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Effects of earthworms, arbuscular mycorrhizae, and phosphate rock on setaria grass (*Setaria splendida*) and phosphorus availability in soil

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Abstract

Phosphate rock (PR) is essentially insoluble in water. Dissolution of PR under acidic soil conditions is a necessary pre-requisite for uptake of phosphorus (P) by plant. Improvement in P dissolution could be achieved through the interaction of micro- and macro-organisms in soil. A greenhouse pot experiment was conducted with epigeic earthworms (W) (*Pontoscolex corethrurus* M.), arbuscular mycorrhizae (AM) fungi (*Glomus mosseae* Tul), and gafsa phosphate rock (GPR) to evaluate their effects on dry matter (DM), root colonization, and nutrient accumulation of setaria grass (*Setaria splendida*) and availability of P in the soil. Earthworms significantly increased DM yield (23.3 g pot^{-1}) and P accumulation (16 mg P pot^{-1}) of setaria grass. The AM colonization on inoculated plants was high (81%) compared to earthworms. Accumulation of P, N, K, Ca^{2+} and Mg^{2+} in grass were significantly higher in soils contained earthworms, compared to other treatments. Presence of worm (W), AM, and GPR significantly increased phosphorus utilization efficiency (PUE) of setaria grass. The residual P was lower in the soils treated with worm or AM compared to non-treated (control) soil which might be due to higher P uptake by setaria grass. However, plant's available P increased under AM or W treatment. There was a significant interaction effect between AM, W and GPR on P accumulation of setaria grass indicating, efficiency of grass in taking up phosphorous. Thus, it could be concluded that presence of W, AM and GPR have efficiency to increase the amount of plant available P in soil.

Keywords: Phosphorus availability, *Setaria splendida,* Phosphate rock, Arbuscular Mycorrhizae, Earthworms.
Abbreviations: PR_phosphate Rock; W_earthworm; AM_arbuscular mycorrhizae; GPR_gafsa phosphate rock; DM_dry matter; PUE_phosphorus utilization efficiency; +AM, -AM_with and without arbuscular mycorrhizae; +GPR, -GPR_with and without gafsa phosphate rock; +W,-W_with and without earthworm.

Introduction

Tropical soils are mostly phosphorus (P) deficient due to inherent low levels of P and its fixation (Buresh et al., 1997). One unique characteristic of P is its low availability due to slow diffusion and high fixation in soils. Therefore, P can be a major limiting factor for plant growth (Shen et al., 2011). Application of phosphate fertilizer and inoculation with arbuscular mycorrhiza (AM) are two methods to alleviate this problem. The main strategy to solve soil P deficiency in the tropics is addition of fertilizers, either in the form of synthetic fertilizer or in the form of phosphate rock (PR) (Cardoso and Kuyper, 2006). For sustainable crop production, full supply of P is imperative (UNEP, 2011). The acid reaction of tropical soils is a pre-requisite for P dissolution from PR (Kanabo and Gilkes, 1987; Hanafi et al., 1992). For efficient P management in tropical soils, application of PR instead of water-soluble P should be environmentally friendly with minimal P losses (UNEP, 2011).

The capability of AM fungi to increase host-plant uptake of rather immobile nutrients, particularly P, is an acknowledged advantage of mycorrhiza (Cardoso and Kuyper, 2006). Mycorrhizal plants generally contain elevated nutrient contents and growth rate in contrast to the non-mycorrhizal counterparts (Janos et al., 2001). Plants absorb nutrients through roots for their growth. Modifications of root system by mycorrhiza create a positive effect on plant nutrients absorption (Bolan, 1991). The main benefit of AM infestation is the ability to increase the uptake of P, particularly when the infested plants grow in low-P soils (Douds and Nagahashi, 2000). The potential for AM to influence the host species depends on their affinities and effects (Gogoi and Singh, 2011). The AM fungal symbionts are endowed with an incredibly efficient trait to uptake and mining P from huge soil volumes and surmounting depletion in the rhizosphere. This occurs when direct (epidermal) root uptake is faster than replacement from the bulk soil (Smith et al., 2011).

The breakdown of organic materials, such as empty oil palm fruit bunches (EFB), a by-product from oil palm mill processing, is mainly by microorganisms and some macrofauna, such as earthworms (Sabrina et al., 2012). Earthworms are important components of the rhizosphere ecosystem, capable of enhancing plant growth by improving soil physical properties (Baker, 1999) and chemical conditions (Tuffen et al., 2002; Sabrina et al., 2009a; 2011). Earthworm activity has increased the uptake of P of AM fungal (AMF) hyphae and facilitated significant changes of the bio-geochemical status of P (availability, organic phosphorus pool, acid phosphatase activity) in certain hot spots, such as casts and burrow-linings (Le-Bayon and Binet, 2006).

Earthworms and AM are known for their beneficial role in increasing plant growth and P uptake but the major contributing factors are not well understood. The objectives of this study were to evaluate the effects of earth worm, arbuscular mycorrhizae, and gafsa phosphate rock dissolution and availability of P to produce dry matter yield of setaria grass.

