



Full Length Research Paper

Mycorrhizal Development and Phosphorus Concentration in selected Kenyan Sorghum Cultivars

Rasowo Brenda*¹, Ndiema Philip¹, Muthuri Jacinta², Onkware Augustino²

¹University of Eldoret Department of Biological Sciences, P.O.BOX 1125-30100, Eldoret

²Moi University, Department of Biological Science, P.O. Box 3900-30100, Eldoret

*Corresponding authors e-mail: bre.rasowo@gmail.com; Tel: +254720578901

ABSTRACT

Mycorrhizal associations are beneficial to plants and crop production because they enhance nutrient uptake especially phosphorus and micronutrients, such as zinc and copper. They also stimulate growth substances and may reduce stresses, diseases or pest attack. This study was carried out on sorghum genotypes that exhibited significant difference in growth and production on P-deficient soils. The objective was to find out if the genotypes developed different levels of mycorrhizal associations and tissue phosphorus. Results showed that plants in unfertilized soils developed extensive mycorrhizae than those in fertilized soils, no significant differences was observed in the extent of mycorrhizal development among cultivars grown in P-fertilized soils. The cultivars in unfertilized soils responded differently to mycorrhizal colonization, those with high level of mycorrhizal development increased growth compared to those that had low levels of mycorrhizal development in the unfertilized soils. The cultivars also responded differently to phosphorus concentration in their tissues. Phosphorus fertilizer apparently increased number of leaves and sorghum plant height. There was no relationship between phosphorus concentration in soil and level of mycorrhizal development though fertilizer significantly suppressed the level of mycorrhizal development in the plants.

Keywords: Mycorrhiza, cultivars, genotypes, symbiosis

INTRODUCTION

Mycorrhiza is the symbiotic association between fungi and roots of vascular plants (Alexopoulos and Mims, 1979). Mycorrhizal fungi occur in nearly all-natural and agricultural soils and commonly colonize roots of many plant species (Smith and Read, 1997). Unlike root pathogens these fungi can increase plant growth and production by enhancing uptake of mineral nutrients from the soil particularly diffusion limited ions such as phosphate. However, a great deal of variability in host plant responsiveness to mycorrhizal colonization has been reported. For example significant variation in the effect of these fungi on promoting growth has been reported between different plant species (Mosse, 1978), and between genotypes of the same species including citrus (Menge et al 1978; Graham and syvertsen, 1985; Graham et al., 1997), corn (Toth et al., 1990), palm

(Clement Harbte, 1995), pea (Estaun et al., 1987) and wheat (Azcon and Ocampo., 1981; Hetrick et al., 1996).

Several studies have predicted that plants with inherent mechanisms for acquiring phosphorus tend to benefit less from mycorrhizal colonization (Koides., 1991, Koides., 1993). For example, mycorrhizal benefit has been shown to be inversely related to absolute root allocation, root density, root fitness, and root: shoot ratio, or root hairiness (Baylis., 1970; Menge et al., 1978). Characteristics such as root length or root density are often excellent predictors of phosphorus uptake as well. Root absorption capacity [absorption rate per unit mass of root tissue at some standard solution] may tend to influence nutrient acquisition, but because phosphate ions are very mobile in soil, it may be of only secondary importance in determining phosphorus uptake; diffusion

of phosphate ions to the root surface is usually much more limiting (Marschner., 1994). Plant roots may also increase phosphorus acquisition by acidifying the rhizosphere in order to increase the solubility of phosphorus sources or producing phosphates, which solubilize organically bound phosphate. Some investigators have also suggested that, in addition to phosphorus-acquiring mechanisms, plants having inherent mechanisms that reduce their requirements for phosphorus, or the rate in which phosphorus is required will form less mycorrhizal colonization (McGonigle and Fitter, 1988; Koides., 1991, Koides., 1993). Requirements for phosphorus are a function of plant growth capacity and phosphorus utilization efficiency [the amount of biomass produced per unit of phosphorus (Chapin and Van Cleve, 1989)]. Plants with lower growth rates or higher phosphorus use efficiency will require lower rates of phosphorus uptake. Also plants capable of efficiently allocating and reallocating phosphorus for growth and reproduction would require less phosphorus from the soil and will also depend less on mycorrhizal fungi for phosphorus acquisition during various stages of development.

MATERIALS AND METHODS

Experimental Setup

Sorghum seeds

Sorghum cultivars were selected to determine their responsiveness to mycorrhizal colonization. The genotypes included: MCSR-O2, MCSR-P3, MCSR-K4E, MCSR-N4 and MCSR-L6. Cultivar MCSR-O2 is a commercial variety while the others are local varieties. These cultivars differ in their susceptibility to phosphorus acquisition; others are efficient in low phosphorus soils while others are inefficient.

Experimental arrangement

The study was a completely randomized design with six replications. The treatments included (control, 6.4 mg/g P) and application of phosphorus fertilizer (to 66.8mg/g P). The experiment was carried out in plastic pots each containing approximately 4 kg of soil collected from top soils apparently having fungi. The soils were acidic; control soils with a PH 5.6, which decreased to 4.5 with P fertilizer application. The pots were irrigated with tap water regularly to field capacity during growth. The plants were raised in a greenhouse for 90 days.

Assessment of Vegetative Attributes

The plants were thinned to three plants per pot at 30

days after sowing and the number of leaves per plant and height were assessed every 10 days.

Root Staining

Root samples were transferred to modified syringes after washing. The roots were cleared in 2.5% KOH by autoclaving for 15 minutes and rinsed in tap water. They were bleached in 30% H₂O₂/NH₃ solution for 1-2 hours depending on the color and thickness of the root. Thereafter rinsed again and acidified in 1%HCl for a period of 1 hour. They were stained in 0.05% trypan blue in acidic glycerol by autoclaving for 3 minutes. The stained roots were washed out into a Petri dish and mounted on slides for observation.

Assessment of Mycorrhizal Development

The levels of mycorrhizal infection were assessed using the stained roots under a compound microscope at a magnification of x100. A minimum of 5x1 cm stained root fragments were chosen at random from the sample and arranged in parallel on glass slides and gently squashed after placing a cover slip. Then using linear eyepiece reticule with an x10 eyepiece and x10 objective, vesicular mycorrhizae structure including intracellular hyphae, arbuscules and vesicles was assessed at 1mm intervals along the root fragment.

Analysis of Phosphorus

Plants tissues were harvested and the stems, roots and leaves separated dried in the autoclave (70°C) then tissue samples were ground and sub samples digested for 1 hour in a H₂SO₄/H₂O₂ mixture using a digestion block (Technicon, Tarrytown) set at 400°C. The digest was analyzed for total phosphorus using the molybdo-phosphate method (Watanabe and Olsen, 1965).

