Journal of Plant Nutrition

ISSN: 0190-4167 (Print) 1532-4087 (Online) Journal homepage: http://www.tandfonline.com/loi/lpla20

Response of a wild-type and modern cowpea cultivars to arbuscular mycorrhizal inoculation in sterilized and non-sterilized soil

Marjorie Bonareri Oruru, Ezekiel Mugendi Njeru, Remy Pasquet & Steve Runo

To cite this article: Marjorie Bonareri Oruru, Ezekiel Mugendi Njeru, Remy Pasquet & Steve Runo (2018) Response of a wild-type and modern cowpea cultivars to arbuscular mycorrhizal inoculation in sterilized and non-sterilized soil, Journal of Plant Nutrition, 41:1, 90-101, DOI: 10.1080/01904167.2017.1381728

To link to this article: https://doi.org/10.1080/01904167.2017.1381728

Accepted author version posted online: 21 Sep 2017.
Published online: 03 Nov 2017.

JOURNAL OF PLANT NUTRITION
2018, VOL. 41, NO. 1, 90–101
https://doi.org/10.1080/01904167.2017.1381728

Taylor & Francis
Taylor & Francis Group

Response of a wild-type and modern cowpea cultivars to arbuscular mycorrhizal inoculation in sterilized and non-sterilized soil

Marjorie Bonareri Oruru[a,b], Ezekiel Mugendi Njeru[a], Remy Pasquet[b], and Steve Runo[c]

[a]Department of Microbiology, Kenyatta University, Nairobi, Kenya; [b]International Center for Insect Physiology and Ecology, Kasarani, Nairobi, Kenya; [c]Department of Biochemistry and Biotechnology, Kenyatta University, Nairobi, Kenya

ABSTRACT

Cowpea is an important crop that serves as a legume and vegetable source to many smallholder farmers in sub-Saharan Africa. Soil fertility is a significant limitation to its production thus; inoculation with beneficial soil biota such as arbuscular mycorrhizal fungi (AMF) could improve its performance. However, plant–AMF interaction could vary based on crop cultivar hence affecting overall crop production. The present study aimed at determining the effect of AMF inoculation and soil sterilization on root colonization and growth of a wild-type and three modern cowpea cultivars grown by smallholder farmers in Kenya. Potted cowpea plants were inoculated with a commercial AMF inoculum comprising of *Rhizophagus irregularis, Funneliformis mosseae, Glomus aggregatum* and *Glomus etunicatum* and maintained in a greenhouse for 40 days. After harvesting, mycorrhizal colonization, nodule number and dry weight, root and shoot dry weights, nitrogen (N,) phosphorus (P) and potassium (K) content were determined. Interestingly, the modern cultivars showed significantly ($p < 0.001$) higher root colonization, nodulation, shoot P and N compared to the wild-type cultivar. Moreover, a strong positive correlation between AMF root colonization and shoot P ($r^2 = 0.73, 0.90$, $p < 0.001$), AMF root colonization and shoot N ($r^2 = 0.78; 0.89$, $p < 0.001$) was observed in both sterilized and non-sterilized soil, respectively. Soil sterilization affected root colonization and growth parameters with plants grown in non-sterilized soil performing better than those grown in sterilized soil. This study provides major evidence that modern cowpea cultivars are still responsive to mycorrhizal inoculation suggesting that modern breeding programs are not deleterious AMF symbiosis.

ARTICLE HISTORY
Received 16 May 2016
Accepted 28 November 2016

KEYWORDS
arbuscular mycorrhizal fungi; cowpea cultivars; root colonization; smallholder farmers

Introduction

Cowpea is a multipurpose legume that serves as human food, livestock fodder, income source, and is grown in the semi-arid regions of most continents worldwide (Singh and Ajeigbe 2003). Approximately, 5.7 million tons of cowpea was produced across the globe annually on about 11.3 million hectares in 2013, with sub-Saharan Africa (SSA) accounting for 70% of the total global production (FAO 2013). Low soil fertility is a key challenge facing cowpea production, especially among smallholder farmers in SSA due to poor soil health management practices. This has led to an average annual depletion rate of 22 kg of nitrogen (N), 2.5 kg of phosphorus (P) and 15 kg of potassium (K) hectare^{-1} of cultivated land in 37 African countries over the last 30 years (Koohafkan and Altieri 2010). The traditional method of overcoming nutrient depletion

CONTACT Ezekiel Mugendi Njeru njeruezek@gmail.com Department of Microbiology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya.

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/lpla.

is the use of chemical fertilizers, which are unfortunately too costly for the majorly resource-poor smallholder farmers. This necessitates the use of sustainable agricultural practices such as incorporation of beneficial soil biota that promote uptake of limited soil nutrients such as N and P by plants.

Among the key groups of important soil microorganisms are arbuscular mycorrhizal fungi (AMF) that belong to the phylum Glomeromycota and form symbiotic association with the roots of over 80% of wild and cultivated plant species (Barrow et al. 2008). AMF depend on the host plant for the supply of photosynthetic carbohydrates and in return deliver many agroecosystem services including soil aggregation, nutrient uptake and carbon sequestration (Oruru and Njeru 2016) through an extensive extraradical hyphal network that spreads from colonized roots into the soil. Additionally, AMF enhance plant resistance to biotic and abiotic stresses, as well as the synthesis of important plant secondary metabolites, which contributes to the production of safe and high-quality food (Giovannetti et al. 2012). These symbiotic fungi have also been found to increase nodulation and atmospheric N fixation potential in legumes such as cowpea (Turk et al. 2008), since the fungus improves P uptake by the plant, which in turn would avail more energy for N fixation by rhizobia. AMF may form tripartite symbiosis with legumes and rhizobia to stimulate nodulation and plant growth (Xavier and Germida 2002). Other additional effects produced by this interaction include greater number and dry weight of nodules, enhanced symbiotic N fixation and higher N content (Shockley et al. 2004). The beneficial effect of N_2 fixation by AMF colonization has been thought to be caused by increased P supply to the nodules by the symbiotic fungal partner.

Differences in the way plants respond to AMF have been observed among not only plant species, but also cultivar types. Zhu et al. (2001) assessed six wheat varieties and reported that the percentage AMF colonization was lower in modern varieties compared to old varieties (Landraces). Tarawaya (2003) also showed that improved, high yielding wheat cultivars responded less to AMF compared to landrace cultivars. Similar findings were obtained by (Zhu et al. 2003) in barley, where an improved cultivar was less responsive compared to a landrace. While conducting a study to compare the interaction of wild-type, old and modern tomato cultivars with AMF, *Glomus mosseae*, Steinkellner et al. (2012), reported a relatively higher AMF colonization in modern cultivars compared to the wild-type and old cultivars. Similar results were reported by Njeru et al. (2013), where composite cross-maize populations developed through evolutionary breeding had relatively a higher level of AMF colonization compared to modern hybrids. Since very few studies have been done on the response of cowpea to AMF, there is a possibility that different cultivars differing in their genetic makeup or improvement respond differently to AMF inoculation. Moreover, to our knowledge no study has been conducted to compare how different cowpea cultivars maintained by farmers in Kenya interact with AMF although the crop is an important food crop to many smallholder farmers as both a grain legume and vegetable.

