Mycorrhizal Development and Phosphorus Concentration in selected Kenyan Sorghum Cultivars

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ABSTRACT

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.

Key words: Mycorrhiza, cultivars, genotypes, symbiosis

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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% H_2O_2/NH_3 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% trypa 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^{o}c) then tissue samples were ground and sub samples digested for 1 hour in a H_2SO_4/H_2O_2 mixture using a digestion block (Technicon, Tarrytown) set at 400^{0}c. The digest was analyzed for total phosphorus using the molybdo-phosphate method (Watanabe and Olsen, 1965). A standard digestion stock solution was prepared, and then a suitable aliquot of sample solution pipetted into a 50-ml volumetric flask (up-to 40 ml soil extract and 5-ml plant digest) then aliquots of 0-15ml of working standard solution was pipetted into separate 50-ml volumetric flask. This gave a standard range from 0.0 to 0.03 g phosphorus and either digest acid or soil extract was included in each flask to match the sample aliquots. From that point standards and samples were treated in the same way. Water was added to each flask until it was about two-thirds full and 2-ml ammonium molybdate reagent was then added and mixed. Afterwards 2-ml stannous chloride reagent was added, mixed and diluted to volume of 50-ml and after 30 minutes the light absorption was measured at 700-nm using water as reference. A calibration curve was constructed using the standard readings and used to determine the concentration of phosphorus in the sample aliquots using the phosphorus concentration in the original sample calculated.

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 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.

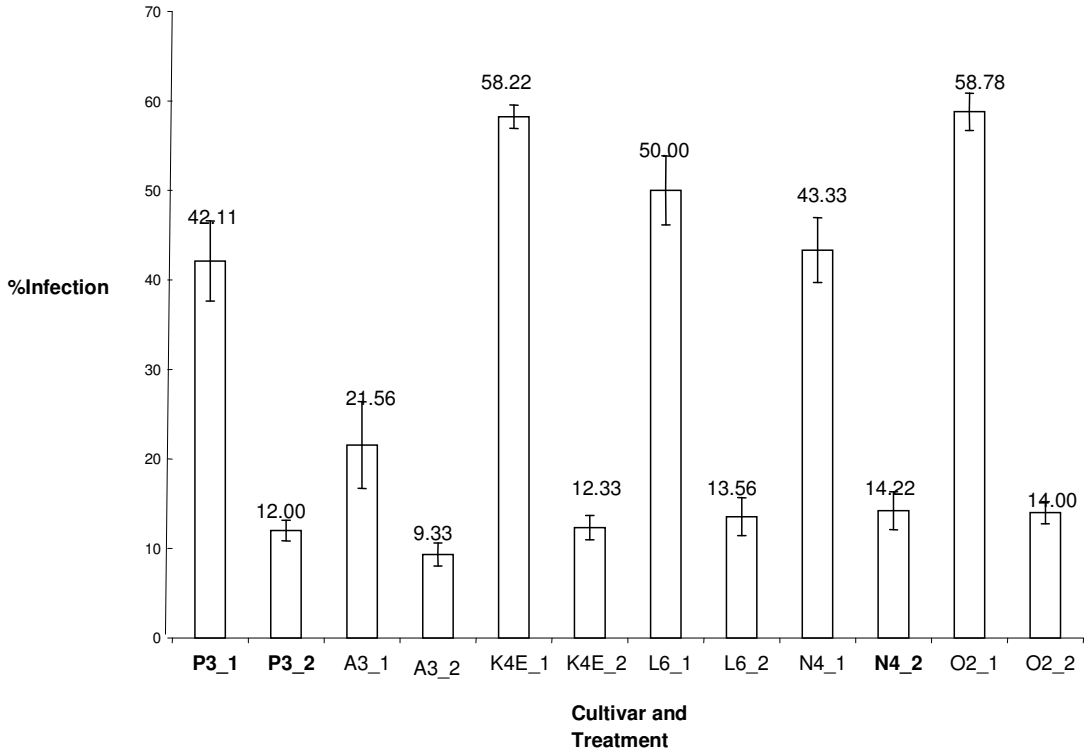

Figure 1: Percentage of Mycorrhizal infection in sorghum cultivars that were grown with or without phosphorus fertilizer application.