Results and Discussion

Dry matter yield

Dry matter (DM) of grass was significantly raised by GPR addition at 4th, 5th, and cumulative harvests and by W at 2nd, 3rd, 4th, and cumulative harvests. Mycorrhizae significantly increased the DM yield at 2nd and cumulative harvests (Table 1). In cumulative and 2nd harvests, DM of grass inoculated with AM was significantly higher than that without AM. There was a significant interaction between AM and W for DM yields at 4th harvest (Fig. 2). The DM yield increased significantly with AM and GPR (5 g) compared to the times that one of the factors was absent. In a study using earthworms and AM, Tuffen et al. (2002) observed no significant ^{32}P transfer between garden leek (*Allium porrum* L.) plants. Hence, inoculation of AM did not affect plant growth, but earthworms caused an increase in shoot and root growth. Earthworms have shown to increase plant growth in 75% of the similar experiments (Blouin et al., 2006). An experiment was conducted on a woody legume (*Leucaena leucocephala* L.) by Ma et al. (2003) revealed that the addition of earthworms increased the production of plants by 10 to 30%.

Nutrient uptake

The contribution of AM to cumulative N accumulation was low (2%) for the +W+GPR treatment as compared to 8, 17, and 14% for the +W–GPR, –W+GPR and –W–GPR treatments, respectively. The contribution of AM to nutrient accumulation was lower for all treatments, compared to W (Fig. 3a).

The contribution of AM to P accumulation was low (2%) for the +W–GPR treatment compared to 4, 11 and 19% for +W+GPR, –W+GPR and –W–GPR treatments, respectively. The contribution of AM to P accumulation by the grass was lower than the contribution of W, except for the –W–GPR treatments (19%), compared to +AM–GPR treatment (13%) (Fig. 3b).

The contribution of AM and W to K accumulation was not significantly different. The highest contribution of AM was for the –W+GPR treatment (27%), and the contribution of W for the –AM–GPR treatment (27 %) (Fig. 3c).

The highest contribution of AM to Ca^{2+} accumulation was observed in +W+GPR treatment (32%), whereas the lowest contribution of AM to Ca^{2+} accumulation was recorded in –W-GPR treatment. A significant increased contribution (34 and 33%) of W to Ca^{2+} accumulation was found in treatments of +AM–GPR and +AM+GPR, respectively. However, the decrease in contribution of W to Ca accumulation was noted under treatment –AM+GPR (Fig. 3d).

The contribution of W to cumulative Mg^{2+} accumulation was higher than that with AM. The greater contribution of AM (22%) to Mg^{2+} accumulation was recorded for –W–GPR treatment. The +W–GPR treatment had a smaller contribution (4%) of AM to Mg^{2+} accumulation. Regarding W contribution to Mg^{2+} accumulation, results revealed that maximum contribution of W (36%) to Mg^{2+} accumulation was found for treatment –AM–GPR, whereas the minimum contribution (20%) of W to Mg^{2+} accumulation was recorded for –AM+GPR (Fig. 3e).

The reduction in the contribution of AM to P accumulation was expected and related to: (i) the carbon (C) supply to AM and (ii) the presence of indigenous AM on un-inoculated grass. The AM is a well-known obligate symbiotic organism. It obtains C from the photosynthesis of host plants and can promote host plant growth by increasing P uptake from soil. The AM demand and obtain C from plants relative to P transfer (Pearson et al., 1994) and can promote host plant growth (Solaiman and Abbott, 2004). However, restrictions in plant growth associated with AM fungi also have been reported (Graham and Abbott, 2000). A suppression can be linked to C and P exchange (Koide and Elliott, 1989). Either growth promotion or restriction depends on a combination of plant-fungal interactions (Johnson et al., 1997) and soil conditions.

The presence of earthworms was expected to increase the low molecular weight compounds comprising soluble sugars, carboxylic acids, and amino acids in the soil, which may affect AM dependency to the host plant in obtaining C. Earthworm casts also contain a considerable amount of P in available forms and might be one of the factors that cause a decline in the contribution of AM to P accumulation. In the gut of earthworms, various enzymes of microbial and earthworm origin are secreted, as well as intestinal mucus (a readily assimilable C source), $CaCO_3$ (if calciferous glands are present), and bacteriostatic and microbicidal substances (Brown, 1995).

The interactive effect between AM and GPR was significant for N and K accumulation in setaria grass. The accumulation of these nutrients also was significantly influenced by inoculation of *Acacia mangium* (black wattle) with AM (Satter et al., 2006). Mycorrhizal mycelia that grow outward into the surrounding soils are a very efficient N scavenger owing to: (i) their capacity to explore a larger soil volume than roots alone, (ii) their ability to provide access to nitrogenous reserves contained in organic horizons, and (iii) their greater capacity for uptake of nitrogenous compounds (Hodge et al., 2000). Although generally considered essential for P uptake, AM can also enhance ammonium and nitrate uptake (Johansen et al., 1993; Mengel and Kirkby, 2001). Such enhancement in plant nutrition by AM is of particular importance in nutrient deficient soils (Mengel and Kirkby, 2001).