Here, we tested the hypotheses that the susceptibility of AMF colonization differs between the wild-type and the cultivated cowpea cultivars and that AMF inoculation would enhance shoot nutrition and other growth parameters. The specific aims of the study were (1) to compare the susceptibility of a wild-type vs. three cultivated cowpea cultivars to AMF colonization, (2) to determine the effect of fungal root colonization in the wild-type and the cultivated cultivars and compare their effect on different growth parameters, and shoot nutrition and (3) to determine the effect of soil sterilization on AMF colonization and growth of different cowpea cultivars.

Materials and methods

Fungal inoculum

Myco Apply custom super concentrate powder, comprising of four mycorrhizal species, *Funneliformis mosseae, Glomus aggregatum, Glomus etunicatum* and *Rhizophagus irregularis*, was used as the inoculum. The super concentrate powder contains 220,200 mycorrhizal propagules gram^{-1}. This

commercial product was provided by Symyco, a joint venture between Symbiotic Sciences based in New Delhi, India and Mycorrhizal Application Inc., based in Grants Pass, Oregon, USA.

Experimental soil

The soil used for the greenhouse experiment was obtained from four farms in Embu County, which lies about 120 km north east of Nairobi, on the South Eastern side of Mount Kenya. Based on FAO soil classification, the soil used belongs to the phaeozems category, characterized by well-developed subsoil with a blocky structure and richness in organic matter. Phaeozems are also porous, fertile soils that make excellent farmland. Crop rotation and use of organic manure are management practices used by farmers in the four farms. Agriculture is the main economy driver in this county with more than 70% of the population accounting for smallholder farmers. Cowpea is among the major food crops produced in the county, although its production is quite lower than staples such as maize. Substantial rainfall in received in the county with the average annual precipitation being 1,206 mm. Ten sub-samples of about 900 g were collected from each of the four farms by removing soil core from 5 to 20 cm depth using a soil auger. The experimental soil characteristics were; pH (water) 5.20, organic carbon (C), 1.82%, N 0.15%, exchangeable K 0.50 $Cmolkg^{-1}$, sodium (Na) trace $Cmolkg^{-1}$, calcium (Ca) 6.50 $Cmolkg^{-1}$, magnesium (Mg) 6.00 $Cmolkg^{-1}$, cation exchange capacity (CEC) 13.40 $Cmolkg^{-1}$and available P (CAL) 14.40 mg kg^{-1}.

Experimental design

The experiment had three factors (soil sterilization, cowpea genotype and mycorrhizal inoculation) factorial in Randomized Complete Block Design with four replicates. The greenhouse experiment was repeated in space for comparison and validity purposes. Cowpea genotypes included three cultivars (Katumani 80, KenKunde 1 and Kunde Mboga) commonly grown by smallholder farmers in Kenya and one wild-type cultivar. Katumani 80 and KenKunde 1 are dual-purpose genotypes suitable for both vegetable and grain production, while Kunde Mboga is a local vegetable that plays a vital role in restoration of soil fertility. The wild-type cowpea was accession SP 219, which belongs to *Vigna unguiculata* (L.) Walp. subsp. unguiculata var. spontanea (Schweinf.) Pasquet. The accession comes from the South Coast of Kenya.

Planting and maintenance of greenhouse bioassays

Sterilized soil and sand (silica) were mixed (1:1 by volume) and mycorrhizal inoculation done by mixing the inoculum with soil at a depth of 3–6 cm prior to sowing as per the manufacturer's instruction. All the pots were also supplied with a filtrate obtained by sieving an aliquot of non-sterilized soil through a 40-μm sieve to provide the substrate with an equivalent soil microbiota (Njeru et al. 2014). Two cowpea seeds were planted per pot, 4 inches apart and each seed approximately 1–2 inches deep. The soil was watered immediately and then regularly after seed germination.

Plant sampling and analyses

The plants were sampled 40 days after planting and gently washed under running tap water to preserve the root system. The roots, shoots and nodules were then separated. About 100 pieces of root segments from each treatment were thoroughly washed, placed in falcon tubes and then cleared using 10% potassium hydroxide (KOH) placed in 80°C water bath for 10–15 min, neutralized in 2% aqueous hydrochloric acid (HCl), and stained with 0.05% trypan blue in lactic acid. Root colonization was assessed under a dissecting microscope at ×25 or ×40 magnification by the gridline intersect method (Giovannetti and Mosse 1980).

The nodules detached from the root system were then counted, followed by dry weight of nodules, roots and shoots obtained after oven drying at 65°C to a constant weight. To extract plant biomass, 0.025 g of oven dried, milled plant tissue was weighed and placed in a clean and dry digestion specimen

tubes prior to ashing them in the muffle furnace for $4^1/_2$ hr and then allowed to cool. 0.5 ml of the digestion mixture of acids and hydrogen peroxide was added and placed on the hot plate to evaporate to dryness. 25 ml of 0.05 ammonium chloride (NH_4Cl) was added to re-dissolve the digested sample and allowed to stand for 5 hr after cooling. Shoot total N content was determined by Kjedahl method, which involves the transformation of organic N to ammonium ions (Bremner 1982). The shoot P content was determined by colorimetric method, while readily exchangeable, water soluble K was determined in neutral NH_4 acetate extractant following sulfuric/perchloric acid digestion (Page et al. 1982).

Data analyses

All data were tested for homogeneity of variance by Bartlett test before analyses. The % data were arcsine ($\sqrt{x}$) transformed wherever necessary to fulfill the assumptions of analysis of variance (ANOVA). The data reported in tables and graphs were back transformed. Data were analyzed by two-way ANOVA as a completely randomized block design. Pearson correlation coefficient was used to determine the relationship between shoot dry weight, N, P and K and mycorrhizal colonization. Wherever applicable, post hoc test was performed using Tukey's HSD test ($p < 0.05$). All statistical analyses were performed with the SPSS (version 20.0 software).