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

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

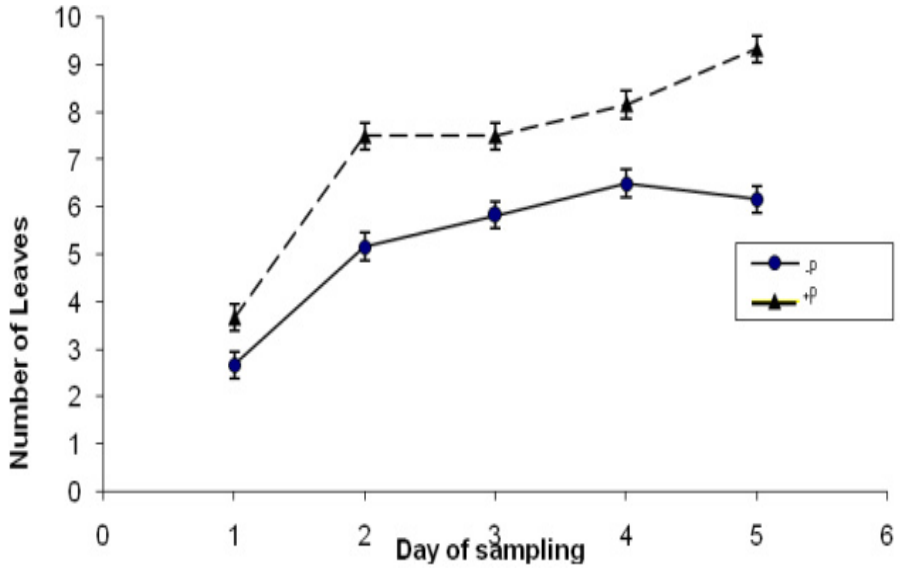

Fig2: Effect of phosphorus fertilizer application on the number of leaves per plant of sorghum cultivar MCSR-P3

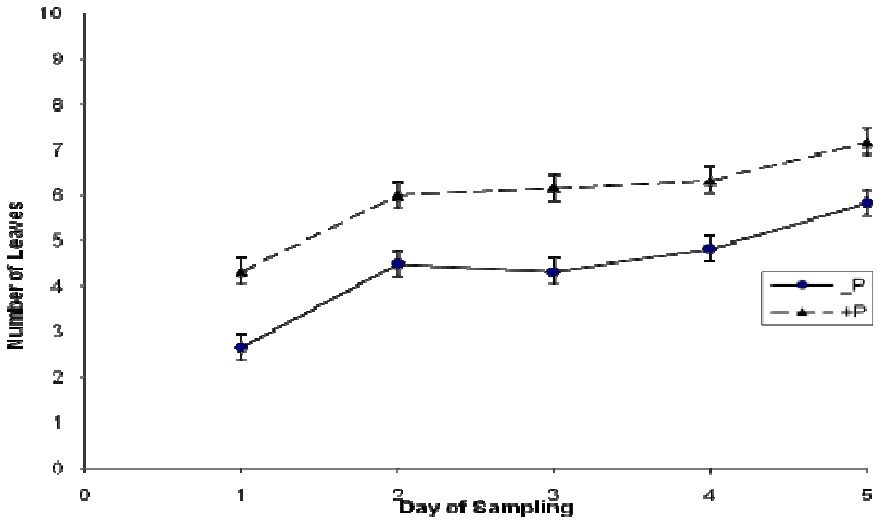

Fig 3: Effect of phosphorus fertilizer application on the number of leaves per plant of sorghum cultivar MCSR-A3

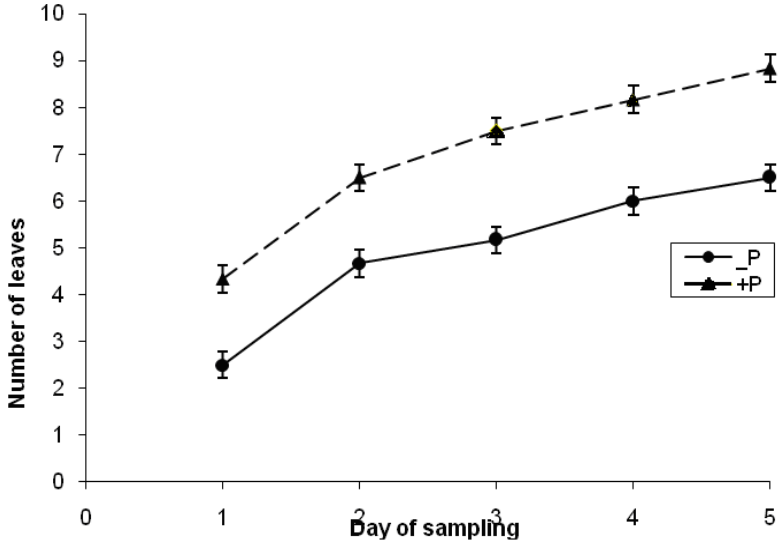

Fig 4: Effect of phosphorus fertilizer application and on the number of leaves per plant of sorghum cultivar MCSR-K4E