The role of earthworms in increasing nutrients uptake by plants is through changing the form of nutrients into forms easily absorbed by plants. In this study, *Pontoscolex corethrurus* is a soil- feeding earthworm. It selectively ingests fine particles in the soil and produces fresh surface casts, which are more dispersible than the control and non-ingested soils. Phosphatase is; therefore, more easily extractable in casts than in the non-ingested soils. Mineralization of organic P may occur during gut transit and

Table 1. Effect of arbuscular mycorrhizae (AM), earthworm (W), and gafsa phosphate rock (GPR) on dry matter yield of *setaria* grass.

Harvest	Factors						LSD (0.05)
	AM		W		GPR		
	With	Without	With	Without	With	Without	
	g pot $^{-1}$						
Cumulative	22.4 a	19.4 b	23.3 a	18.7 b	22.5 a	19.6 b	0.5
5th	4.7 a	4.5 a	4.7 a	4.5 a	5.1 a	4.1 b	1.0
4th	4.0 a	3.4 a	4.5 a	3.0 b	4.4 a	3.1 b	0.7
3rd	3.9 a	3.2 a	4.1 a	3.0 b	3.8 a	3.3 a	1.0
2nd	5.3 a	4.5 b	5.3 a	4.5 b	5.1 a	4.7 a	0.9
1st	4.5 a	5.2 a	5.0 a	4.7 a	5.1 a	4.6 a	1.6

Abbreviations: AM= arbuscular mycorrhizae; W= earthworm; GPR= gafsa phosphate rock. In each row, means followed by common letter are not significantly different at ($P < 0.05$) based on Duncan's multiple range test

possibly continues for a few hours after egestion of the casts (Chapuis-Lardy et al., 1998). Earthworm mucus, casts, and even their channel wall consist of microorganisms in significant numbers, compared to soil (Syers and Springett, 1984). Furthermore, Ma et al. (2003) reported that earthworms slightly improved available N and P in soil. They amplified around 10% additional P accumulation to above-ground plant tissues. Previous studies (Derouard et al., 1997; Blouin et al., 2006; Eriksen-Hamel and Whalen, 2007) on influence of earthworms in various crops also reported similar findings. The utilization of P by AM was not significantly different from the utilization by non- AM plants. Without the application of W, the effect of AM on P utilization efficiency was 0.9 g DM mg^{-1} P, whereas without W and AM, PUE was 0.8 g DM mg^{-1} P (Fig. 4).

Root colonization by AM

Root colonization for the +AM+GPR treatment was significantly higher (85%) than that of the –AM+GPR treatment (36%). Root colonization of the +AM–GPR treatment was 78%. Meanwhile, root colonization by –AM–GPR (51%) was higher than –AM+GPR (Fig. 5a). Plant roots treated with +W–GPR was colonized by AM (80%), and was higher compared to roots of plants treated with +W+GPR. Root colonization of plants treated with +W+GPR (68%) was higher than those treated with –W–GPR (48%) (Fig. 5b). A mycorrhizal colonization was also decreased in fertile soils (Thomson et al., 1986). A decline in arbuscule frequency was observed in field grown desert wheatgrass (*Agropyron desertorum* Fisch. ex Link) roots under a nutrient-rich microsite (Duke et al., 1994). In this study, mycorrhizal colonization was suppressed by GPR. On the other hand, studies have shown that PR significantly increased AM colonization of maize roots (Asmah, 1995) and Leucaena (Manjunath et al., 1989). Arbuscular M colonization can be suppressed at higher levels of P (Stribley et al., 1980; Mosse, 1981) possibly by increasing P concentration in roots (Menge et al., 1978).

Total nutrients in soil

The presence of W increased the concentration of P and K in soil (12 and 237 mg kg^{-1}, respectively), compared to soils without W (7 and 141 mg kg^{-1}, respectively). An opposite result was observed for Mg^{2+}, being higher (37 mg kg^{-1}) in treatment without W and lower (23 mg kg^{-1}) in the soil with

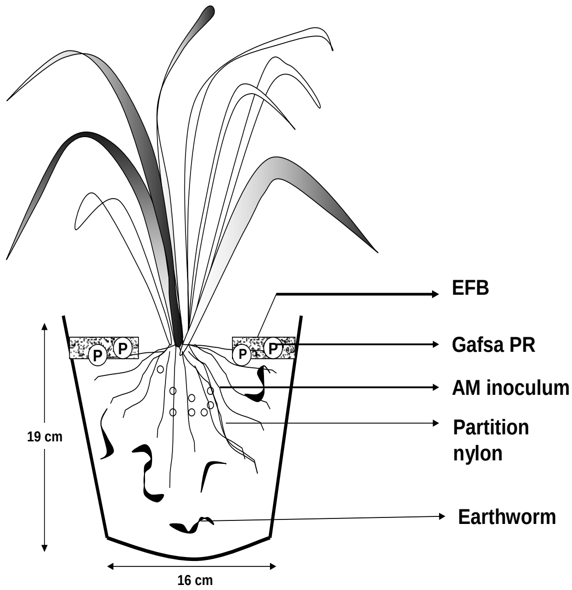

Fig 1. Pot containing arbuscular mycorrhizae (*Glomus mosseae* Tul), epigeic earthworms (*Pontoscolex corethrurus* M.), and oil palm empty fruit bunch.

