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Full Length Research Paper

The effect of intercropping *Sclerocarya birrea* (A. Rich.) Hochst., millet and corn in the presence of arbuscular mycorrhizal fungi

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***Sclerocarya birrea* (A. Rich.) Hochst. (marula) is native to Africa occurring in the semi-arid, deciduous savannas of much of sub-Saharan Africa. It has multiple uses, including the fruits, kernels, oil, bark, wood and leaves which make it a key species to support the development of rural enterprises. Enhancing positive interactions between marula and other crops is key to successful introduction of marula into the farming systems in the arid and semiarid areas of Africa. The objective of the study was to determine the influence of various combinations of marula, *Pennisetum glaucum* (L.) R. Br. (millet) and *Zea mays* (corn) with one another when inoculated with arbuscular mycorrhizal (AM) fungi. A three-chambered acrylic root boxes were used. One outer chamber contained seedlings of *S. birrea* while the other contained millet or corn or bare soil. The central chamber was either inoculated with an AM fungus (*Gigaspora margarita* Baker and Hall) or uninoculated. Inoculation in the presence of the two crops enhanced both biomass production and height growth of marula seedlings. Both hyphal density and number of spores in marula compartments were increased under intercropping system compared to marula monoculture. The study demonstrated that intercropping marula with millet or corn could help in the propagation of AM fungi spores in the soil which would enhance marula establishment especially in soil with low phosphorous and moisture scarcity.**

Key words: Arbuscular mycorrhizal, corn, intercropping, millet, *Sclerocarya birrea*.

INTRODUCTION

Sclerocarya birrea (A. Rich.) Hochst. (marula) is a widespread species throughout the semi-arid, deciduous savannas of much of sub-Saharan Africa occupying 29 countries. It is widely used by rural populations in most countries in which it is found. It has multiple uses, including the fruits, kernels, oil, bark, wood and leaves. Wine made from the fruits is sold commercially in the international market. Because of the widespread occurrence, potentially high fruit production and uses, marula has potential to provide food security and to alleviate poverty and malnutrition in dry land Africa. It has frequently been identified as a key species to support the development of rural enterprises based and therefore as a species for potential domestication.

Increasing human activities and livestock grazing in the arid and semiarid areas has put a pressure on the fragile ecosystem leading to land degradation and loss of genetic resources. Marula is particularly, vulnerable due to its dioecious nature. The importance of conserving marula has been recognized over the past decade. One way to conserve the species is by integrating it into the local farming systems. Leakey and Newton (1994) noted that the integration and evaluation of indigenous trees is necessary to protect biological diversity and provide an opportunity for rural communities to have adequate food. For the species to be successfully integrated into the farming systems, they need to fit into the system with minimum disruption to the local agro-eco system. Understanding the ecological and economic interaction between the various components of the agro forestry system is therefore of utmost importance.

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Intercropping is an age-old practice of crop production in tropical agriculture. Scientific investigations to evaluate this system have reported many advantages associated with intercropping (Burner, 2003; Hazra and Saha, 2000). For example, introduction of intercropping may benefit the plants by improving soil texture, preventing soil erosion, promoting better water penetration, and supplying organic matter as well as propagation of some symbiotic microorganisms such as indigenous arbuscular mycorrhizal fungi in the soil. Association between soil microorganisms such as arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi are important component of agro forestry systems but the interactions involved are poorly understood.

Benefits of AM fungi to plants includes enhanced nutrient uptake, especially phosphorus (P) and Zinc (Zn) has been reported (Al-Karaki, 2000; Bolan, 1991; Kahiluoto and Vestberg, 1998). In addition, formation of mycorrhizal roots enables plants to obtain more moisture from the surrounding soil than non-mycorrhizal plants (Stahl et al., 1998). Due to adverse factors such as low soil moisture and low vegetation cover that prevails in soils of arid and semi-arid areas of the tropics the soils have natural population of AM fungi. Ishii (2000) found natural populations of AM fungi in Kenyan soils were less than 200 spores/25 g soil as compared to about 1000 spores/25 g soil in soils collected in Japan. The main species of these spores collected in Kenyan soils were *Acaulospora* sp., *Gigaspora margarita*, *Glomus caledonium*, *Glomus etunicatum* and *Glomus fasciculatum* (Ishii, 2000). For full benefits of AM fungi to be realized, there needs to maintain high and viable natural populations of AM fungi in the soil to increase the chances of root colonization. Douds and Schenck (1991) noted that most soils are not conducive to keeping AM fungi viable without host plants, because AM fungi are obligate symbionts.