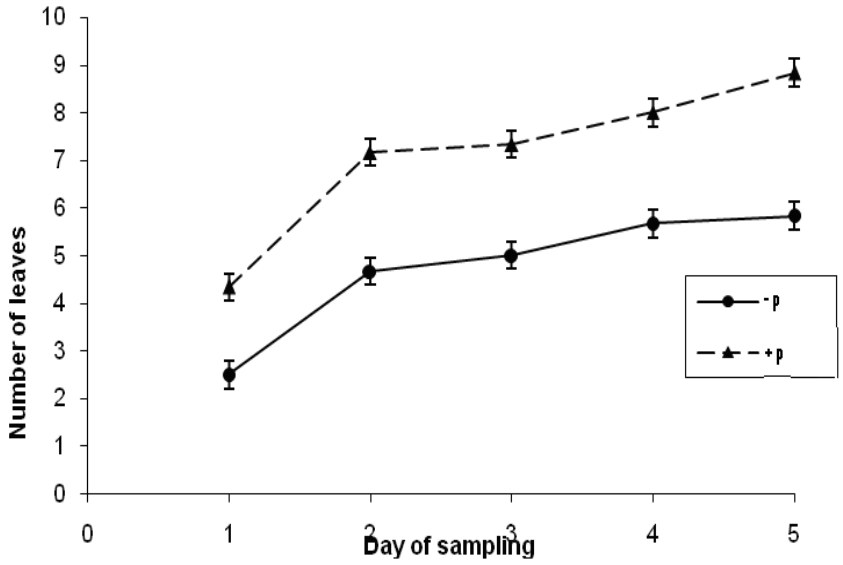

Fig 5: Effect of fertilizer application on the number of leaves per plant of sorghum cultivar MCSR-L6

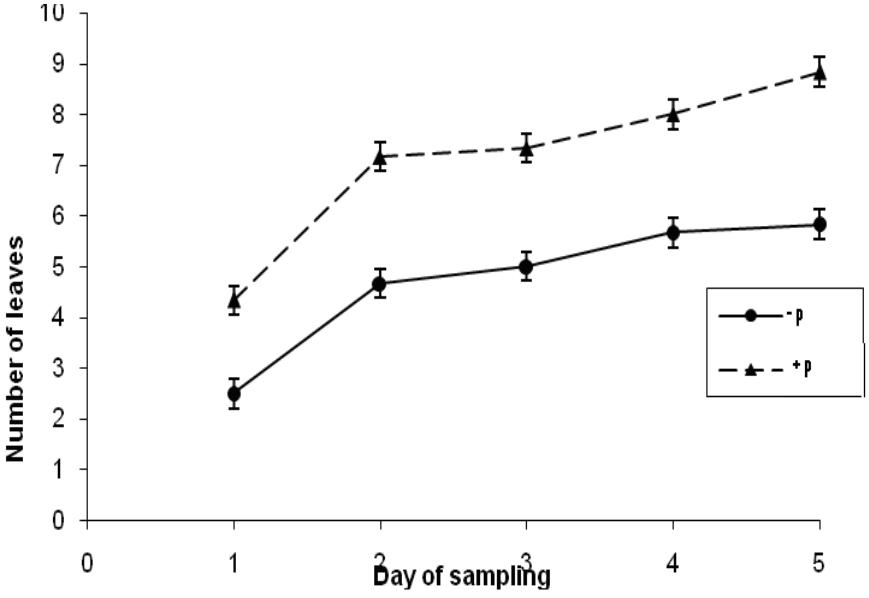

Fig 6: Effect of fertilizer application on the number of leaves per plant of sorghum cultivar MCSR-N4