Statistical Analysis

Each measured variable in the experiment such as number of leaves, plant height and mycorrhizal development was subjected to analysis of variance with cultivars and phosphorus as the fixed factors. Mean separations were done using Duncan's multiple-range test at P < 0.05 (Zar, 1984).

RESULTS

Mycorrhizal Development

All the sorghum plants developed vesicular mycorrhizae although the plants that had no fertilizer application developed more extensive mycorrhizae than those

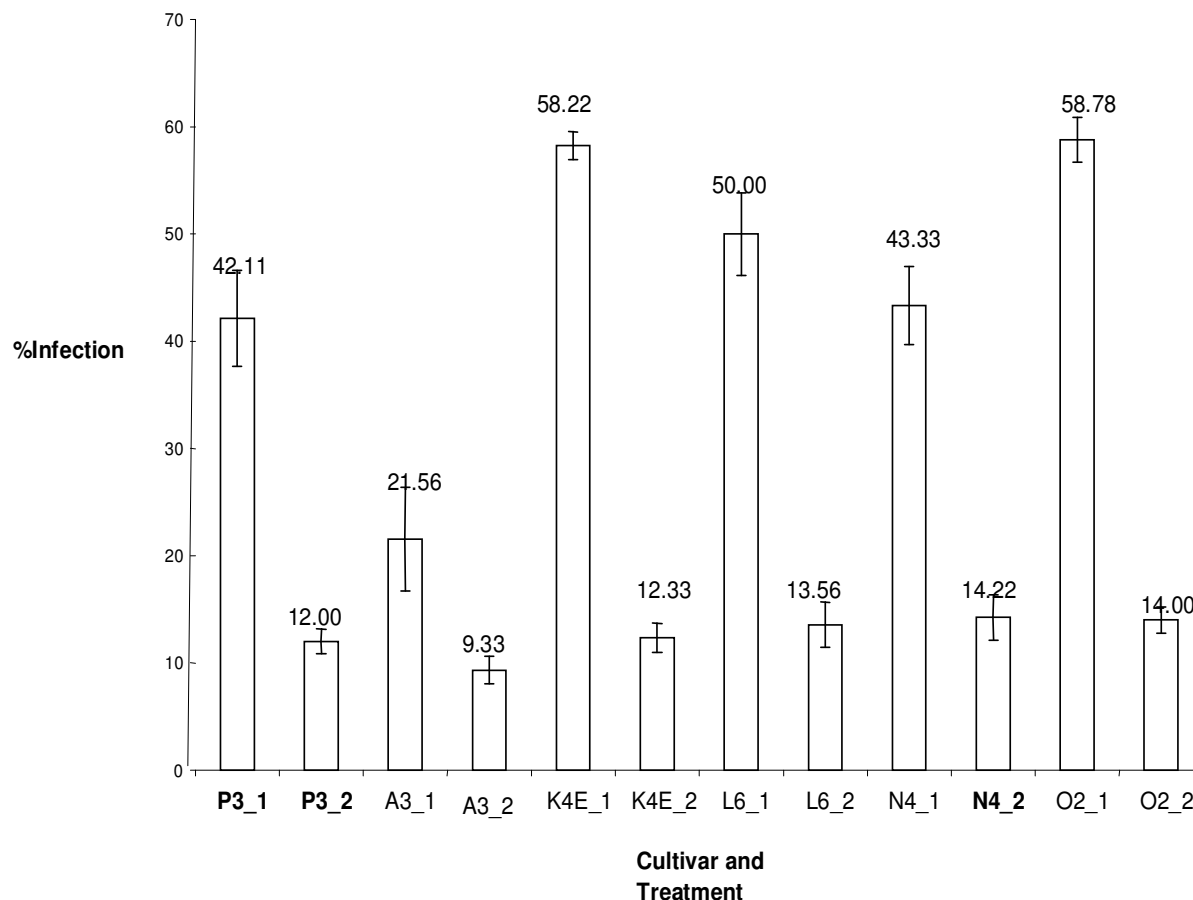


Figure 1. Percentage of Mycorrhizal infection in sorghum cultivars that were grown with or without phosphorus fertilizer application.

KEY: 1=without phosphorus, 2=with phosphorus applied.

growing in fertilized soils. There were significant differences in the extent of mycorrhizal development among the sorghum cultivars, especially in the absence of P-fertilizer application and MCSR-A3 had the lowest mycorrhizal development whereas MCSR-O2 had the highest level of development.

There were no significant differences in the extent of mycorrhizal development among the cultivars when they were grown in P-fertilized soils.

The subsets indicated which cultivars were statistically different or at same level with each other. From the different means of mycorrhizal colonization we saw that cultivar MCSR-A3 had the least mean level of infection in both P-efficient and P-inefficient plants therefore there is no statistical significance in the effect of mycorrhiza in the cultivar because it performs poorly with or without fertilizer.

Plant Height and Number of Leaves

There were significant responses of the sorghum cultivars to phosphorus application. Application of phosphorus increased number of leaves and plant height in all the cultivars. Plants growing on P-fertilized soils were taller compared to plants in unfertilized soils. Cultivar MCSR-L6 were the tallest in soils supplied with P-fertilizer whereas MCSR-O2 were the tallest in P-deficient soils therefore being the best performing cultivar in unfertilized soils. There was no significant difference in number of plant leaves in fertilized soils.

Phosphorus application caused an increase in the number of leaves

Phosphorus application caused an increase in the number of leaves are shown in Figures 2-13 below.