W. With regard to total N, none of the treatments recorded significantly different values (Table 2). The interaction (Fig. 6) between +W+GPR significantly increased total Ca^{2+} in soil (74 mg kg^{-1}), compared to other interaction combinations. However, total Ca^{2+} for the +W–GPR treatment was similar to the –W+GPR treatment (42 mg kg^{-1}). In the absence of either W or GPR it caused the same effect on total Ca^{2+} in the soil (Fig. 6b). The interaction between –AM + GPR increased total Ca^{2+} in soil (68 mg kg^{-1}), which was significantly higher than other interaction combinations (Fig. 6c). In the absence of AM and GPR, total Ca^{2+} (32 mg kg^{-1}) was lower than for other treatments. In this investigation, earthworms significantly increased plant nutrient concentration, especially P. It is well known that earthworms are beneficial in improving soil physico-chemical properties by supplying nutrients through their burrowing actions, which ultimately creates pores in the soil, nutrient cycling, and in turn, plant growth (Lal, 1991; Scheu, 2003). It has been reported that the earthworm castings contained three-fold more available potassium than the surrounding soil (Basker et al., 1993). The contents of ammonium in the earthworm castings increased during gut passage of the ingested soil

Table 2. Effect of arbuscular mycorrhizae (AM), earthworm (W), and gafsa phosphate rock (GPR) on total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) in soil at the end of experiment.

Nutrients (mg kg^{-1})	Factors						LSD (0.05)
	AM		W		GPR		
	With	Without	With	Without	With	Without	
N	1300 a	1400 a	1300 a	1400 a	1400 a	1400 a	0.7
P	10 a	9 a	12 a	7 b	11 a	9 a	4.7
K	187 a	192 a	238 a	141 b	201 a	178 a	26
Ca	45 a	50 a	58 a	37 b	58 a	37 b	9
Mg	29 a	31 a	23 a	37 a	30 a	30 a	6

Abbreviations: AM= arbuscular mycorrhizae; W= earthworm; GPR= gafsa phosphate rock. In each row, means followed by common letter are not significantly different at ($P < 0.05$) based on Duncan's multiple range test.

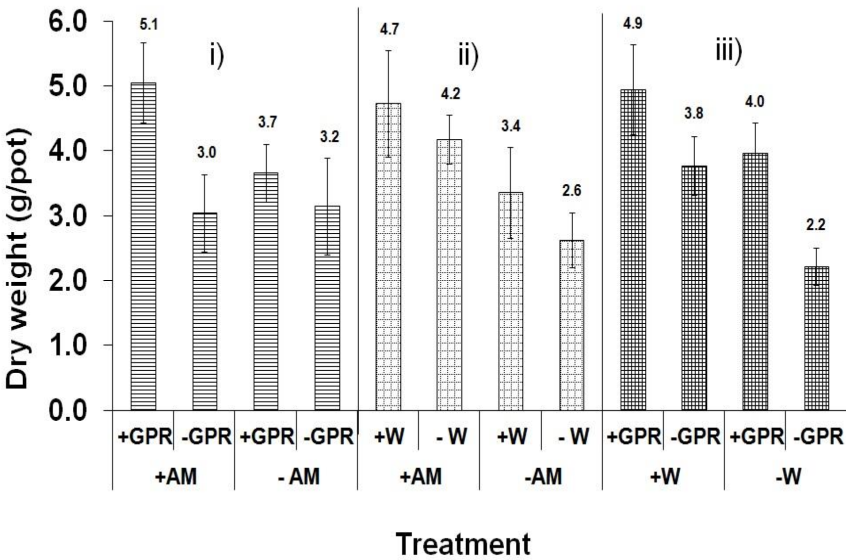

Fig 2. Interactive effect between i) AM and GPR, ii) AM and W, and iii) W and GPR on DM yield (g pot^{-1}) of *Setaria splendida* S. at the 4th harvest. Abbreviations: +AM, -AM, with and without arbuscular mycorrhizae; +GPR, -GPR, with and without gafsa phosphate rock; +W,-W, with and without earthworm. Vertical line on each bar indicates standard error value.