Intercropping is known to have the potential to keep high and viable natural population of AM fungi in soils because of the higher diversity of plants involved. Harinikumar et al. (1990) reported that intercropping system between maize and soybean stimulated proliferation of AM fungi as compared to a monoculture system. Intercropping in fruit orchard of *Citrus reticulata* (satsuma mandarin) and sod culture using *Paspalum notatum* Flugge (bahiagrass) improved mycorrhizal colonization in the roots of satsuma mandarin as compared to monoculture culture (Ishii et al., 1996).

Under intercropping systems, it has been reported that AM hyphae distributed in soils may connect two or more different plant species promoting a network system among the plants. This network made by AM interconnections may bring benefits to the plant such as extension of root longevity (Tommerup and Abbott, 1981) and also provide channels to allow nutrient transfer between plants (Martins and Cruz, 1997; Xiaolin and Zhang, 1997). Establishment of AM symbiosis with host plants is complex interaction which involves both physical and biochemical changes which acts as stimulators (Gemma and Koske, 1988). Ishii et al. (1997) identified stimulatory compounds in bahia grass roots such as eupalitin, a flavonoid. Gianinazzi-Pearson et al. (1989) reported that some root exudates of AM host plants increased *in vitro* spore germination and hyphal growth of *G. margarita* compared to those of non-AM host plants. Cruz et al. (2002) demonstrated that root exudates of *Poncirus trifoliata* (trifoliate orange), bahia grass, and *Pennisetum glaucum* L. R. Br. (millet) stimulated the hyphal growth of *G. margarita in vitro*.

Millets has been one of the important staples in the semi-arid tropics of Asia and Africa for centuries. These crops are still the principal sources of energy, protein, vitamins and minerals for millions of the poorest people in these regions (Codex Alimentarius Commission, 1990). Millets are grown in harsh environments where other crops grow or yield poorly. They are grown with limited water resources and usually without application of any fertilizers.

Marula is emerging as one of the important indigenous fruit tree species for introduction into the dry land agro forestry systems of Africa (Muok et al., 2000; Mulilo, 2004). Where it naturally occurs the tree is found left standing in the cropland in the vast fields of Africa. At the moment no information is available on the integration of marula with other crops in agro forestry systems when inoculated with arbuscular mycorrhizal hyphae. The goal was to determine the influence various combinations of marula, millet and corn have on one another when planted in a three chambered pot configuration and inoculated with AM fungi. The study is based on the hypothesis that inoculation enhances growth marula, millet and *Zea mays* (corn) under intercropping system

MATERIALS AND METHODS

Acrylic root boxes were constructed and divided into three compartments. Each of the two outer compartments was 3 cm wide, 45 cm long and 15 cm deep. The middle small compartment measured 3 cm wide, 5 cm long and 15 cm deep (Figure 1). The small middle compartment were separated from the two outer compartments, one on each side, by a barrier made of a nylon screen of 37 µm mesh that allows the AM hyphae to penetrate but not the plant roots. The boxes were covered with aluminum foil to block the light and prevent the formation of algae. Each compartment was filled with sterile fine sand.