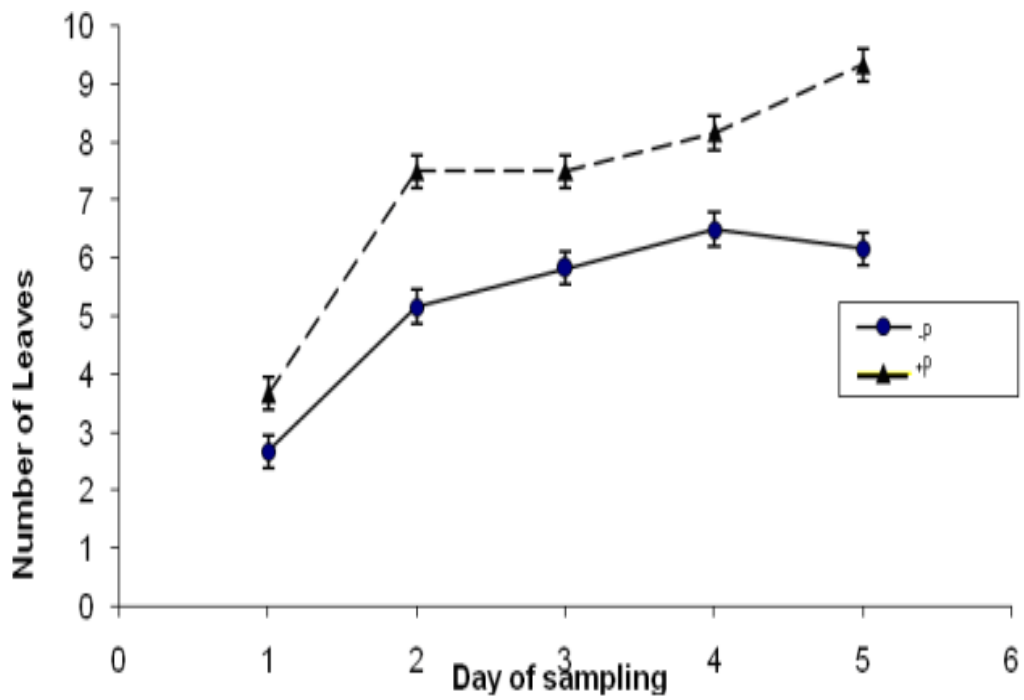


Figure 2. Effect of phosphorus fertilizer application on the number of leaves per plant of sorghum cultivar MCSR-P3

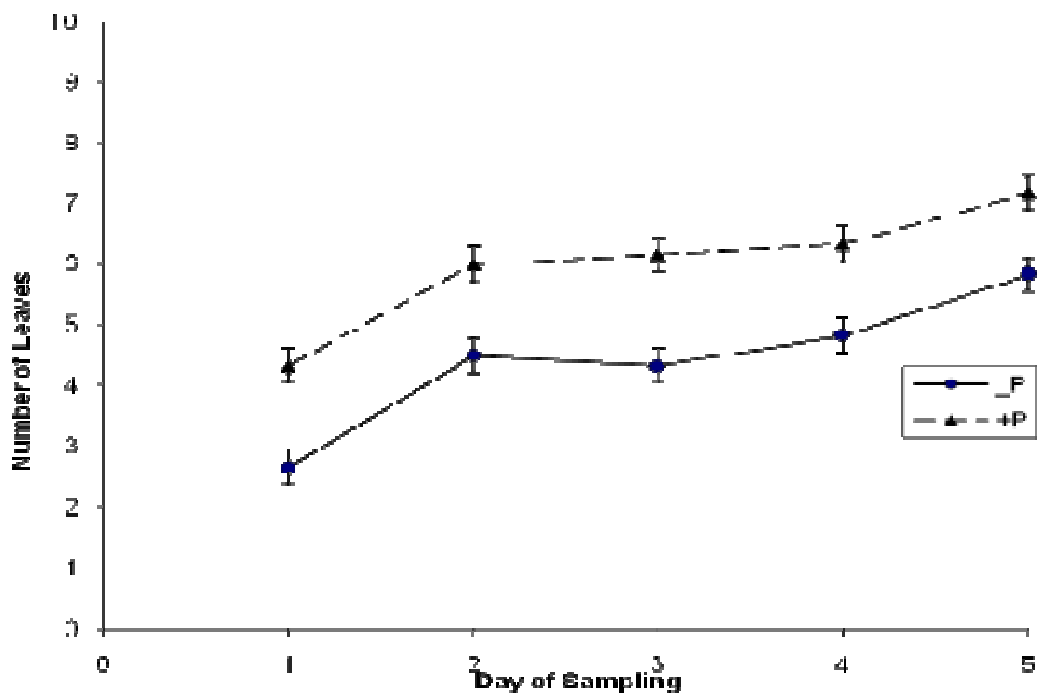


Figure 3. Effect of phosphorus fertilizer application on the number of leaves per plant of sorghum cultivar MCSR-A3

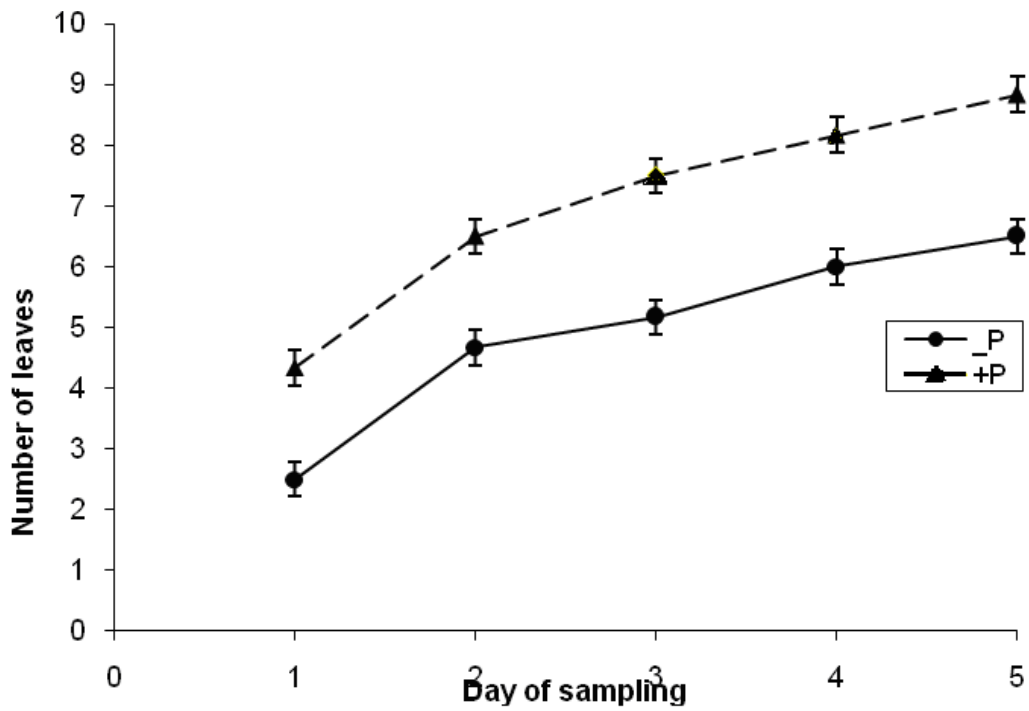


Figure 4. Effect of phosphorus fertilizer application and on the number of leaves per plant of sorghum cultivar MCSR-K4E