(Elliot et al., 1990). The soil passed through the earthworms gut hase more available nutrients to be taken up by plants (Abbot and Parker, 1981). Earthworms also had shown significant effects on the amount of extractable nitrate, which are increased with time (Bohlen and Edwards, 1995). Similar to our results, regarding P increase due to earthworms, Kuczak et al. (2006) also reported similar findings that earthworm casts have higher levels of organic hydroxide P than surrounding soils. Inorganic P was increased by fertilization, and organic P was increased by earthworm gut passage and/or selection of ingested materials, which increased available P.

Plant-available and residual-P in soil

The +W+AM+GPR treatment resulted in 28 mg P kg^{-1} and was significantly higher compared to other treatments for plant available P. Meanwhile, the lowest available P resulted from the soil treated with –W –AM –GPR. The interaction effect showed that available P for the –AM+W+GPR treatment was lower than for the –AM+W–GPR treatment (Fig. 7).

Residual P for the -W-AM treatment was higher (244 mg P kg^{-1} soil) than residual P for the +W+AM treatment (41 mg P kg^{-1} soil) (Table 3). A similar trend for available P in the soil was also noted as in the case of residual P. The results showed that P for the treatments without W was not readily available for uptake by the grass in the earlier harvest period. Therefore, the amount of P, remaining in the soil without W, was high at the end of experiment. The presence of worms increased the availability level of P in its casts (Mackay et al., 1983), and the casts had higher enzyme and microbial activities according to other researchers (Mulongoy and Bedoret, 1989). The results are in agreement with our previous study (Sabrina et al., 2009b), which showed that earthworms cast played a vital role in the availability of P. Available P content in earthworms cast was five-fold higher than that of the soil.

Materials and Methods

Media preparation, earthworms, and setaria grass

Empty oil palm fruit bunches (EFB) were collected from Universiti Putra Malaysia's (UPM) experimental farm. The samples were ground with a micro-hammer mill to pass through a 2- mm sieve. Fresh cow dung was obtained from the Animal Science Department, Faculty of Agriculture UPM's experimental farm. The cow-dung was air-dried (moisture content 20% w/w), undigested grass removed and sieved (2-mm sieve) to remove earthworms or cocoons. An acid Ultisol, Bungor soil (Typic Kandiudult) with a pH of 4.6, CEC of 5.9 cmol (+) kg^{-1}, fine sandy clay texture, fine structure, 0.97% C and 0.07% N was used as plant growth

Table 3. Effect of arbuscular mycorrhizae (AM) and earthworm (W) on changes in 0.5M NaOH extractable (ΔP), P accumulation (P_s), and available P (P_b) in the soil

Treatment	ΔP (mg P kg^{-1})	ΔP_s (mg pot^{-1})	ΔP_b (mg P kg^{-1})
+ W+ AM	41c	19a	2b
+ W – AM	43c	16a	4ab
– W + AM	63b	7b	3b
–W – AM	244a	3c	5a
LSD (0.05)	18	3.1	1.4

Abbreviations: AM= arbuscular mycorrhizae; W= earthworm; GPR= gafsa phosphate rock. In each column, means followed by common letter are not significantly different at (*P< 0.05*) based on Duncan's multiple range test