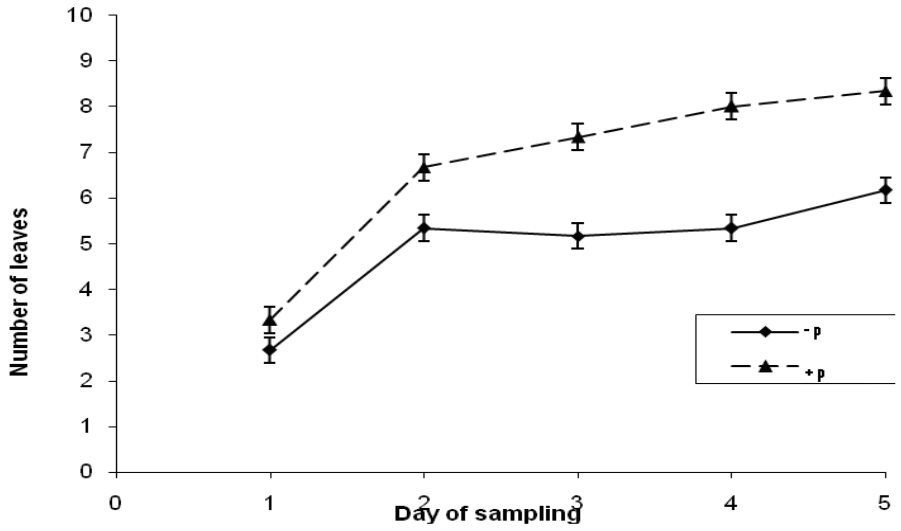

Fig 7: Effect of fertilizer application on the number of leaves per plant of sorghum cultivar MCSR-02

Application of phosphorus caused a significant increase in the height of sorghum cultivar