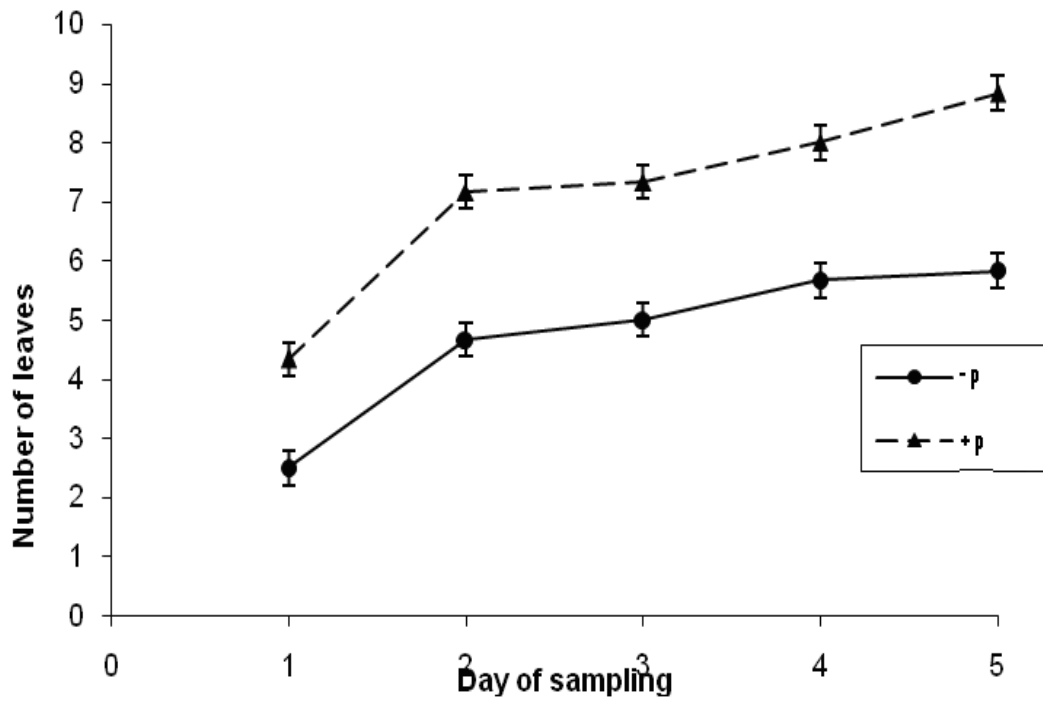


Figure 5. Effect of fertilizer application on the number of leaves per plant of sorghum cultivar MCSR-L6

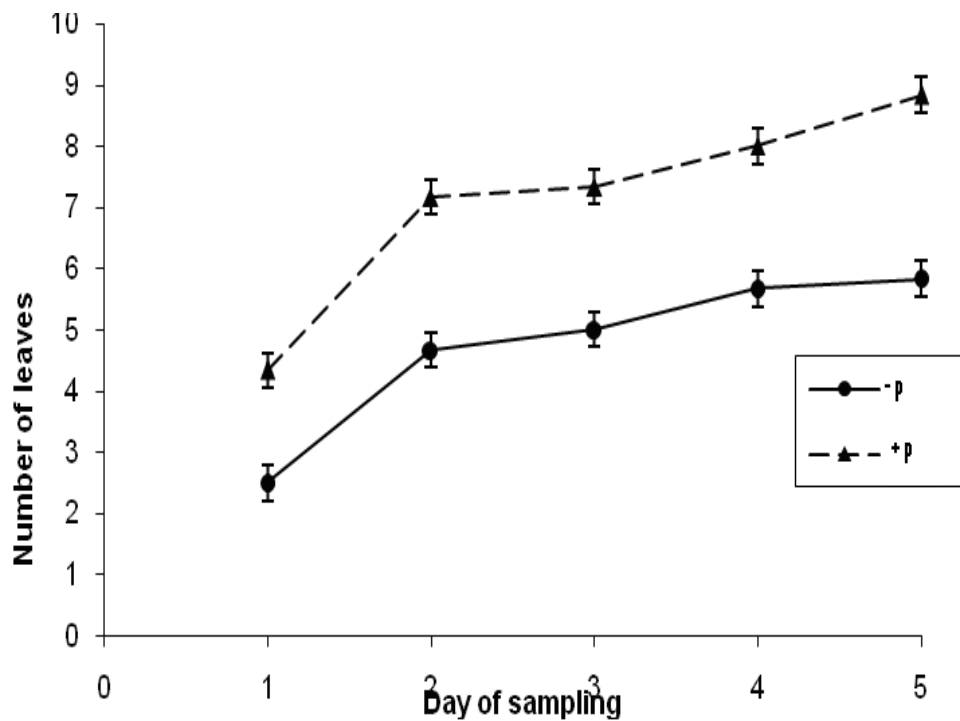


Figure 6. Effect of fertilizer application on the number of leaves per plant of sorghum cultivar MCSR-N4

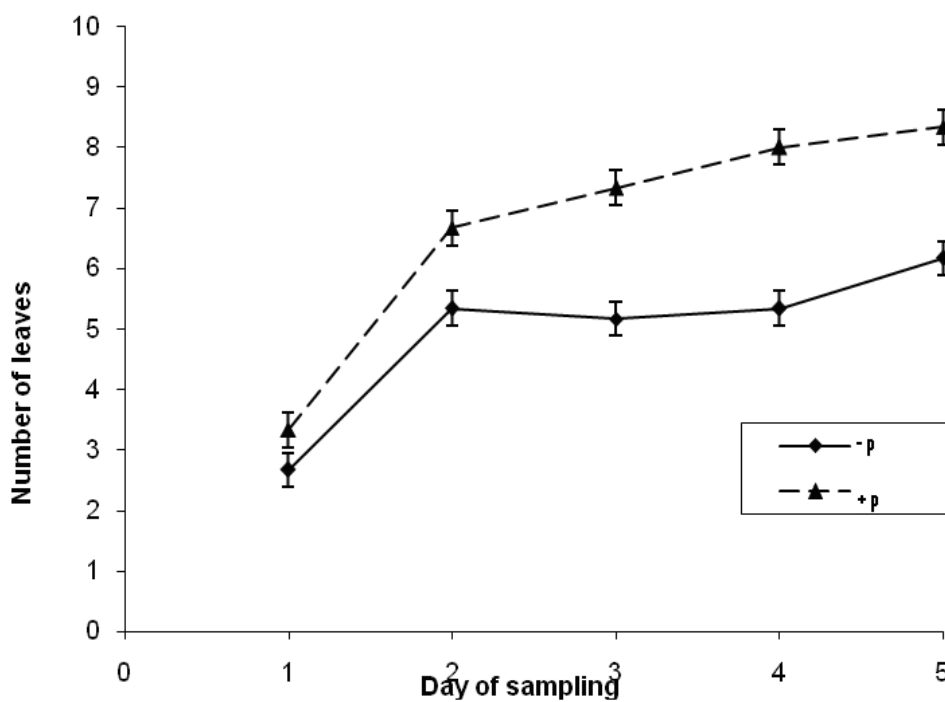


Figure 7. Effect of fertilizer application on the number of leaves per plant of sorghum cultivar MCSR-02
Application of phosphorus caused a significant increase in the height of sorghum cultivar