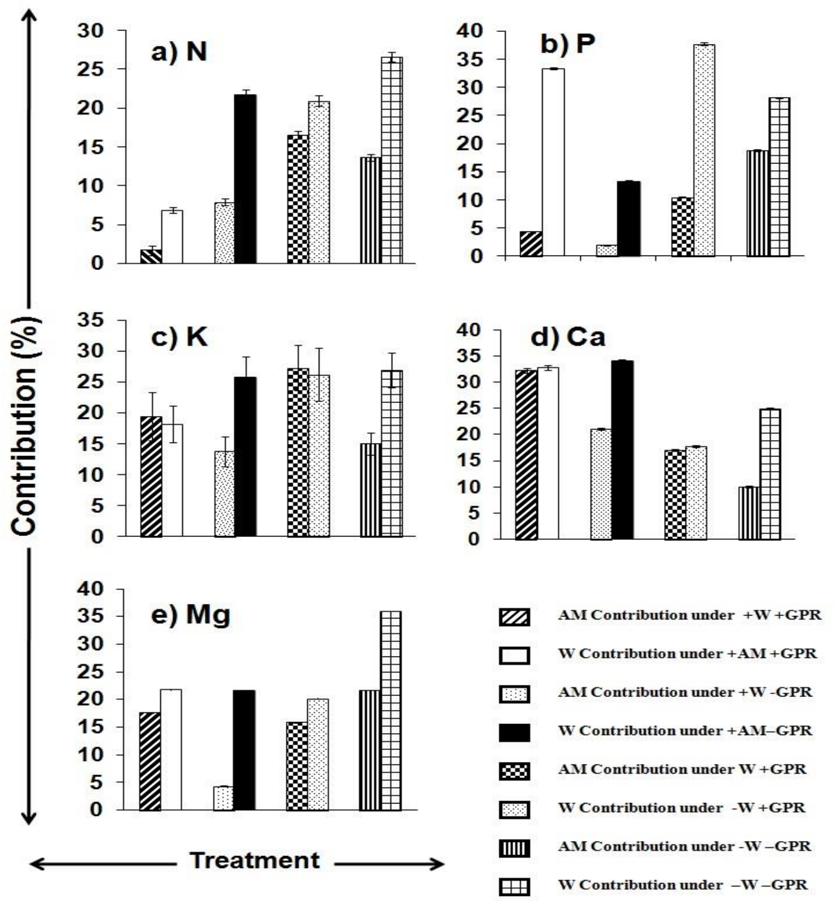

Fig 3. The contribution (%) of AM (arbuscular mycorrhizae) and W (earthworm) to a) Nitrogen (N), b) phosphorus (P), c) potassium (K), d) calcium (Ca) and e) magnesium (Mg) concentration in *Setaria splendida*. Abbreviations: +W, -W, with and without earthworm; +GPR, -GPR, with and without gafsa phosphate rock; +AM, -AM, with and without arbuscular mycorrhizae. Vertical line on each bar indicates standard error value.

media. The soil was air-dried and sieved to pass through a 2 mm sieve size before use. The soil was not sterilized and it contained 4.0 ± 0.5 g spores of *Glomus* spp $soil^{-1}$.

The AM inoculum containing *Glomus mosseae* Gerd & Trappe UK 118 was obtained from the International Culture Collection of VAM (INVAM) fungi, which consisted of spores, external hyphae, and infested root fragments. The inoculum was propagated on sorghum (*Sorghum bicolor* L.) for 4 months in pot cultures in a glasshouse using the method described by Feldmann and Idczak (1991). The inoculum was used later for the experiments. Enumeration of the infective VAM propagules of fungi was carried out by the most probable number (MPN) method as described by Sieverding (1991), and resulted in 88 infective propagules 100 g^{-1} inoculum. The spore number was 560 spores 100 g^{-1} inoculum using the wet sieving method (Gerdemann and Nicolson, 1963) and 20 g pot^{-1} of AM inoculum was used.

An epigeic, *Pontoscolex corethrurus* M. earthworm, the only species occurring in oil palm plantations based on our previous survey (Sabrina et al., 2009b) was used in this study. The earthworms were obtained from the stock culture maintained in the laboratory.

The test crop, setaria grass (*Setaria splendida*) cuttings (15 cm height) was obtained from the grass museum of the Animal Science Department, Faculty of Agriculture, Universiti Putra Malaysia.

Experimental design

A completely randomized design using a 2^3 factorial structure was used for this experiment with each treatment replicated

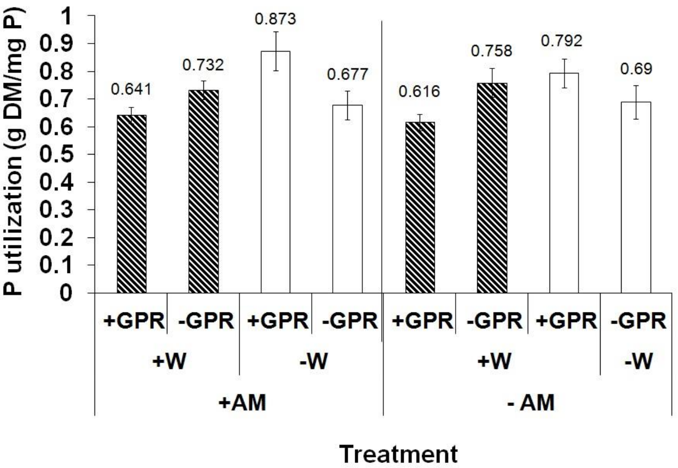

Fig 4. Phosphorus utilization efficiency (g DM mg^{-1} P) by AM, W, and GPR. Abbreviations: +AM, -AM, with and without arbuscular mycorrhizae; +W, -W, with and without earthworm; +GPR, -GPR, with and without gafsa phosphate rock. Vertical line on each bar indicates standard error value.

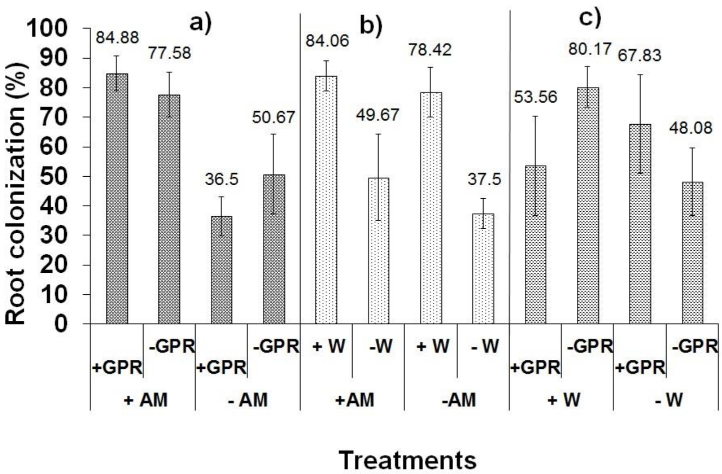

Fig 5. Interactive effect between a) AM and GPR, b) AM and W, and c) W and GPR on AM root colonization (%) of *Setaria splendida*. Abbreviations: +AM, -AM, with and without arbuscular mycorrhizae; +GPR, -GPR, with and without gafsa phosphate rock; +W, -W, with and without earthworm. Vertical line on each bar indicates standard error value.

three times. The factors were W, AM, and GPR. In each treatment, one of the three factors was either included (+) or excluded (─). The experiment was conducted in a greenhouse at the Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia located at 2º59′N, 101º42′E and about 45 m above sea level.