Results

Effect of AMF inoculation, cowpea genotype and soil sterilization on root colonization and growth parameters

Two way ANOVA data showed that root colonization was significantly affected by both the genotype ($F = 9.56$, $p < 0.001$) and AMF inoculation ($F = 231.75$, $p < 0.001$) with modern cultivars having higher root colonization compared to the wild-type cultivar (Table 1). Moreover, significant differences in AMF colonization were observed within the modern cultivars. Katumani 80 and Kunde Mboga had higher level root AMF colonization compared to KenKunde 1, while non-inoculated plants were not colonized. Plants grown in non-sterilized soil had significantly higher ($F = 52.77$, $p < 0.001$)

Table 1. ANOVA results for the effects of soil sterilization, cowpea genotype and AMF inoculation, and their interaction on root mycorrhizal colonization, nodule number, nodule dry weight, root dry weight and shoot dry weight of cowpea. The mean standard errors are presented in parentheses.

	%MC	NN	NDW (mg plant^{-1})	RDW (mg plant^{-1})	SDW (mg plant^{-1})
Soil					
Non-sterilized	30.34 (1.99)a	21.15 (0.94)a	13.86 (1.24)a	162.06 (10.19)a	917.98 (57.62)a
Sterilized	18.47 (3.29)b	19.38 (1.18)b	10.01 (1.23)b	145.27 (10.51)b	803.71 (58.71)b
Cowpea genotype					
K	29.22 (4.65)a	22.31 (1.10)a	14.13 (1.96)a	188.55 (14.27)a	1048.24 (78.80)a
KM	27.41 (4.46)a	20.31 (1.41)ab	12.98 (2.00)ab	166.76 (16.20)b	921.55 (90.68)b
KK	22.95 (3.88)ab	21.00 (1.76)a	11.65 (1.77)b	143.04 (12.56)c	829.59 (77.04)c
Wild	17.45 (3.06)b	17.44 (1.59)b	8.98 (1.26)c	116.31 (9.36)d	644.00 (51.83)d
AMF inoculation					
M	37.17 (1.27)a	24.84 (0.63)a	18.24 (0.71)a	202.5 (6.83)a	1141.13 (36.43)a
NM	11.34 (2.20)b	15.69 (0.77)b	5.62 (0.45)b	104.83 (4.28)b	580.56 (24.13)b
p values of the main factors and interaction					
Soil	<0.001	0.0294	<0.001	<0.001	<0.001
Genotype	<0.001	0.0006	<0.001	<0.001	<0.001
AMF In	<0.001	<0.001	<0.001	<0.001	<0.001
Genotype × AMF In	0.3592	0.0023	<0.001	<0.001	<0.001

Values followed by the same letter in a column within each treatment are not significantly different at $p < 0.05$ (Tukey's HSD test)
%MC: Mycorrhizal colonization percentage, NN: Nodule number, NDW:Nodule dry weight, RDW:Root dry weight, SDW:Shoot dry weight, K-Katumani 80, KM:Kunde Mboga, KK:KenKunde 1, M:Mycorrhizal, NM:Non-mycorrhizal AMF In: Arbuscular mycorrhizal fungi inoculation.

Table 2. The effect of genotype × AMF inoculation interaction on mycorrhizal colonization, nodule number, nodule dry weight, root dry weight and shoot dry weight of cowpea with the mean standard errors shown in parentheses.

Cowpea genotype	AMF In	NN	NDW	RDW	SDW
K	M	21.25 (1.15)a	24.22 (4.79)a	242.21 (4.79)a	1344.46 (26.09)a
	NM	7.13 (1.07)d	13.48 (5.19)c	134.88 (5.19)c	752.01 (29.33)cd
KM	M	20.25 (1.09)ab	22.74 (6.11)ab	227.44 (6.11)ab	1261.03 (33.05)ab
	NM	5.71 (0.89)de	10.68 (5.95)cd	106.08 (5.95)cd	582.08 (34.99)d
KK	M	18.14 (0.94)b	18.97 (6.01)b	189.68 (6.01)b	1123.46 (12.36)b
	NM	5.16 (0.77)de	9.65 (4.57)d	96.49 (4.57)d	535.73 (24.72)de
Wild	M	13.48 (0.74)c	15.07 (3.21)bc	150.74 (3.21)bc	835.58 (15.49)c
	NM	4.49 (0.72)e	8.19 (5.13)d	81.89 (5.13)d	452.43 (28.02)e

Values followed by the same letter in a column within each treatment are not significantly different at $p < 0.05$ (Tukey's HSD test).
NN: Nodule number, NDW:Nodule dry weight, RDW:Root dry weight, SDW:Shoot dry weight, K:Katumani 80, KM: Kunde Mboga, KK: KenKunde 1, M:Mycorrhizal, NM:Non-mycorrhizal, AMF In: Arbuscular mycorrhizal fungi inoculation.

percentage colonization compared to those grown in sterilized soil. The interaction between genotype × AMF was not significant.

Cowpea genotype significantly affected nodule number ($F = 6.69$, $p = 0.0006$), where Katumani 80 had the highest mean nodule number, while the wild-type had the lowest nodule number (Table 1). Mycorrhizal plants had a higher nodule number ($F = 132.13$, $p < 0.001$) compared with the non-inoculated plants. Moreover, soil sterilization has a significant effect in the number of nodules ($F = 5.00$, $p = 0.0294$) with plants grown in non-sterilized soil having higher nodule number compared to those grown in sterilized soil. Additionally, there was a significant ($F = 5.46$, $p = 0.023$) genotype × AMF interaction on nodule number (Table 1). The highest increase in nodulation after AMF inoculation was observed in Kunde Mboga (69.1%) followed by KenKunde 1 (68.9%), while Kunde Mboga and Katumani 80 had the same percentage increase (63.4%) (Table 2).

Correspondingly, the nodule dry weight was significantly affected by the genotype ($F = 28.99$, $p = <0.001$), where Katumani 80 had the highest nodule dry weight and the wild-type having the lowest weight (Table 1). AMF inoculation ($F = 930.58$, $p < 0.001$) and soil sterilization ($F = 87.23$, $p = <0.001$) similarly affected nodule dry weight, with AMF inoculation and unsterilized soil producing higher nodule dry weight compared non-inoculated plants and sterilized soils. Besides, a significant genotype × AMF interaction ($F = 9.32$, $p < 0.001$) on the nodule dry weight was observed. In this case, AMF inoculated Kunde Mboga (52.03%) showed the highest increase in nodule dry weight followed by KenKunde 1 (48.4%), while the wild-type and Katumani 80 showed a similar same percentage increase (43.4%) after inoculation (Table 2).

The root and shoot dry weights varied significantly ($F = 111.85$, $p < 0.001$) in all the cowpea cultivars with Katumani 80 having the highest root and shoot dry weights, while the wild-type had the lowest root and shoot dry weights per plant. Mycorrhizal plants had significantly ($F = 1105.94$, $p < 0.001$) higher root and shoot dry weights than non-mycorrhizal plants. Soil sterilization also had a significant ($F = 32.72$, $p < 0.001$) effect with plants grown in non-sterilized soil having higher weights compared to those grown in sterilized soil. The effect of genotype × AMF interaction on the root and shoot dry weights was significant ($F = 14.54$, $p < 0.001$). The highest increase in root and shoot dry weights after inoculation was observed in Kunde Mboga (57.1% and 58.1%), while Katumani 80 had the lowest increase (45.5% and 47.2%) (Table 2).