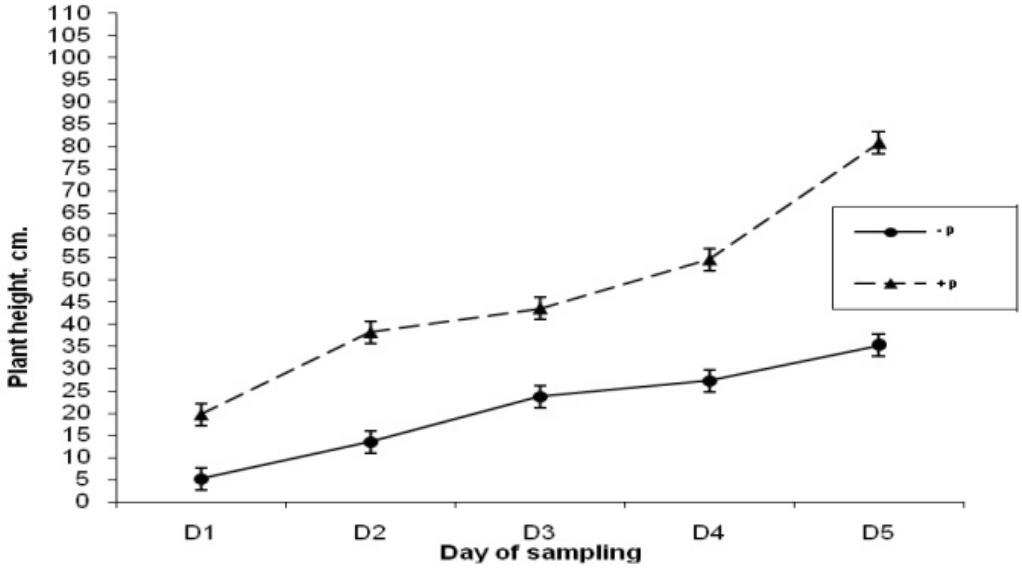

Fig 8: Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR-P3

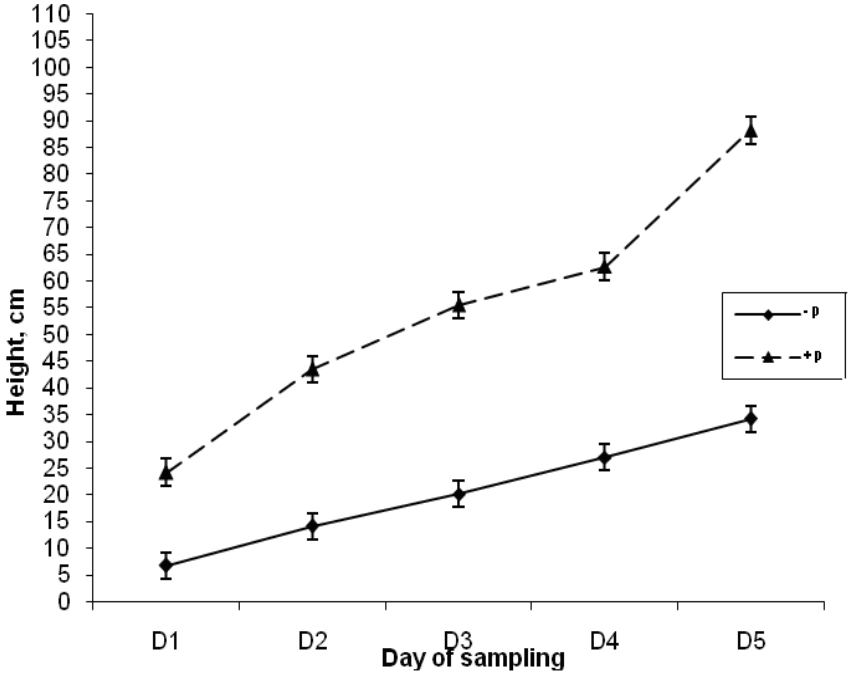

Fig 10: Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR- K4E

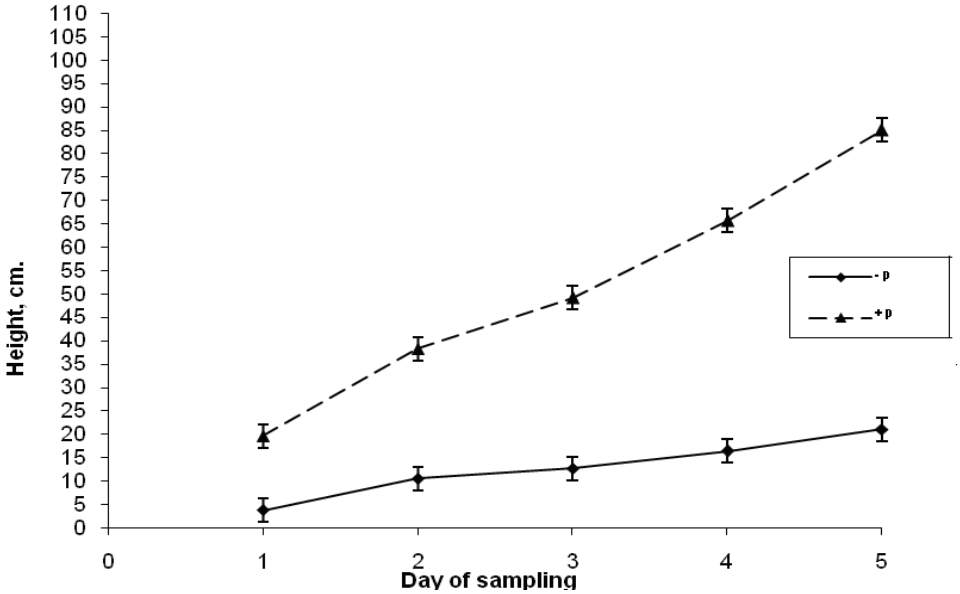

Fig 9: Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR-A3

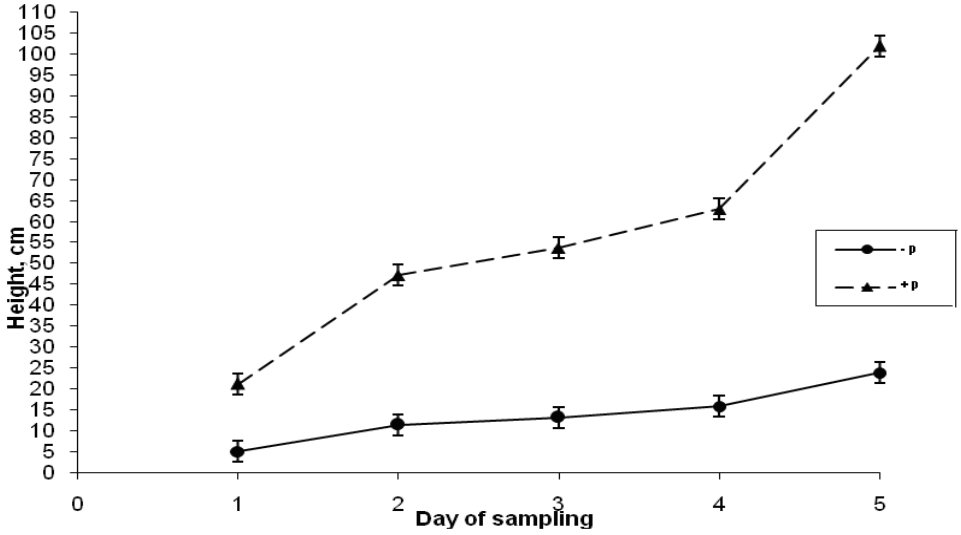

Fig 11: Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR-L6

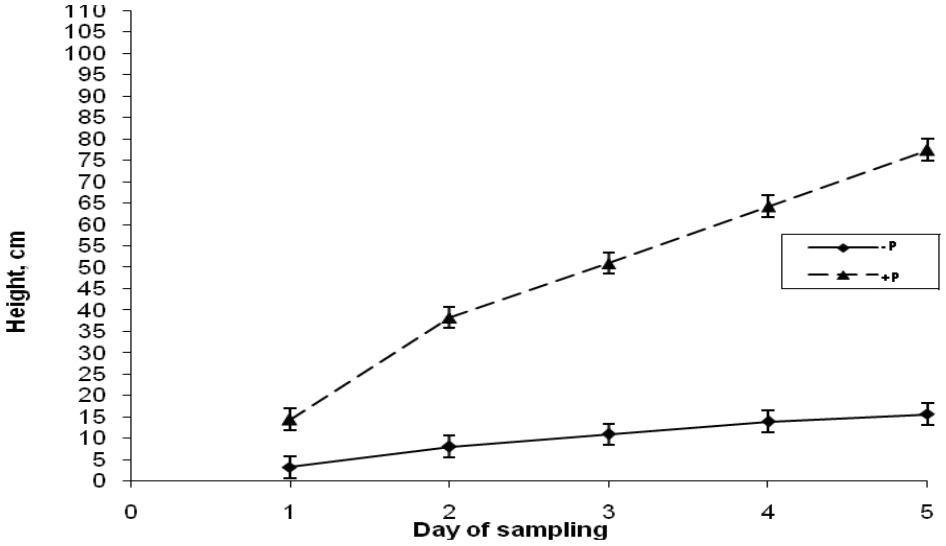

Fig 12: Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR-N4

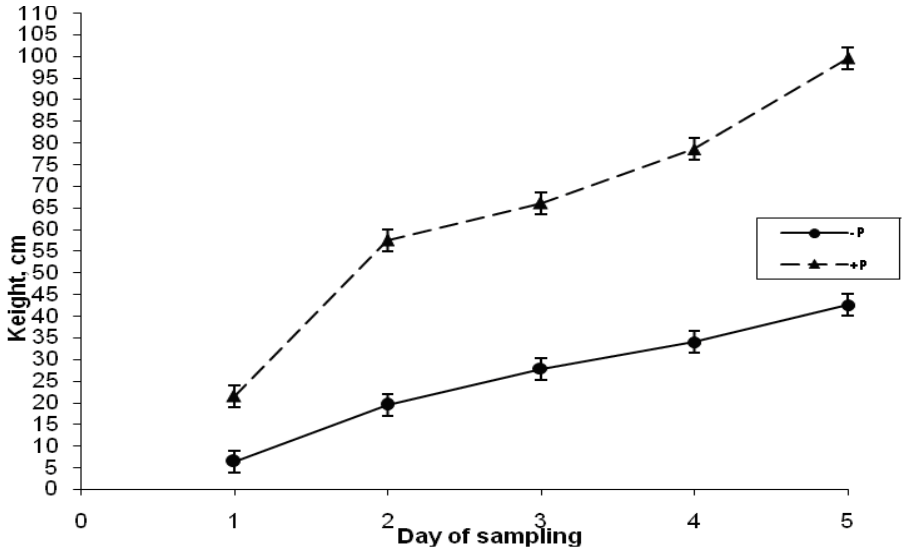

Fig13. Effect of phosphorus fertilizer on growth of sorghum cultivar MCSR-O2

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

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 applicatio

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
	+P	0.126	1.05		0.091	2.69		0.126	2.51
MCSR-A3	-P	0.152	0.95		0.109	2.20		0.110	2.52
	+P	0.156	1.09		0.142	2.85		0.083	2.17
MCSR-K4E	-P	0.115	0.89		0.107	2.59		0.094	2.42
	+P	0.144	1.11		0.137	2.61		0.075	2.47
MCSR-L6	-P	0.110	0.79		0.095	2.10		0.115	2.68
	+P	0.120	0.92		0.101	2.88		0.079	2.22
MCSR-N4	-P	0.135	0.93		0.086	2.09		0.089	2.65
	+P	0.105	0.84		0.127	2.62		0.091	2.53
MCSR-O2	-P	0.097	0.91		0.103	2.45		0.095	2.59

Discussion

As expected from earlier studies (Bryla and koides 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 unfertilzed 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 uptak

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