A modified version of growth chamber pots for earthworms designed by Tuffen et al. (2002) was used in this experiment with the following dimensions: rim= 12.7 cm, base= 9.2 cm and height= 7.6 cm. Each pot was separated into two parts with a 0.5 mm pore size nylon cloth, which allowed fungal hyphae and roots to pass through (Fig. 1). The outer part of each pot was filled with soil to about 1/3rd of the pot volume, then the inside part of the pot placed on top. Both pots were then filled with soil up to about 5 cm from the top. Five earthworms (0.4 g $worm^{-1}$) were added to the soil, a number which is equivalent to the mean population of earthworm found in an earlier survey (Sabrina et al., 2009b). The GPR (150 kg P_2O_5 ha^{-1}) was thoroughly mixed with EFB (36.5 ton ha^{-1}) and placed on the soil surface of outer section. Cow dung (0.5 mg/earthworm/ day) was added to the media at the beginning of the experiment as food supplement for the earthworms.

The AM inoculum was introduced into the inner pot under the setaria grass cuttings. Basal fertilizers, urea (46% N) and KCl (50% K) were applied at 60-day intervals, in solution form, at rates equal to 100 kg N ha^{-1} and 50 kg K ha^{-1} in the field (Hanafi and Syers, 1994). The grass was watered twice a day in the morning (08:00 h) and in the afternoon (18:00 h), for 5 minutes using a sprinkler irrigation system installed in the greenhouse to get the surface of the pot wet.

Harvesting and root sampling

The grass shoot was cut above previous cuttings height using secateurs every month. Dry matter (DM) yield and P accumulation by setaria were determined at 30 day intervals for 5 months. The mycorrhizal root colonization was evaluated using procedures of Koske and Gemma (1989). Randomly selected root samples were excised from each plant, cut into 1.5 cm pieces, cleaned, and immersed in 20 mL of 10% KOH. The root pieces were boiled at 100°C in water for 30 minutes. The cleansed root samples were then washed thoroughly by gently running tap water and acidified with 1N HCl to enhance staining. Acidified roots were stained in an acidic glycerol solution (500 mL glycerol, 450 mL 1% HCl) containing 0.05% trypan blue for 15 minutes. The stained roots were mounted onto glass slides and observed for infections with microscope. A total of 20 root pieces were used per sample, and five random readings were taken per root piece. The presence of fungal bodies (mycelium, spores, arbuscules, and vesicles) in the root tissues indicated positive infection. The percent root infection was calculated as follows:

$$\text{Root infection (\%)} = \frac{N(+ve)*100}{N}$$

Where, N (+ ve) = Number of AM positive segments and N = Total number of segments observed.

Phosphorus utilization efficiency (PUE) was calculated using the following equation:

$$\text{PUE (g DM mg}^{-1}\text{ P)} = \frac{DM}{PU}$$

Where, DM = Dry matter yield pot^{-1} (g); PU = P accumulation pot^{-1} (mg).

The contribution of the experimental factors (W, AM, and PR) to nutrients accumulation (%) = $\frac{(A-B)*100}{A}$

A = P accumulation by treated plants (mg); B = P accumulation by untreated plants (mg).

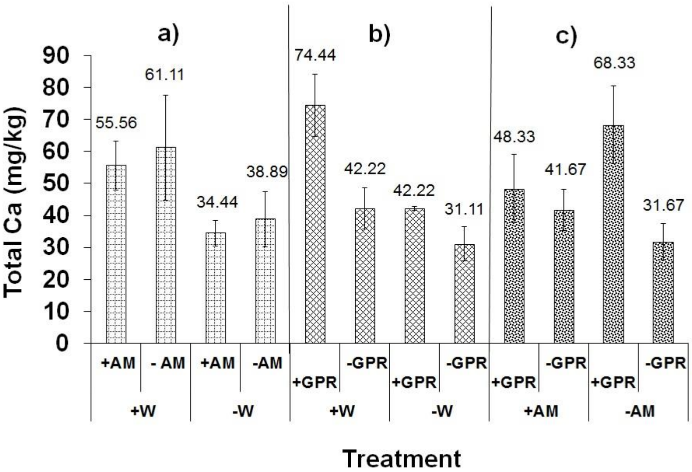

Fig 6. Interactive effect between a) W and AM, b) W and GPR, and c) AM and GPR on total Ca (mg kg^{-1}) in soil. Abbreviations: +W, -W, with and without earthworm; +AM, -AM, with and without arbuscular mycorrhizae; +GPR, -GPR, with and without gafsa phosphate rock. Vertical line on each bar indicates standard error value**.**