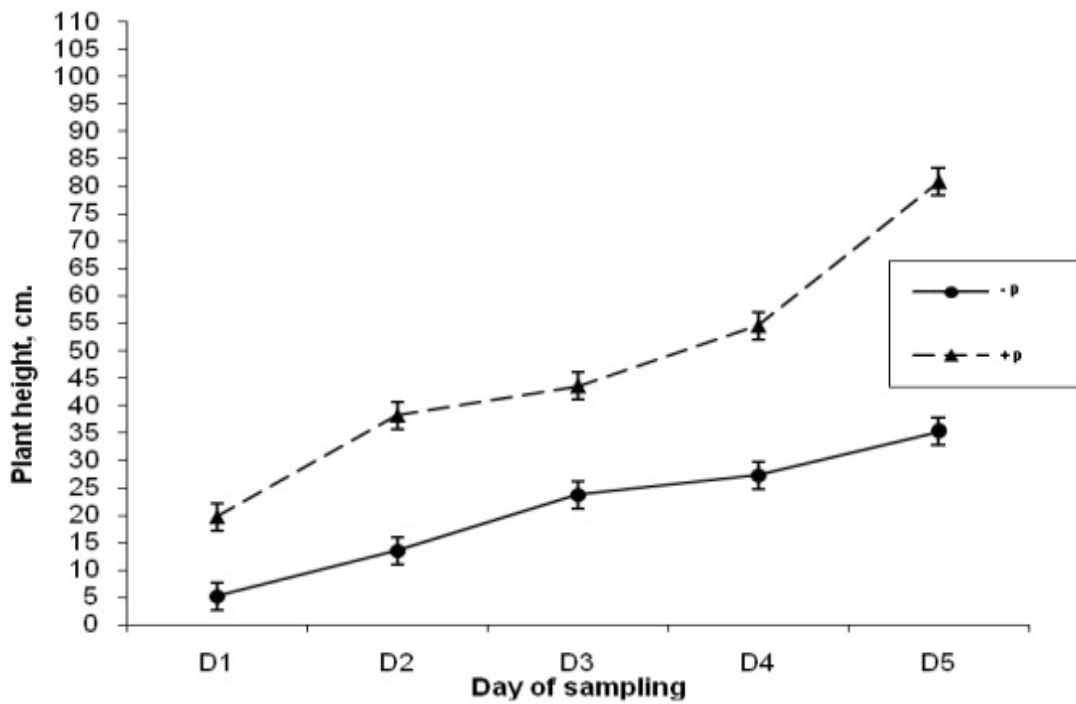


Figure 8. Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR-P3

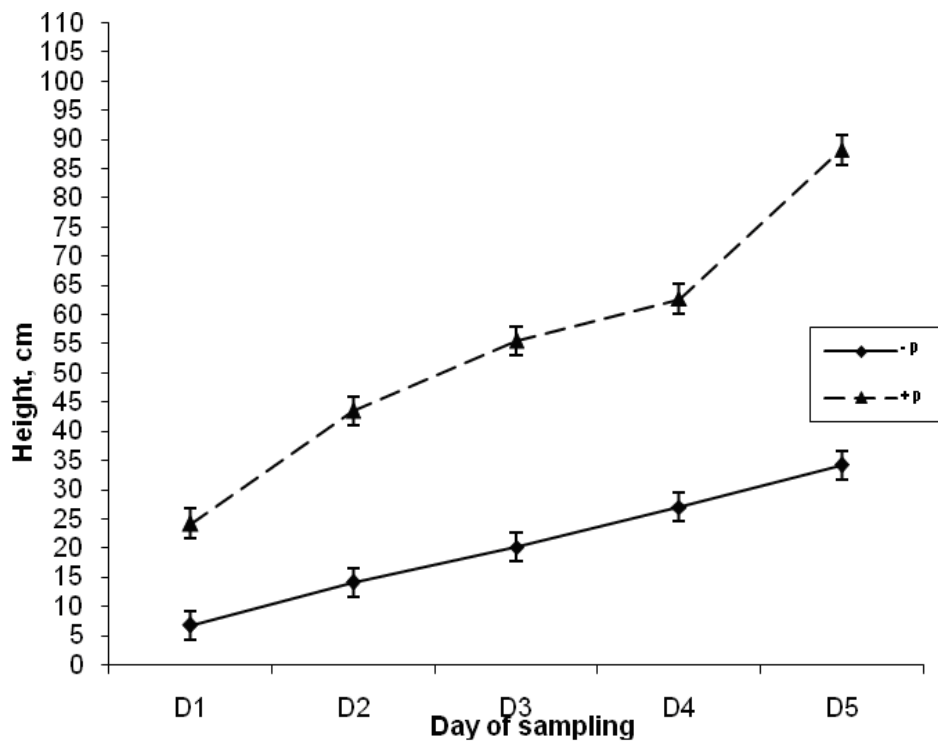


Figure 9. Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR-K4E

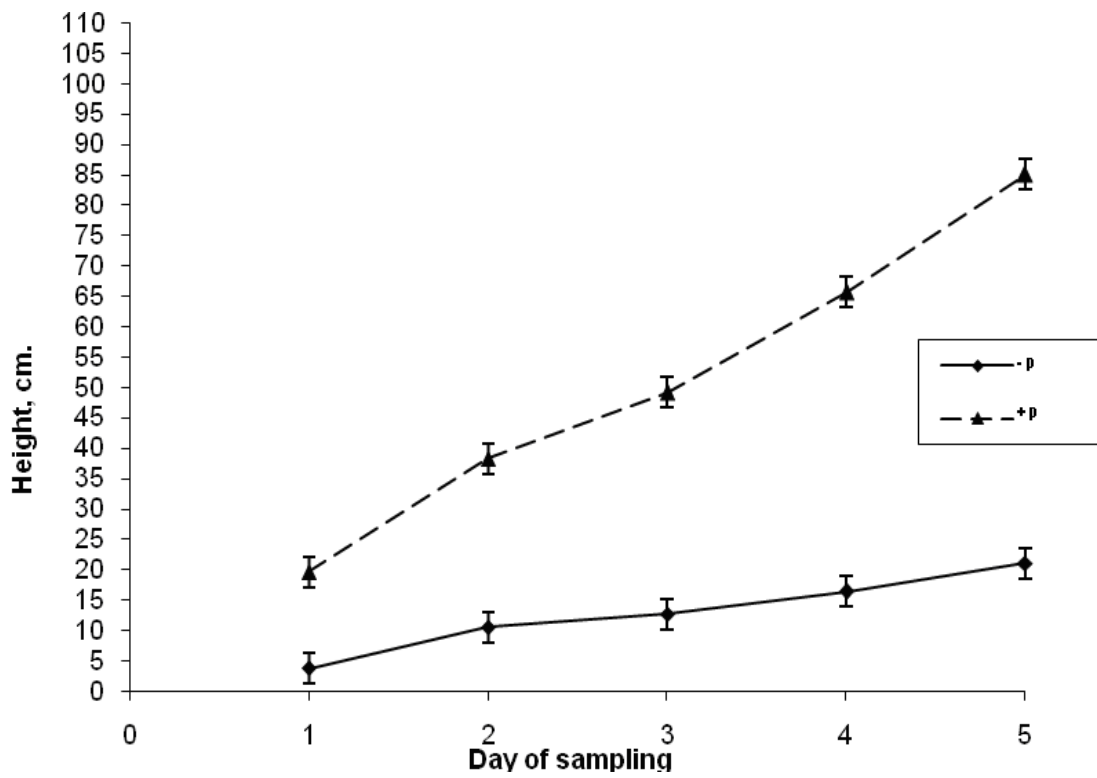


Figure 9. Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR-A3

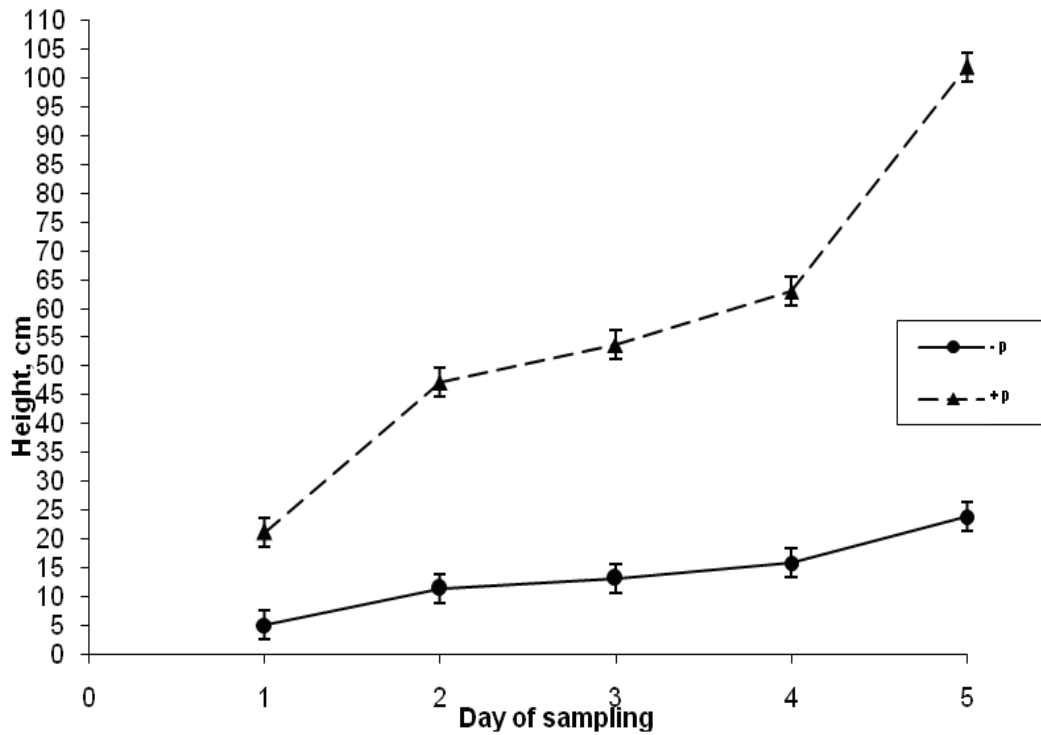


Figure 11. Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR-L6

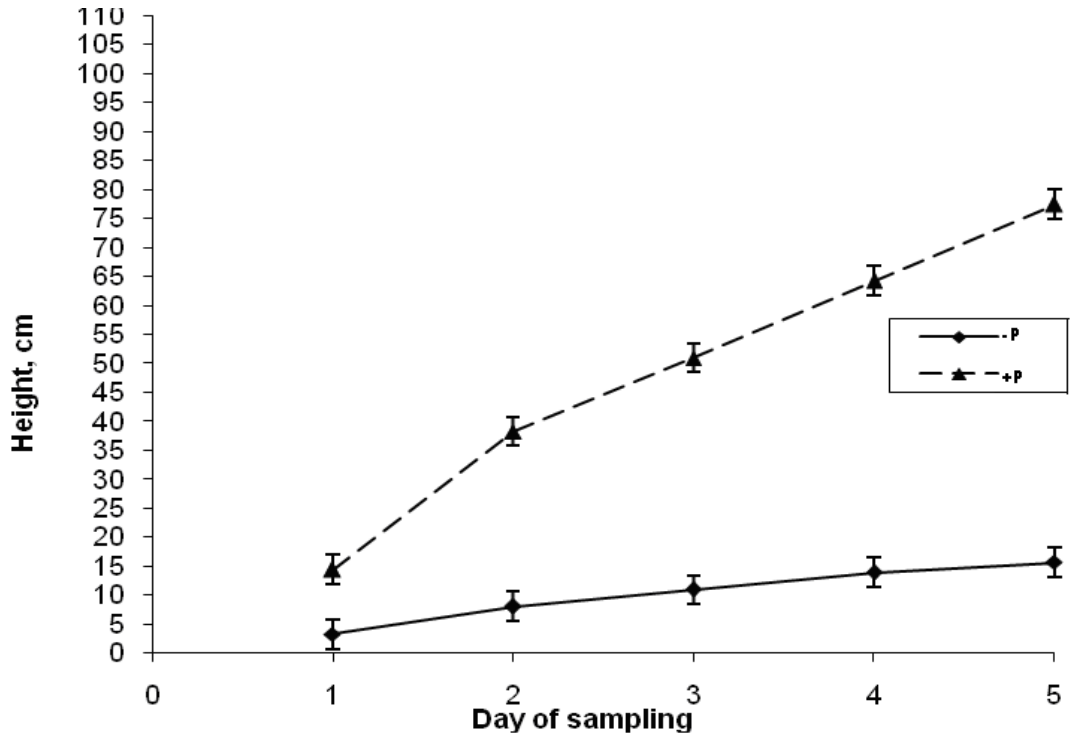


Figure 12. Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR-N4

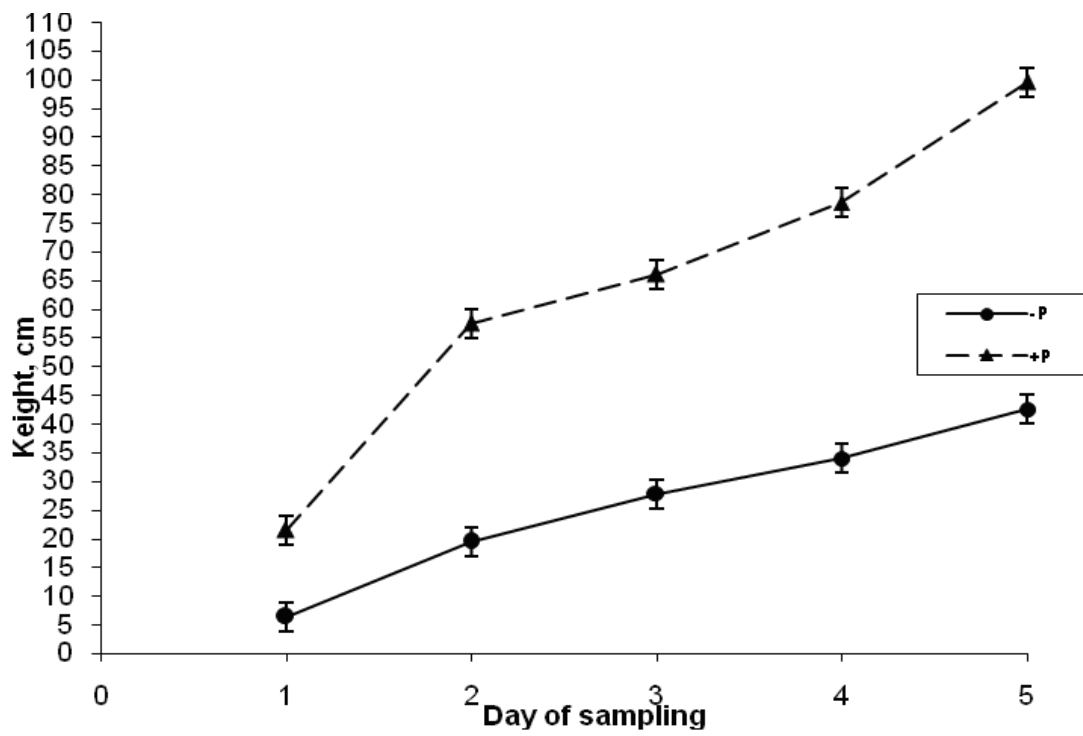


Figure 13. Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR-O2

Table 1. Phosphorus and Nitrogen concentration in the root, stem and leaves of selected sorghum cultivars that were grown with (+P) or without (-P) phosphorus fertilizer application

Cultivar	Treatment	Roots		Stems		Leaves	
		%P	%N	%P	%N	%P	%N
MCSR-P3	+P	0.100	0.70	0.148	2.37	0.093	2.33
	-P	0.147	1.11	0.096	2.41	0.107	2.69
MCSR-A3	+P	0.126	1.05	0.091	2.69	0.126	2.51
	-P	0.152	0.95	0.109	2.20	0.110	2.52
MCSR-K4E	+P	0.156	1.09	0.142	2.85	0.083	2.17
	-P	0.115	0.89	0.107	2.59	0.094	2.42
MCSR-L6	+P	0.144	1.11	0.137	2.61	0.075	2.47
	-P	0.110	0.79	0.095	2.10	0.115	2.68
MCSR-N4	+P	0.120	0.92	0.101	2.88	0.079	2.22
	-P	0.135	0.93	0.086	2.09	0.089	2.65
MCSR-O2	+P	0.105	0.84	0.127	2.62	0.091	2.53
	-P	0.097	0.91	0.103	2.45	0.095	2.59

Nutrient Concentration in Plant Tissue

Stem tissue tended to have more phosphorus concentration than any other part of plant tissue. These data did not portray a clear relationship between level of mycorrhizal development and phosphorus concentration in plant tissues

DISCUSSION