Fruits of marula were collected using random sampling techniques from four locations in Kenya (Kitui, Baringo, Mbeere, Nyanza). Freshly collected seeds were extracted after which equal samples of seed were combined to give the bulk population sample from which sub samples were taken for germination. Before sowing, seed were surface sterilized in 10% sodium hypochlorite solution for 30 min and the operculum, which covers the embryo removed before sowing. Seed were germinated in pots containing sterile vermiculite in a growth chamber. A month later, healthy looking seedlings of uniform height (average 12 cm) were transferred to a green house and transplanted according to the pre-determined experimental design in acrylic root boxes. Millet and corn were sur-

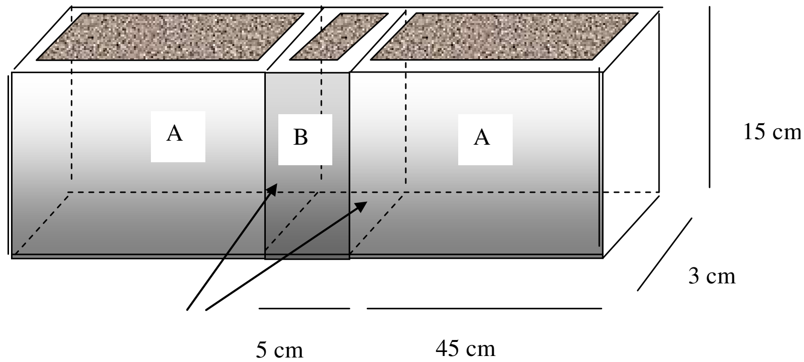

Figure 1. A 3-chamber acrylic root box. A: Compartment containing seedlings one of the three intercropping plant seedlings or bare sand; B: Compartment is either inoculated, with AMF spores or uninoculated. Arrows: nylon screen.

Table 1. Effect of intercropping marula with millet and corn, when planted in a 3- chambered pot configuration and inoculated with AM fungi, on total biomass and height of marula seedlings.

AM fungi combination	Total dry weight (g)	Height (cm)
Bare-AM[z]	20.7±1.1a[y]	33.7± 1.6a
Bare+AM[x]	26.9±1.7b	40.9± 2.3a
Millet-AM	16.9±1.3a	35.2± 2.5a
Millet+AM	32.5±0.8c	42.3± 3.1a
Corn-AM	17.6±0.5a	35.9± 3.1a
Corn+AM	29.3±0.4bc	40.1± 2.8a

[z]Uninoculated.
[x]inoculated.
[y]Mean±standard error (SE) (n = 4).
Numbers in the same column followed by the same letters are not significantly different using Dancan's multiple range test P<0.5.

face sterilized by dipping in 5% sodium hypochlorite solution for 5 min and pre-germinated in sterile vermiculite two weeks before the start of the experiment. Sand was sterilized by autoclaving and root boxes were filled with sterilized sand before transplanting. Root boxes filled with sand were watered to field capacity just before transplanting was done.

There were 12 treatments marula monoculture, marula with millet intercrop, marula with corn intercrop, millet monoculture, millet with corn intercrop and corn monoculture. Each of the treatment set was inoculated or uninoculated sets. Four replicates were included per treatment. In the monoculture treatments only one compartment contained plants while the opposite side was bare soil. A week after transplanting, inoculation was done with 5 g of inoculum's (approximately 250 spores of *G. margarita*) (Central Glass Co. Ltd, Tokyo, Japan). Number of spores was determined according to Ishii et al. (1996). Inoculation was done by spreading a thin layer of the inoculums on the sand surface and watered lightly. The boxes were shuffled weekly at random to reduce biasness which could be caused by angle of the sun light reaching the seedlings.

The experiment was maintained for three months during which time regular watering was done. Each seedling in the experiment was drenched once a week with Hoagland's nutrient solution (Millner and Kitt, 1992) modified by halving the concentrations of P and Zn at a rate of 100 ml per plant. Watering was done daily using tap water. The study was conducted in a greenhouse at Kyoto Prefectural University, Japan in summer (June to August). The greenhouse was maintained under natural conditions with normal day length and no air conditioner.