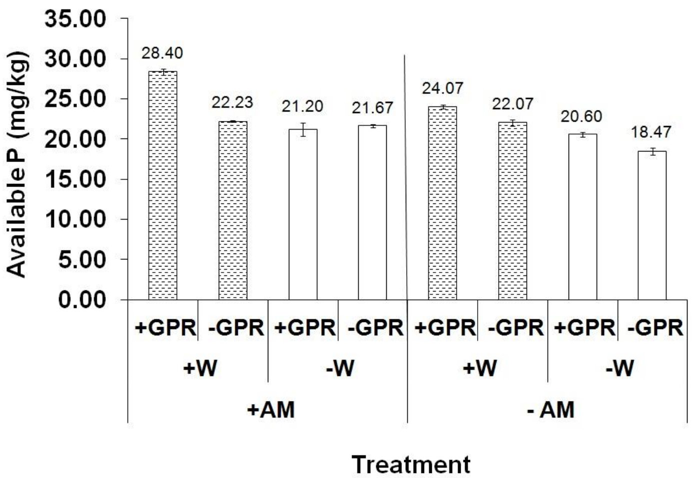

Fig 7. Interactive effect between AM, W, and GPR on plant-available P (mg kg^{-1}). +AM, -AM, with and without arbuscular mycorrhizae; +W, -W, with and without earthworm; +GPR, -GPR, with and without gafsa phosphate rock. Vertical line on each bar indicates standard error value.

Soil and plant sampling

Soil was sampled at the end of the experiment and analyzed for total N, P, K, Ca^{+2}, and Mg^{+2}, plant-available P, pH, and the amount of residual P dissolved from PR. Micro-Kjedhal method was used for determining total nitrogen (Bremner, 1960). The total P, K, Ca^{+2}, and Mg^{+2} were measured after digesting the sample using HNO_3 and HCl (1:3 ratio) (Mehlich, 1953); plant availability of P in soil was determined using Bray 2 method (Bray and Kurtz, 1945). Due to the PR application, plant available P in soil for the glasshouse experiment was determined using 0.5M $NaHCO_3$ at pH 8.5 using a ratio of 1:20 (soil-reagent) based on the method of Olsen (Olsen et al., 1954). The exchangeable K, Ca^{+2}, Mg^{+2} and the CEC were measured after leaching the sample with neutral 1 M ammonium acetate (Blakemore et al., 1987). The concentrations of N, P and K in solution were determined using an auto-analyzer (Technicon Industrial Systems, 1977), and Ca^{+2} and Mg^{+2} were measured using an atomic absorption spectrometer (Perkin-Elmer, 5100 pc, Perkin Elmer) in the presence of 1000 mg $SrCl_2$ L^{-1} as an ionization suppressant (Isaac and Kerber, 1971). Soil pH was measured with a glass electrode pH meter (PHM210, Metrolab) in a 1:2.5 soil-water suspension. The DM yield and N, P, K, Ca and Mg uptake by setaria were determined at 30-days intervals for five months.

Plant analysis was done by digesting samples with 5 mL concentrated H_2SO_4 and further oxidized with H_2O_2 (30% reagent grade) using the method of Thomas et al. (1967). The digest was mixed thoroughly with distilled water and made up to 250 mL. The solution was allowed to stand overnight to permit precipitation of silica. Then, the solution was analyzed for N, P, K, Ca^{+2}, and Mg^{+2}. Nitrogen, P, and K were measured using an auto-analyzer (Technicon Industrial Systems, 1977), while Ca^{+2} and Mg^{+2} elements were measured using an atomic absorption spectrometer (Perkin-Elmer, 5100 pc, Perkin Elmer) (Isaac and Kerber, 1971) after adding of 1000 µg L^{-1} $LiCl_2$ to eliminate any interference during measurement of Ca^{+2}.

Statistical analyses

A two-way analysis of variance was used for all the data. For the percentage root colonization by AM, the data were square root transformed before analyses (Gomez and Gomez, 1981). Treatments means separation was done using Duncan's Multiple Range Test at 5% level. The data were analysed using the statistical analysis system (SAS) version 8e (SAS, 1999).

Conclusions

In this study no interaction between W, AM, and GPR to increase DM yield at any harvest time was observed; however, these factors had individual effect. For nutrients accumulation, large variations in AM were noted, which indicated no response of AM to setaria grass for these traits. However, contribution of W to N, P, and Mg^{+2} accumulations was significantly higher than other factors. The presence of W and AM and their interactions significantly enhanced root colonization whereas; application of GPR reduced root colonization. Overall, findings showed that the presence of W, AM and GPR increased the amount of plant available P.

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