As expected from earlier studies (Bryla and Koide 1990 a, b), the different sorghum cultivars examined in this study responded very differently to mycorrhizal development and also to phosphorus concentration in the soil. This was seen from the microscopic observation of stained roots and the results indicated that there was variation in mycorrhizal development levels.

There is extensive information on interaction between the mycorrhizal legume host and phosphorus levels in the environment, and it is accepted that the beneficial effects of vesicular mycorrhiza decrease as the supply of phosphorus increases (Abbott and Robson 1982). The results of this study also suggested that phosphorus inefficient plants had high levels of mycorrhizal infection compared to P-efficient plants and we can deduce that phosphorus significantly suppressed level of mycorrhizal infection.

Several other studies have shown that mycorrhizal and phosphorus responsiveness is related (Graham and Syvertsen, 1985; Koide et al., 1988; Hetrick et al., 1996). In the present study, I found that changes in responsiveness to mycorrhizal colonization and phosphorus supply occurred with plant development. Mycorrhizal fungi level positively affected growth characteristics such as heights, leaf number and seed

development in the different sorghum cultivars in the unfertilized soils also phosphorus improved the cultivars performance significantly for example, the cultivar MCSR-O2 was the best performing without phosphorus since it had a high level of mycorrhizal colonization whereas MCSR-N4 and MCSR-A3 which had low level of mycorrhizae performed poorly in unfertilized soil with thin stems, slender leaves and were the first to show symptoms of wilting. Therefore there was a strong relationship between level of development of mycorrhizae and plant growth. Also MCSR-K4E and MCSR-L6 showed good performance in soil without phosphorus though MCSR-L6 flowered earlier than