species (Smith and Read 2008). Comparable differences in shoot and root dry weight between the control and inoculated plants showed a clear contribution of AMF to the growth of the plant. AMFs are well known to have the ability to improve growth of plants under phosphate-limiting conditions (Elbon and Whalen, 2014) due to their abilities to enhance phosphate uptake from the soil, thereby increasing phosphate nutrient supply to the plant (Bücking et al., 2012). In tomatoes, AMFs have been shown to improve tomato seedling growth and nutrient uptake under low levels of fertilization (Ortas et al., 2013).

The phosphate content in soil is a major factor affecting root colonization by AMF and it is widely recognised that phosphate fertilization often negatively affects root colonization of many host plants by AMF (Smith and Read 1997). In this experiment, the addition of phosphate significantly reduced root colonization compared to treatments without phosphate addition. Increased colonization, due to phosphate omission however, did not translate to better growth, as the treatments with the highest root and shoot weights were the ones inoculated with either of the AMFs and supplied with phosphate.

In this study, all four treatments under phosphate addition showed higher phosphate concentration in the leaves compared to plants without phosphate addition. When phosphate was omitted from the nutrient solution, *G. mossea* and *G. intraradices* inoculated plants had the highest phosphate concentration in the plant leaves. This is an indication of the role of AM fungi in enhancing uptake of phosphorus which in turn leads to increased plant growth. In most agricultural production systems, phosphorus has been identified as the most frequently occurring essential element deficiency limiting crop yields, hence, its recommended addition in substantial quantities to the growing medium. Symbiosis with AMF can increase phosphate uptake in phosphate-limited growth media, thereby improving plant growth (Smith and Read, 2008).

The beneficial effect when phosphate was omitted from the nutrient solution was only observed with inoculation of the plants with either *G. intraradices* or *G. mossea*. Combined

application however resulted in a significantly lower plant growth. In an ecological context, it is normal to observe multiple AMF colonizing a single host plant, as a single root system is capable of accommodation more than one AMF species (van Tuinen et al., 1998). The phenomenon of co-colonization is poorly understood, and it remains unclear whether such colonization results in competitive, synergistic, or antagonistic interaction (Alkan et al., 2006).

## CONCLUSION

Plant inoculation with either *G. intraradices* or *G. mossea* had the highest root and shoot dry weights with or without the addition of phosphate to the nutrient solution. Inoculation with either AMFs in the absence of phosphate produced higher shoot dry weight compared to non-inoculated treatment with added phosphate. Furthermore, plants inoculated with either AMFs showed the highest leaf phosphorus concentration when phosphate was omitted from the nutrient solution, suggesting that both AMFs enhanced nutrient uptake from the growth media. In conclusion, the results from this study suggest that it may be possible to reduce phosphate application while increasing the growth of tomato plants by inoculating plants with AMF. The observations confirmed the overall benefit of plant inoculation with AMF in improving plant growth, especially in the phosphate-limiting growth media. Thus, it is suggested that the inoculation of growing media with AMF exerts a beneficial effect on plant growth.

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