Effect of AMF inoculation, cowpea genotype and soil sterilization on cowpea shoot nutrition

The effect of cowpea genotype on the level of shoot N was statistically significant ($F = 220.08$, $p < 0.001$) with Katumani 80 having the highest percentage of shoot N and the wild-type having the lowest (Table 3). Mycorrhizal plants had significantly ($F = 2418.61$, $p < 0.001$) higher level of shoot total N than the non-inoculated plants (Table 3). Similarly, soil sterilization significantly ($F = 104.23$, $p < 0.001$) affected shoot N content with plants grown in non-sterilized soil having a higher N content compared to those grown in sterilized soil. Additionally, genotype × AMF interaction showed

Table 3. Means and *p* values from ANOVA of soil sterilization, cowpea genotype, AMF inoculation, and interactions on shoot N, K and P nutrition of cowpea. The mean standard errors are presented in parentheses.

	%N	K (ppm)	P (ppm)
Soil			
Non-sterilized	2.77 (0.17)a	2599.81 (27.61)b	1195.28 (65.91)a
Sterilized	2.42 (0.18)b	2859.09 (24.27)a	1057.47 (64.20)b
Cowpea genotype			
K	3.16 (0.24)a	2705.06 (44.10)ab	1396.00 (65.58)a
KM	2.77 (0.27)b	2804.19 (49.74)a	1189.69 (95.48)b
KK	2.51 (0.23)c	2606.94 (61.20)b	1060.69 (81.91)c
Wild	1.95 (0.16)d	2801.63 (11.95)a	859.13 (54.24)d
AMF inoculation			
M	3.44 (0.11)a	2702.94 (29.09)a	1423.22 (45.42)a
NM	1.76 (0.07)b	2755.97 (39.29)a	829.53 (31.99)b
P values of the main factors and interaction			
Soil	<0.001	<0.001	<0.001
Genotype	<0.001	<0.001	<0.001
AMF In	<0.001	0.0762	<0.001
Genotype x AMF In	<0.001	0.0493	<0.001

Values followed by the same letter in a column within each treatment are not significantly different at $p < 0.05$ (Tukey's HSD test). K: Katumani 80, KM:Kunde Mboga, KK:KenKunde 1, M:Mycorrhizal, NM:Non-mycorrhizal (control), AMF In: Arbuscular mycorrhizal fungi inoculation.

comparable results ($F = 30.01$, $p < 0.001$), where the highest increase in percentage N after inoculation was observed in Kunde Mboga (51.2%) followed by KenKunde 1 (50.1%) then the wild-type (43.4%). Katumani 80 had the lowest increase (42.1%) in percentage N (Table 4).

The level of shoot P was significantly affected by the genotype ($F = 428.14$, $p < 0.001$) with Katumani 80 cultivar having the highest shoot P content, while the wild-type cultivar had the lowest shoot P level. Also, the effect of AMF inoculation on shoot P was significant ($F = 2969.74$, $p < 0.001$), with mycorrhizal inoculated plants having a higher shoot P content compared to the non-inoculated ones. Soil sterilization's effect was significant ($F = 160.02$, $p < 0.001$) with the plants grown in non-sterilized soil having higher P content compared to those grown in sterilized soil. Besides, a significant genotype × AMF interaction ($F = 37.59$, $p < 0.001$) in shoot P was observed where inoculated Kunde Mboga showed the highest increase in shoot P (45.3%), while Katumani 80 and the wild-type showed the lowest increase (35.5%) (Table 4).

Shoot K differed significantly ($F = 10.22$, $p < 0.001$) in all the cowpea cultivars with Kunde Mboga and the wild-type having the highest level of shoot K. However, AMF inoculation did not significantly affect ($F = 3.26$, $p = 0.0762$) shoot K content. Nonetheless, soil sterilization had a significant effect ($F = 78.04$, $p < 0.001$) with plants grown in sterilized soil having a higher content of shoot K compared to those grown in non-sterilized soil. The effect of genotype × AMF interaction on shoot K was significant ($F = 2.78$, $p = 0.0493$) with the highest increase in shoot K after inoculation observed in

Table 4. The effect of genotype × AMF inoculation interaction on shoot N, K and P nutrition of cowpea. The mean standard errors are presented in parentheses.

Cowpea genotype	AMF In	%N	P (ppm)	K (ppm)
K	M	4.04 (0.072)a	1714.25 (29.74)a	2723.75 (51.90)ab
	NM	2.28 (0.10)cd	1077.75 (39.53)d	2686.38 (74.44)b
KM	M	3.79 (0.10)ab	1545.75 (41.34)b	2802.75 (64.10)a
	NM	1.79 (0.11)d	833.63 (33.76)de	2805.63 (80.58)a
KK	M	3.39 (0.04)b	1372.75 (20.11)c	2513.25 (29.71)c
	NM	1.63 (0.08)d	748.63 (22.94)e	2700.63 (112.54)ab
Wild	M	2.53 (0.05)c	1060.13 (9.89)d	2772.00 (1.67)ab
	NM	1.37 (0.09)e	658.13 (31.10)e	2831.25 (18.93)a

Values followed by the same letter in a column within each treatment are not significantly different at $p < 0.05$ (Tukey's HSD test). K:Katumani 80, KM:Kunde Mboga, KK:KenKunde 1, M:Mycorrhizal, NM:Non-mycorrhizal, AMF In: Arbuscular mycorrhizal fungi inoculation.

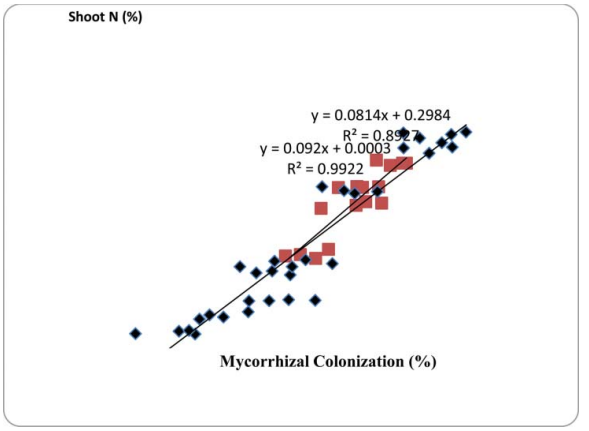

Figure 1. Relationship between AMF root colonization and shoot N (%) in sterilized soil (■) ($r^2 = 0.78$, $y = 0.09x + 0.02$) and non sterilized soil (■) ($r^2 = 0.89$, $y = 0.08x + 0.30$) following AMF inoculation.