MCSR-K4E and their leaves were average in size. Generally plants in phosphorus fertilized soils had more leaves and were taller than plants in unfertilized soils; this clearly showed that addition of fertilizer favored increased growth performance in all the cultivars. When observing at the effect of phosphorus on growth, genotype MCSR-N4 showed poor performance in both fertilized and unfertilized soils since it had the shortest height in both cases and had fewer leaves compared to other genotypes though it tended to recover later in its development. This illustrates the importance of examining not only early stages of plant growth when assessing the impact of phosphorus and fungal species on mycorrhizal responsiveness, but later stages including reproduction as well (McGonigle and Fitter, 1988; Bryla and Koide, 1990 a)

Mycorrhiza enhances plant growth by increasing uptake of minerals, phosphorus and soil nitrogen (Marschner et al., 1994; Barea et al., 1987; Bowen and Smith, 1981). Unlike the previous observations this study could not explain the link between level of mycorrhizal and phosphorus concentration on plant tissues in unfertilized soils. The inconsistency with other studies

could have been due to errors during cleaning of the roots or maybe mycorrhiza produced other hormones for growth. This suggests that mycorrhizae may affect plant reproduction in ways other than those directly related to phosphorus acquisition and utilization (Koide and Lu, 1992). Nye, 1977 reported that the uptake of nitrogen, phosphorus and potassium is limited by the rate of diffusion of each nutrient through the soil. It is more likely vesicular mycorrhiza in soil not inoculated with phosphorus increased nutrient uptake by shortening the distance of the nutrients diffused through the soil to the roots.