At the termination of the experiments, plant height (from the sand surface to the tallest tip of the terminal shoot), total dry weight (as sum of shoot and root dry weight) was recorded. Aluminum foil cover around the root boxes were removed and hyphal density determined as an average of six random observation on an area of 12.5 x 7.5 mm in each compartment using a camera with a 0.5 inch, 900,000 pixel Charge Coupled Device (CCD) image sensor (Keyence VH-6300, Osaka, Japan). On a computer screen, this area was divided into 192 squares and the density of hyphae calculated according to the following equation: Density of hyphae (%) = (Number of squares with hyphae / total number of squares (192)) x 100 (Cruz et al., 2002). Shoots were severed from the roots and fresh weights of shoots and roots were recorded. Roots were rinsed and samples taken for estimation of AM root colonization according to Ishii and Kadoya (1994), while number of spores were determined according to Ishii et al. (1996). Root samples for SEM were prepared according to (Ishii and Kadoya, 1984) and observed by a scanning electron microscope (SEM) (Nihon Denshi JXA-840, JEOL, Tokyo). Root catalase and SOD activities were analyzed by the methods of Aebi (1974) and McCord and Fridorich (1969), respectively. Total protein content in the roots was measured according to the procedure of Lowry et al. (1951). Collected data was subjected statistical analysis using the analysis of variance (ANOVA) procedure and differences between the mean determined by Duncan's multiple range test (DMRT) at 95% significant level.

RESULTS

As shown in Table 1, mycorrhizal marula seedlings recorded significantly higher total biomass production than non mycorrhizal seedlings. Inoculated marula seedlings under millet intercrop recorded higher total biomass production as compared to seedlings under monoculture. There was no significant difference between mycorrhizal marula seedlings under millet and corn intercrop. Height growth in marula seedlings were not affected by inoculation.

Both millet and corn seedlings showed improved total biomass production due to inoculation. There was no difference in total biomass or height growth attributed to the type of the intercrop species in both millet and corn

Table 2. Effect of intercropping millet with marula and corn, when planted in a 3- chambered pot configuration and inoculated with AM fungi, on total biomass and height of millet seedlings.

AM fungi combination	Total dry weight(g)	Height (cm)
Bare-AM	50.8±3.2a^y	83.8± 2.7a
Bare+AM	66.0±2.4b	89.1± 4.4a
Marula-AM	49.2±4.1a	82.3± 2.8a
Marula+AM	64.0±3.6b	86.5± 2.3a
Corn-AM	47.9±4.1a	81.8± 2.7a
Corn+AM	62.7±3.2b	84.5± 2.1a

^{z}Uninoculated.
^{x}inoculated.
^{y}Mean±SE (n = 4).
Numbers in the same column followed by the same letters are not significantly different using DMRT P<0.5.

Table 3. Effect of intercropping corn with marula and millet, when planted in a 3- chambered pot configuration and inoculated with AM fungi, on total biomass and height of corn seedlings.

AM fungi combination	Total dry weight (g)	Height (cm)
Bare-AM^z	43.3±1.7a^y	52.7± 3.8a
Bare+AM^x	48.9±1.0b	58.2± 4.0a
Marula-AM	43.9±2.2a	51.9± 4.2a
Marula+AM	45.0±1.9b	57.0± 2.3a
Millet-AM	43.2±1.3a	51.7± 3.2a
Millet+AM	46.2±1.3b	56.0± 2.4a

^{z}Uninoculated.
^{x}inoculated.
^{y}Mean±SE (n=4).
Numbers in the same column followed by the same letters are not significantly different using DMRT P<0.5.

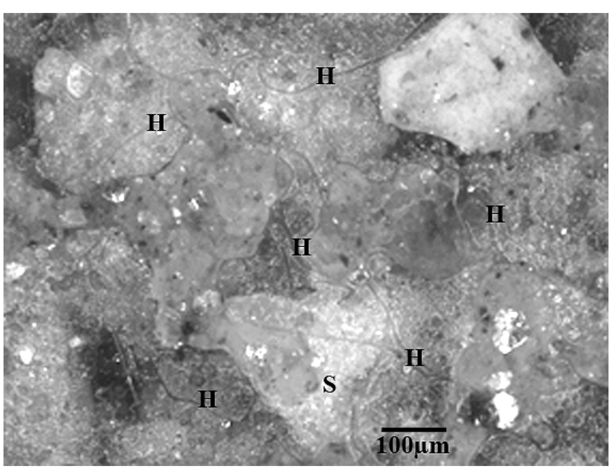

Figure 2. A CCD image of AM hyphal growth in the compartment of marula with millet. H: hypha, S: spore.