Katumani 80 (5.3%). On the contrary, marginal decrease in shoot K content was observed in the other three cultivars (Table 4).

There was a strong positive correlation between root AMF colonization and shoot N in both sterilized and non-sterilized soil ($r^2 = 0.78$, $p < 0.001$ and 0.89, $p < 0.001$, respectively) (Figure 1). An increase in percentage AMF corresponded to increased levels of shoot N and vice versa. Similarly, there was a strong positive correlation between root AMF colonization and shoot P nutrition ($r^2 = 0.73$, $p < 0.001$ and 0.90, $p < 0.001$ in sterilized and non-sterilized soil, respectively) (Figure 2). A high percentage of AMF colonization corresponded to high levels of shoot P. On the contrary, the correlation between root AMF colonization and shoot K content was not significant.

Discussion

AMF responsiveness of cowpea genotypes

In this work, modern cowpea cultivars were more susceptible to AMF inoculation compared to the wild type that accordingly resulted to better root colonization and growth in the modern cultivars. Such findings are consistent to previous studies that have shown variation in AMF colonization among different plant genotypes (Zhu et al. 2001; Tawaraya 2003). In an extensive comparison of several lines of maize, modern hybrids showed a significantly higher percentage mycorrhizal colonization than

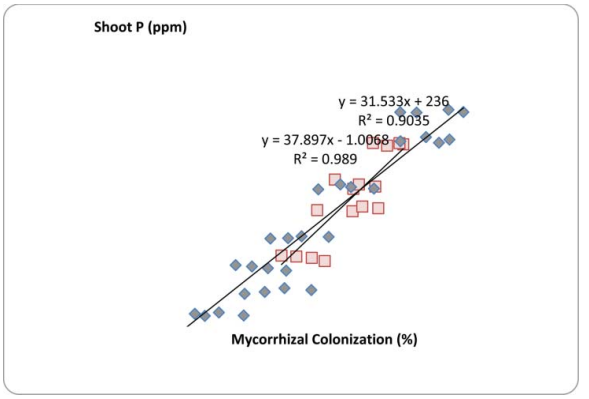

Figure 2. Relationship between AMF root colonization and shoot P (ppm) in sterilized soil (■) ($r^2 = 0.73$, $y = 39.88x + 73.64$) and non sterilized soil (■) ($r^2 = 0.90$, $y = 31.53x + 236.00$) following AMF inoculation.

older landraces and inbred lines (An et al. 2010). A study of mutants of soybean and *Lotus japonicas* revealed accelerated AMF colonization and increased arbuscule formation compared to wild-type plants (Solaiman and Senoo 2005).

In contrast, other studies have shown higher AMF colonization in wild-types. For instance, studies conducted by (Yücel et al. 2009), led to the finding that old wheat cultivars relied more on mycorrhizal symbiosis than the modern wheat cultivars. Hence, old wheat cultivars had higher AMF colonization and infection than the modern cultivars. Besides, Steinkellner et al. (2012) demonstrated varying susceptibility of tomato cultivars to AMF colonization although such differences were not linked to cultivar age.

Genotypic variation to AMF responses may arise from differences in the degree of fine root development (Lebrón et al. 2012). In this case, the wild cowpea cultivar might allocate less photosynthate to AMF, which might limit their ability to grow into the soil, and absorb nutrients. The differences could also be a reflection of the inherent traits of the cultivars, the rhizosphere, or differences in soil nutrients' availability. The plant genotype determines the effect of AMF by influencing AMF development and consequently the AMF populations flourishing in the soil. Some AMF that are good colonizers on other species may even be denied access in some species (Sanders et al. 2003) and fail to reproduce while other crop varieties are highly compatible with AMF, resulting to improved crop nutrient and water-use efficiency as demonstrated by (Singh et al. 2012) in durum wheat. The genetic differences in compatibility of durum wheat with AMF led to notable effects in straw biomass production, straw P, grain P and straw K contents. This differential colonization accounts for the genotypic differences observed with respect to parameters such as nodulation, dry matter and nutrition.

AMF colonization and cowpea growth

Shoot and root dry matter increased significantly across all the cowpea cultivars following inoculation with AMF. Such findings suggest that mycorrhizal inoculation has a positive effect on plant height, leaves and roots, which consequently results to increased dry weight. These results are in line with the findings of (Sharif et al. 2009), which showed that the root and shoot dry matter of wheat increased after inoculation with AMF. The dry matter differences between mycorrhizal and non-mycorrhizal plants were due to beneficial effects derived from mycorrhizal association. Al-Karaki et al. (1998) studied the effects of AMF inoculation on two wheat genotypes and the findings revealed that AMF inoculated genotypes had higher root and shoot dry matter than non-inoculated plants. Similar findings were reported by (Jan et al. 2014), indicating a positive correlation between root dry weights and shoot P in wheat samples inoculated with AMF, results that are consistent with our findings. According to (Mandou et al. 2015), AMF inoculation increases the shoot and root dry matter of micropropagated banana plantlets, which may enhance photosynthesis rate and nutrient uptake. It has been noted that enhanced growth effects on mycorrhizal plants are due to improved water relations, resulting from enhanced P nutrition.

In this study, AMF inoculation increased the nodule number and nodule dry weight in all the cowpea genotypes with mycorrhizal plants having a higher nodule number and dry weight compared to the non-inoculated plants. This is in line with studies that have shown that different AMF species are able to increase nodulation and N fixation. Tajini et al. (2012), found that common bean (*Phaseolus vulgaris* L.) plants inoculated with *Glomus intraradices* and *Rhizobium* had higher number of nodules and higher nodule dry weight than non-inoculated plants. This suggested that combined inoculation of plants with both AMF and rhizobia increases the P use efficiency for symbiotic N fixation. Our results are also consistent to those obtained by Goicoechea et al. (2004) on *Anthylis cytisoides* L., a drought-tolerant legume that can form symbiosis with both mycorrhizal and rhizobial microsymbionts. The study demonstrated that AMF inoculation increased nodulation and N fixation, which in turn improved N nutrition. Similarly, a study done by Huang et al. (2014), on white clover (*Trifolium repens*) found a significant correlation between percentage AMF colonization and number of nodules. Nonetheless, contrary findings were obtained in alfalfa (Catford et al. 2003), whereby colonization of roots with AMF systematically inhibited further mycorrhization as well as nodule formation.