CONCLUSION

The sorghum cultivars responded differently to mycorrhizal development and the control plants in unfertilized soils developed extensive mycorrhiza than plants in fertilized soils. There was no significant difference in the extent of mycorrhizal development among the cultivars that were grown on P-fertilized soils. Those cultivars in unfertilized soils that had extensive development of mycorrhiza had increased growth compared to those that developed few mycorrhizae. The sorghum cultivars also responded differently to phosphorus concentration in the soils and application of phosphorus significantly increased growth and number of leaves. There was no apparent clear relationship between level of mycorrhizae development and phosphorus concentration in plant tissues.

RECOMMENDATION

More sorghum cultivars should be studied and a more sustainable solution is to screen and adopt cultivars that form effective mycorrhizal symbiosis and hence enhance phosphorus uptake.

REFERENCES

- Abbot LK, Robson AD (1982). The role of vesicular-ArbuscularMycorrhizal
- Alexopolus CJ, Minns CW (1979). Introductory Mycology 3rdED. John Wiley and Sons, Newyork.
- Azcon R, Ocampo JA (1981). Factors affecting the vesicular- arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. New phytologist 87: 677-685
- Barea JM, Azcon-Aguilar C, Azcon R (1987). Vesicular-arbuscularmycorrhizas improve both symbiotic N₂ fixation and N uptake from soil as assessed with a 15N technique under field conditions. New Phytol.95, 381-396.
- Baylis GTS (1970). Root hairs and PhycomycetousMycorrhiza in Phosphorus Deficient Soil Plant and Soil 33: 713-716
- Bowen GD, Smith SE (1981). The effects of mycorrhizas on nitrogen uptake by plants. InTerrestrial Nitrogen Cycle: Processes, Ecosystem Strategies and Management Impacts. Eds. F.E Clark and T Rosswal. Pp 237-247. Ecological Bulletin No.33, Swedish Natural Science Research Council, Stockholm.
- Bryla DR, Koide RT (1990 a). Regulation of reproduction in Wild and cultivated LycopersiconesculentumMill. By vesicular-arbuscularmycorrhizal infection. Oecologia 84:74-81.
- Bryla DR, Koide RT (1990 b). Role of mycorrhizal infection in the growth and reproduction of the wild vs. cultivated plants. II.Eight wild occasions and two cultivars of LycopersiconesculentumMill.Oecologia 84:82-92.
- Chapin FS, K.Van Cleve (1989). Approaches to studying nutrient uptake, use and loss in plants. Pages 185-207
- Clement CR, Habte M (1995). Genotypic Variation in Vascular ArbuscularMycorrhizal Dependence of Pejibaye Palm. J. Plant Nutr. 18: 1907- 1916
- Estaun V, Calvet C, Hayman DS (1987). Influence of Plant Genotype on Mycorrhizal Fungi in Agriculture and the Selection of Fungi for Inoculation. Aust J. Res, 33:389
- Graham JH, Syvesten JP (1985). Host determination of mycorrhizal dependency of citrus rootstock seedlings, new phytologist 101: 667-676
- Graham JH, Duncan LW, Eissenstat DM (1997). Carbohydrate allocation pattern in citrus genotypes as affected by phosphorus nutrition, mycorrhizal colonization and mycorrhizal dependency new phytologist 135: 335- 343
- Hetrick BAD, Kitt DG, Wilson GWT, Todd TC (1996). Mycorrhizal response in wheat cultivars relationships to phosphorus. Can. J. Bot. 74: 19-25
- Koide RT, Li M, Lewis J, Irby C (1988). Role of mycorrhizal infection in the growth and reproduction of wild vs.cultivated plants. I. Wild vs.cultivated oats. Oecologia 77:537-543.
- Koide RT (1991). Nutrient supply nutrient demand and plant response to mycorrhizal infection new phytologist 117:365- 386
- Koide RT (1993). Physiology of mycorrhizal plant, in: Tomerurp IC, Ed advance in plant pathology volume 9. Mycorrhizal synthesis. New York: academic press, 33-54
- Marschner H, Dell B (1994). Nutrient uptake in mycorrhizal symbiosis. Plant and soil 159; 89-102
- McGonigle TP, Fitter AH (1988). Growth and phosphorus inflows of Trifoliumrepen L. with a range of indigenous vesicular-arbuscularmycorrhizalinfection levels under field conditions. New Phytologist 108:59-65.
- Menge JA, Johnson ELV, Platt RG (1978). Mycorrhizal dependency of several citrus cultivars under three nutrient regimes new phytologist 81: 553-559
- Mosse B (1978). Mycorrhizae and plant growth in Freyden A.H.J Woldendorp J.W, eds, structure and functioning plant populations London: north Holland publishing co. 269- 298
- Nye PH (1977). The rate- limiting step in plant nutrient absorption from soil. Soil science 123: 292-297
- Smith SE, Read D (1997). Mycorrhizal symbiosis 2nd Ed San Diego Academic Press
- Toth R, Toth D, Starke D, Smith DR (1990). Vesicular - arbuscularmycorrhizal colonization in zea mays affected by breeding resistance of fungal pathogens. Canadian Journal of Botany 68: 1039- 1044
- Watanabe FS, Olsen SR (1965). Test of an ascorbic acid method for determining phosphorus in water and NaHCO₃ extracts from soil. Proceedings of the soil science society of America 29: 677-678.
- Zar JH (1984). Biostatistical analysis. 2ndedn. London: Prentice-Hall.

How to cite this article: Rasowo B., Ndiema P., Muthuri J., Onkware A. (2014). Mycorrhizal Development and Phosphorus Concentration in selected Kenyan Sorghum Cultivars. Int. Res. J. Microbiol. 5(4):62-72