(Tables 2 and 3). Comparison of non-mycorrhizal marula seedlings showed that marula monoculture had slightly higher biomass production than non-mycorrhizal seedlings with millet or corn intercrops but the difference was not significant.

Observation of the various compartments showed presence of AM hyphae in the compartments of millet, corn and marula and no hyphae in bare compartments (Figure 2 and Table 4). Marula compartment under both intercrops recorded significantly higher hyphal densities compared to compartments under monoculture. There was no significant difference between hyphal densities of marula compartment under millet and corn intercrop. Comparison of the compartments containing the two crops under marula intercrop showed that the compartments containing millet had higher hyphal density than those containing corn (Table 4).

As shown in Table 4, marula seedlings under the two intercrops (millet and corn) recorded higher root colonization compared to marula monoculture. Between the two intercrops, marula seedlings growing with millet intercrop recorded higher root colonization (53.4%) compared to those under corn intercrop with recorded 49.5% root colonization rate. Millet compartments recorded the highest root colonization rate. No significant difference was recorded in the colonization rate of millet under intercropping system (73.3%) and monoculture (72.4%). Corn compartments also did not show any significant difference in rate of root colonization under intercrop and monoculture.

DISCUSSION

Marula trees and products have long formed an integral part of the lives, food security and spirituality of indigenous communities living within the distribution of this highly valued and versatile species (Fox and Norwood-Young, 1983; Shackelton et al., 2000). Indeed, archaeological evidence indicates that this species has been used from the earliest of times (Shone, 1979). Increased human activities, frequent drought due to climatic change as well as over grazing by livestock has lead to decline of the marula populations in the natural habitat. This not only affect the ecosystem but also humans and animals that has been depending on the species over the years. To enhance sustainable production of the two species, there needs an understanding of the basic silvicultural requirements as well as interaction with other crops in the farming system.

Presence of AM hyphae and spores in the outer compartments showed that AM fungi had grown from the central compartments to outer compartments containing millet, corn or marula. The results demonstrated that inoculation in the presence of millet or corn intercrop enhances the benefits of AM inoculation to marula seedlings. The effect of millet and corn intercrop seems to be related to the ability of the two crops to induce mul-

Table 4. Influence of various combinations of marula, millet and corn on hyphal density, number of spores and root colonization when planted in a 3-chambered pot configuration and inoculated with AM fungi.

Intercrop system			Hyphal density (%)	No. of spores/25 g soil	Root colonization (%)
Marula intercrop system					
	Marula mono.	Marula comp.	37.8±1.5a[y]	84.5 ± 6.4a	37.2±1.6a
	With millet	Marula comp.	51.8±1.5b	166.6 ± 4.9c	53.4±1.2c
		Millet comp.	67.8±1.2d	212.2 ± 1.7d	73.3±1.3e
	With corn	Marula comp.	50.0±1.8b	144.2±16.2b	49.5±1.0b
		Corn comp.	60.7±1.0c	193.5 ± 3.2d	67.6±1.2d
Millet intercrop system					
	Millet mono.	Millet comp.	67.2±0.9d	202.6 ± 4.4d	72.4±1.1e
	With corn	Millet comp.	68.2±1.7d	214.7 ± 5.2d	73.8±1.2e
		Corn comp.	62.7±1.3c	198.1 ± 4.0d	68.2±1.0d
Corn intercrop system					
	Corn mono	Corn comp.	61.8±1.6c	193.7 ± 1.5d	68.3±0.6d