Larimer et al. (2014) investigated the synergistic effects of AMF and rhizobia on growth and nodulation of a prairie legume, *Armorpha canescens*. Strong synergistic effects between both symbionts were found on plant biomass production and nodulation, which were dependent on nutrient level. AMF infection increased root nodule number and mass, while rhizobia inoculation decreased AMF hyphal root colonization. It was further noted that the relative benefits of each combination of symbionts were determined by the P level. P is the key nutrient utilized by legumes for nodulation process. The combinational effect of rhizobia and a mixture of AMF (*Aculospora laevis, Glomus geosporum, Glomus mosseae* and *Scutellospora armeniaca*) was a significant increase in nodulation, N fixation and growth of *Vicia faba* under alkalinity stress (Abd-Alla et al. 2014). In our study, AMF inoculation resulted to increased P uptake, and this could account for increased nodulation.

As expected, AMF inoculation increased shoot N and P across all the cowpea cultivars although this was not reflected on K uptake. Interestingly, shoot nutrient content however varied significantly based on the cultivar type, with wild-type recording the lowest shoot N and P content. Besides, we observed a strong positive correlation between AMF colonization and shoot N and P content. These results are consistent with those obtained by Yaseen et al. (2011), who found out that nutrient uptake in two cowpea varieties inoculated with AMF was higher than that in non-inoculated plants. Our results are also in line with those of (Ghazala 2005; Singh and Gogoi 2012 and Sharma et al. 2013), who reported that mycorrhizal plants had higher nutrient uptake compared to non-mycorrhizal plants.

Other studies that have shown enhanced P nutrition following AMF inoculation have been done on cowpea (Yaseen et al. 2011), maize (Antunes et al. 2009; Miransari 2011; Njeru et al. 2014), tomato (Cavagnaro et al. 2006; Cavagnaro and Martin 2010; Abdel Latef and Chaoxing 2011) and cucumber (Ortas 2010). Although P is critical for plant growth and makes up about 0.2% of dry mass, it is one of the most difficult nutrients for plants to acquire (Habibzadeh 2015). It may be present in relatively large amounts in soil, but much of it is poorly available because of the very low solubility of phosphates of aluminum, iron and Ca, or very low mobility (Ryan et al. 2005). The increase in P uptake is one of the most dramatic effects of mycorrhizal infection on the host plant (Bai et al. 2008), and this is because mycorrhizal fungi have the ability to absorb phosphate from soil and transfer it to the host plant. Our results are further supported by the statement that AMF improve uptake of immobile nutrients such as P and Zn (Balakrishnan and Subramanian 2012). The extensive AMF hyphal network alters the physiochemical properties of soil and directly or indirectly contributes to the release of phosphates from inorganic complexes that have low solubility (Finlay 2008).

The AMF cytoplasm may serve as a host to bacterial endophytes, particularly, plant growth promoting rhizobacteria. Although, there is a need to clarify the role of endophytes living within AMF spores, some evidence has shown that they could be involved in nutrient exchange between the partners (Varennes and Goss 2007). Tripartite symbiosis, which incorporates N fixation by rhizobia, could explain why there was increased N uptake by AMF inoculated plants in the current study. Enhanced N uptake through AMF symbiosis has been shown in maize (*Zea mays* L.) (Miransari 2011), melon plant (*Cucumis melo* L.) (Martínez-Medina et al. 2011) and Long pepper (*Piper longum* L.) (Singh and Gogoi 2012). Nonetheless, contradictory results have been reported by Reynolds et al. (2005), who found that AMF did not enhance N acquisition and growth of old-field perennials under low N supply.

Although limited studies have been done to investigate the role of AMF in K uptake, a few studies have reported increased uptake of the nutrient. Studies done on tomato (*Lycopersicon esculentum*) (Abdel Latef and Chaoxing 2011), maize (*Zea mays*) (Miransari 2011) and melon plant (*Cucumis melo* L.). Martínez-Medina et al. (2011) showed that K uptake was enhanced following AMF inoculation. These results are contrary to those from the current study where there was no significant relationship between K uptake and AMF colonization.

Effect of soil sterilization on AMF colonization and growth

In the current study, soil sterilization had a significant effect on root AMF colonization and different growth parameters. Plants grown in non-sterilized soil not only had higher colonization but they also performed better than those grown in sterilized soil. In this case, the increased growth observed in

unsterilized soil could be associated to AMF colonization since all the pots in had been provided with equivalent soil microbiota except AMF.

These findings are consistent to those obtained by Farzaneh et al. (2009), which showed that AMF colonization level was higher in chickpea that additional inoculation increased colonization in non-sterilized soil. Without sterilization, the soil contained indigenous AMF populations which were able to colonize chickpea, thereby enhancing its performance. This could explain why the cowpea plants that were grown in non-sterilized soil in this study had a higher root colonization, nodulation and nodule dry weight compared to those grown in sterilized soil. Our findings are contrary to those obtained by Nelly and Heung-Kyu (2013), where soil sterilization increased the growth and survival of *Kalopanax septemlobus* microplants during the acclimatization period.

Conclusion

In this study, AMF might improve cowpea production by enhancing the uptake of nutrients, particularly P and N. AMF inoculation had a positive effect on different cowpea growth parameters including nodule number, nodule dry weight and root and shoot dry weights. Thus, the study further revealed that modern cultivars were more responsive to AMF inoculation since they had higher root colonization, dry matter and shoot P and N than the wild-type cultivar. Although modern breeding programs may suppress AMF colonization since plant cultivars are usually bred in nutrient-rich environment, this was not the case in our study. Hence, there is the need to screen different cowpea cultivars for AMF symbiosis, which should be extended to other crops. Considering the differences revealed in terms of responses of different plant genotypes to AMF, as demonstrated in this and other studies, future studies should elucidate appropriate genotype-AMF combinations in order to obtain the optimal benefit from mycorrhizal symbiosis.

Acknowledgment

This work was supported by the International Centre for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya and the Department of Microbiology, Kenyatta University. The authors wish to thank Symyco Inc. and Mycorrhizal Applications, Inc (Grants Pass, Oregon, U.S.) for providing with AMF inoculum.

ORCID

Ezekiel Mugendi Njeru http://orcid.org/0000-0002-9104-808X

References

Abd-Alla, M. H., A. W. El-Enany, N. A. Nafady, D. M. Khalaf, and F. M. Morsy. 2014. Synergistic Interaction of Rhizobium leguminosarum Bv. viciae and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (Vicia faba L.) in alkaline soil. *Microbiological Research* 169:49–58. doi:10.1016/j.micres.2013.07.007.

Abdel Latef, A. A., and H. Chaoxing. 2011. Effect of arbuscular mycorrhizal fungi on growth,mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. *Science Horticulture* 127:228–33. doi:10.1016/j.scienta.2010.09.020.

Al-Karaki, G. N., A. Al-Raddad, and R. B. Clark. 1998. Water stress and mycorrhizal isolate effects on growth and nutrient acquisition of wheat. *Journal of Plant Nutrition* 21:891–902. doi:10.1080/01904169809365451.

An, G. H., S. Kobayashi, H. Enoki, K. Sonobe, M. Muraki, T. Karasawa, and T. Ezawa. 2010. How does arbuscular mycorrhizal colonization vary with host plant genotype? *An Example Based on Maize (Zea mays) Germplasms. Plant Soil. Plant and Soil* 327:441–53.

Antunes, P., A. Koch, K. Dunfield, M. Hart, A. Downing, M. Rillig, and J. Klironomos. 2009. Influence of commercial inoculation with glomus intraradices on the structure and functioning of an AM fungal community from an agricultural site. *Plant and Soil* 317:257–66. doi:10.1007/s11104-008-9806-y.

Bai, J. F., X. G. Lin, R. Yin, H. Y. Zhang, J. H. Wang, X. M. Chen, and Y. M. Luo. 2008. The influence of arbuscular mycorrhizal fungi on As and P uptake by maize (*Zea mays* L.) from As contaminated soils. *Applied Soil Ecology* 38:137–45. doi:10.1016/j.apsoil.2007.10.002.

Balakrishnan, N., and K. S. Subramanian. 2012. Mycorrhizal symbiosis and bioavailability of micronutrients in maize grain. *Maydica* 57:129–38.

Barrow, J. R., M. E. Lucero, I. Reyes-Vera, and K. M. Havstad. 2008. Do symbiotic microbes have a role in plant evolution, performance and response to stress? *Communicative & Integrative Biology* 1:69–73. doi:10.4161/cib.1.1.6238.

Bremner, J. M. 1982. Inorganic Nitrogen. In: *Methods of soil analysis part 2: Chemical and mineralogical properties*, eds. A. L. Page, R. H. Miller, and D. R. Keeney, Agronomy Monograph No. 9 ASA-SSSA Madison.

Catford, J. G., C. Staehelin, S. Lerat, Y. Piché, and H. Vierheilig. 2003. Suppression of arbuscular mycorrhizal colonization and nodulation in split-root systems of alfalfa after pre-inoculation and treatment with nod factors. *Journal of Experimental Botany* 54:1481–87. doi:10.1093/jxb/erg156.

Cavagnaro, T. R., L. E. Jackson, J. Six, H. Ferris, S. Goyal, D. Asami, and K. M. Scow. 2006. Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. *Plant and Soil* 282:209–25. doi:10.1007/s11104-005-5847-7.

Cavagnaro, T. R, and A. W Martin. 2010. The role of mycorrhizas in plant nutrition: Field and mutant based approaches. *Plant and Soil* 287:148–51.

FAO. 2013. http://faostat.fao.org/site/567/.

Farzaneh, M., S. Wichmann, H. Vierheilig, and H.-P. Kaul. 2009. The effects of arbuscular mycorrhiza and nitrogen nutrition on growth of chickpea and barley. *German Journal of Agronomy* 13(1):15–22.

Finlay, R. D. 2008. Ecological aspects of mycorrhizal symbiosis: With special emphasis on the functional diversity of interactions involving the extraradical mycelium. *Journal of Experimental Botany* 59:1115–26. doi:10.1093/jxb/ern059.

Ghazala, N. 2005. Role of symbiotic soil fungi in controlling roadside erosion and in the establishment of plant communities. *Caderno Pesquisa Serie Biologia* 17:119–36.

Giovannetti, M., and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 84:489–500. doi:10.1111/j.1469-8137.1980.tb04556.x.

Giovannetti, M., L. Avio, R. Barale, N. Ceccarelli, R. Cristofani, A. Iezzi, F. Mignolli, P. Picciarelli, B. Pinto, D. Reali, C. Sbrana, and R. Scarpato. 2012. Nutraceutical value and safety of tomato fruits produced by mycorrhizal plants. *British Journal of Nutrition* 107:242–51. doi:10.1017/S000711451100290X.

Goicoechea, N., S. Merino, and M. Sańchez-Dıaz. 2004. Contribution of arbuscular mycorrhizal fungi (AMF) to the adaptations exhibited by the deciduous shrub anthyllis cytisoides under water deficit. *Physiologia Plantarum* 122:453–64. doi:10.1111/j.1399-3054.2004.00421.x.

Habibzadeh, Y. 2015. The effects of arbuscular mycorrhizal fungi and phosphorus levels on dry matter production and root traits in cucumber (*Cucumis sativus* L.). *African Journal of Environmental Science and Technology* 9:67–70.

Huang, Y. Z., M. Zhong, W. Wu, L. H. Sui, C. Zhang, and X. W. Hao. 2014. Effects of arbuscular mycorrhizal fungi isolated from white clovers (*Trifolium repens* L.) on soil bacteria and fungi. *Chemistry and Ecology* 30. doi:10.1080/02757540.2013.841892.

Jan, B., M. Sharif, F. Khan, and J. Bakht. 2014. Effect of arbuscular mycorrhiza fungal inoculation with compost on yield and P uptake of wheat in alkaline calcareous soil. *American Journal of Plant Sciences* 5:1995–2004. doi:10.4236/ajps.2014.513213.

Koohafkan, P, and M. A. Altieri. 2010. *Globally important agricultural heritage systems: A legacy for the future*. Rome: UN-FAO.

Larimer, A. L., K. Clay, and J. D. Bever. 2014. Synergism and context dependency of interactions between arbuscular mycorrhizal fungi and rhizobia with a prairie legume. *Ecology* 95:1045–54. doi:10.1890/13-0025.1.

Lebrón, L, D. J. Lodge, and P. Bayman. 2012. Differences in arbuscular mycorrhizal fungi among three coffee cultivars in puerto rico. *Agronomy* 2012:53–78.

Mandou, M. S., A. M. Ze, F. Etoa, and S. Declerck. 2015. Effects of extraradical mycelium network of an arbuscular mycorrhizal fungus on the growth of banana plantlets. *Journal of Plant Biology Research* 4:22–32.

Martínez-Medina, A., A. Roldán, and J. A. Pascual. 2011. Interaction between arbuscular mycorrhizal fungi and *trichoderma harzianum* under conventional and low input fertilization field condition in melon crops: Growth response and fusarium wilt biocontrol. *Applied Soil Ecology* 47:98–105. doi:10.1016/j.apsoil.2010.11.010.

Miransari, M. 2011. Arbuscular mycorrhizal fungi and nitrogen uptake. *Archives of Microbiology* 193:77–81. doi:10.1007/s00203-010-0657-6.

Nelly, A., and M. Heung-Kyu. 2013. The effects of soil sterilization, mycorrhizal inoculation, and rates of phosphorus on growth and survival of *kalopanax septemlobus* microplants during the acclimatization period. *Plant Biotechnol Reports* 7:71–82.