[y]Mean ± SE (n = 4).
Mono: Monoculture.
Comp: Compartment.
Numbers in the same column followed by the same letters are not significantly different using DMRT P<0.5.

tiplication of AM spores in the soil resulting in higher marula root colonization. This was demonstrated by the fact that marula compartments with both millet and corn intercrops had higher number of spores, hyphal density and root colonization than marula monoculture. No record was available on the marula, millet and corn intercropping to support our view but Cruz et al. (2002) reported that root exudates of millet stimulate the hyphal growth of *G. margarita in vitro*. Another possibility could be that increased hyphal density in marula intercropping treatments due to higher root density in the intercropping treatments than in the monoculture treatments. The reason corn and millet did not show an increase in colonization with intercropping, while marula did, could be because corn and millet form inherently vigorous associations with *G. margarita* while marula appears to form only a weak mycorrhizal association. The results show that having two fast growing, strongly mycorrhizal species along with the relatively slow growing, weakly mycorrhizal marula increases hyphal and spore proliferation through out the 3-chambered system, promoting increased colonization in the marula side chamber.

Since the compartments were separated with a nylon screen, which plant roots could not penetrate, mycorrhizal hyphae formed the only link between the crops and marula roots. Previous study of other plants has demonstrated that the movement of the hyphae between the compartments provides channels to allow nutrient transfer between plants (Martins and Cruz, 1997; Xiaolin and Zhang, 1997). Compared with corn, millet is more drought tolerant crops. In the poor soils of the tropics, millet is used to multiply AMF spores for bulking up crops seeds. Millet is widely cultivated under traditional agro forestry systems in the arid and semi arid regions of Africa where it is grown under *Acacia albida* (*Faidherbia albida*) trees (Pearson et al., 1995). *A. albida* being N-fixing tree, the system has mainly focused on the benefits of nitrogen fixed by *A. albida* to the companion crops and little attention given to the benefits of millet or other companion crops to the tree component.

It has been noted that the current intense exploitation of natural forests in the sub humid to arid tropics is leading to degradation of stable ecosystems. The resulting changes in abiotic and biotic soil properties hamper the reestablishment of vegetation. The biotic changes include a decrease in the density AM spores (Michelsen and Rosendahl, 1990). The observed ability of millet to induce propagation of AM spores in the rhizosphere around the roots demonstrated the potential benefit of the companion crops to the tree at least in the early stages of development. In arid and semi arid areas, the early stages of tree establishment are the most critical stage which determines the success of afforestation. Multiplication of AM spores around the root region enhanced AM root colonization in trees thus improving the benefits of AM fungi in trees. It is our hypothesis that given the drought tolerance of millet and the observed benefit of millet – marula intercrop, if drought is anticipated millet-marula system is likely to survive better than marula monoculture.

In the current study there was no indication that marula benefited the companion crops in terms of AM root infection but it showed that marula seedlings had no adverse effect on the growth of millet or corn in the first two months of planting. In the early stages of tree establishment, intercropping is a common practice. The fact that marula seedlings did not negatively affect the growth performance of millet and corn could be due to the fact that in the first two months marula seedlings are still not big enough to out-compete the crops for the growth factors. In the areas where it naturally occurs, it is common among farmers to leave marula standing on the croplands during clearing for cultivation (Maundu et al., 1999). This could be an indication that marula has little negative effect on crops or the benefits of marula outweigh the negative effect it may be having on the crops. As it has been recorded world over, rural people selectively clear woodland when preparing land for cultivation (Rackhan, 1989). The criterion is based on the relative importance attached to the tree species.

The results demonstrate that intercropping marula with millet or corn could help in the propagation of AM fungi spores in the soil which would benefit marula. AM fungi are known to enhance tree establishment in environmental stress conditions such as water stress and salt stress. The study would provide valuable information needed in introducing marula in the farming systems of the arid and semiarid areas of Africa. Further field studies is required to determine the mechanism by which millet and corn seem to encourage propagation of AM spores in the soil as well as the role played by the hyphae which formed a network between the various compartments.

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