Njeru, E. M., L. Avio, G. Bocci, C. Sbrana, A. Turrini, P. Bàrberi, M. Giovannetti, and F. Oehl. 2014. Contrasting effects of cover crops on 'hot spot' arbuscular mycorrhizal fungal communities in organic tomato. *Biology and Fertility of Soils* 51:151–66. doi:10.1007/s00374-014-0958-z.

Njeru, E. M., L. Avio, C. Sbrana, A. Turrini, G. Bocci, P. Bàrberi, and M. Giovannetti. 2013. First evidence for a major cover crop effect on arbuscular mycorrhizal fungi and organic maize growth. *Agronomy for Sustainable Development* 34:841–48. doi:10.1007/s13593-013-0197-y.

Ortas, I. 2010. Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions. *Spanish Journal of Agricultural Research* 8:116–22. doi:10.5424/sjar/201008S1-1230.

Oruru, M. B., and E. M. Njeru. 2016. Upscaling arbuscular mycorrhizal symbiosis and related agroecosystems services in smallholder farming systems. *BioMed Research International* 2016:12. doi:10.1155/2016/4376240.
Page, A. L., R. H. Miller, and D. R. Keeney. 1982. *Methods of soil analysis part 2: Chemical and mineralogical properties*. Wisconsin: Agronomy Monograph No. 9 ASA-SSSA Madison.
Reynolds, H. L., A. E. Hartley, K. M. Vogelsang, J. D. Bever, and P. A. Schultz. 2005. Arbuscular mycorrhizal fungi do not enhance nitrogen acquisition and growth of old-field perennials under low nitrogen supply in glasshouse culture. *New Phytologist* 167:869–80. doi:10.1111/j.1469-8137.2005.01455.x.
Ryan, M. H., A. F. van Herwaarden, J. F. Angus, and J. A. Kirkegaard. 2005. Reduced growth of autumn-sown wheat in a low-P soil is associated with high colonization by arbuscular mycorrhizal fungi. *Plant and Soil* 270:275–86. doi:10.1007/s11104-004-1611-7.
Sanders, I. R., A. Koch, and G. Kuhn. 2003. Arbuscular mycorrhizal fungi: Genetics of multigenomic, clonal networks and its ecological consequences. *Biological Journal of the Linnean Society* 79:59–60. doi:10.1046/j.1095-8312.2003.00187.x.
Sharif, M., M. S. Sarir, J. Bakht, S. Saud, A. Ali, and M. Ahmad. 2009. Response of wheat to the inoculation of arbuscular mycorrhizal fungi in salt affected soil. *Sarhad Journal of Agriculture* 25:209–16.
Sharma, S. B., R. Z. Sayyed, M. H. Trivedi, and T. A. Gobi. 2013. Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus* 2:587. doi:10.1186/2193-1801-2-587.
Shockley, F. W., R. L. McGraw, and H. E. Garrett. 2004. Growth and nutrient concentration of two native forage legumes inoculated with rhizobium and mycorrhiza in Missouri, USA. *Agroforest Systems* 60:137–42. doi:10.1023/B:AGFO.0000013269.19284.53.
Singh, A. K., C. Hamel, R. M. Depauw, and R. E. Knox. 2012. Genetic variability in arbuscular mycorrhizal fungi compatibility supports the selection of durum wheat genotypes for enhancing soil ecological services and cropping systems in Canada. *Canadian Journal of Microbiology* 58:293–302. doi:10.1139/w11-140.
Singh, B, and H. A. Ajeigbe. 2003. Improving the production and utilization of cowpea as food and fodder. *Field Crops Research* 84:169–77. doi:10.1016/S0378-4290(03)00148-5.
Singh, R. K., and P. Gogoi. 2012. Augmented growth of long pepper in response to arbuscular mycorrhizal inoculation. *Journal of Forestry Research* 23:339–44. doi:10.1007/s11676-012-0262-4.
Solaiman, Z., and K. Senoo. 2005. Interactions between *Lotus japonicus* genotypes and arbuscular mycorrhizal fungi. *Journal of Plant Interactions* 1:179–86. doi:10.1080/17429140601010151.
Steinkellner, S., K. Hage-Ahmed, J. M. García-Garrido, A. Illana, J. A. Ocampo, and H. Vierheilig. 2012. A comparison of wild-type, old and modern tomato cultivars in the interaction with the arbuscular mycorrhizal fungus *glomus mosseae* and the tomato pathogen *fusarium oxysporum* F. Sp. *Lycopersici. Mycorrhiza* 22:189–94. doi:10.1007/s00572-011-0393-z.
Tajini, F., M. Trabelsi, and J. Drevon. 2012. Combined inoculation with *glomus intraradices* and *rhizobium tropici* CIAT899 increases phosphorus use efficiency for symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.). *Saudi Journal of Biological Sciences* 19:157–63. doi:10.1016/j.sjbs.2011.11.003.
Tarawaya, K. 2003. Arbuscular mycorrhizal dependency of sifferent plant species and cultivars. *Journal of Soil Science and Plant Nutrition* 49:655–68. doi:10.1080/00380768.2003.10410323.
Turk, M. A., T. A. Assaf, K. M. Hammed, and A. M. Al-Tawahi. 2008. Significance of mycorrhizae. *World Journal of Agricultural Sciences* 2:16–20.
Varennes, A., and M. J. Goss. 2007. The tripartite symbiosis between legumes, rhizobia and indigenous mycorrhizal fungi is more efficient in undisturbed soil. *Soil Biology and Biochemistry* 39:2603–7. doi:10.1016/j.soilbio.2007.05.007.
Xavier, L. J. C., and J. J. Germida. 2002. Response of lentil under controlled conditions to co-inoculation with arbuscular mycorrhizal fungi and rhizobia varying in efficiency. *Soil Biology and Biochemistry* 34:181–88. doi:10.1016/S0038-0717(01)00165-1.
Yaseen, T., T. Burni, and F. Hussain. 2011. Effect of arbuscular mycorrhizal inoculation on nutrient uptake, growth and productivity of cowpea (*Vigna unguiculata*) varieties. *African Journal of Biotechnology* 10(43):8593–98. doi:10.5897/AJB10.1494.
Yücel, C., H. Ozkan, 1. Ortas, and T. Yagbasanlar. 2009. Screening of wild emmer wheat accessions (*Triticum turgidum* Subsp Dicoccoides) for mycorrhizal dependency. *Turkish Journal of Agriculture and Forestry* 33:513–23.
Zhu, Y. G., S. E. Smith, A. R. Barritt, and F. A. Smith. 2001. Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars. *Plant and Soil* 237:249–55. doi:10.1023/A:1013343811110.
Zhu, Y.-G., F A. Smith, and S. E Smith. 2003. Phosphorus efficiencies and responses of barley (*Hordeum vulgare* L.) to arbuscular mycorrhizal fungi grown in highly calcareous soil. *Mycorrhiza* 13:93–100. doi:10.1007/s00572-002-0205-6.