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Original Article

EFFECTS OF VARYING CONCENTRATION OF *GLOMUS CLARUM* ON ROOT BIOMASS AND ROOT NODULES OF FOUR NIGERIAN COWPEA [*Vigna unguiculata* L (WALP)] VARIETIES

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ABSTRACT

The effects of different concentration of *Glomus clarum*, an Arbuscular Mycorrhiza Fungus (AMF), on the root biomass and root nodules of cowpea [*Vigna unguiculata* L (Walp)] varieties were investigated. Four varieties of cowpea, i.e. B301, SAMPEA 7, SAMPEA 10 and IAR-1074 were used for the study. Sterilized soil inoculated with varying concentration (0, 10, 20 and 30g/bag) of *G. clarum* was used for planting of the seeds. The root biomass (RB) and root nodules (RN) of each treatment combination were assessed. The varieties B301 and SAMPEA 10 showed quicker response to *G. clarum* at 4, 6 and 8 weeks after planting (WAP). High root nodules and root biomass were obtained in all the varieties at 8 WAP. At week 8 for example, IAR-1074 and B301 displayed better performance in RN when mycorrhiza was not present (32.33) whereas SAMPEA 7 and SAMPEA 10 tend to increase as the concentration of mycorrhiza increases, there values were statistically different ($P<0.05$) from the control. Therefore, it is evident that certain concentration of *G. clarum* has varying effects on the RN and RB of different cowpea varieties. SAMPEA 7 and SAMPEA 10 responded better to certain concentration and can therefore be used as an alternative source of fertilizer for the varieties.

Keywords: Mycorrhiza, B301, SAMPEA 7, biomass

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INTRODUCTION

Large amounts of mineral fertilizer have been used for enhancing fruit yields. Mycorrhizal fungi are an alternative biofertilizer to enhance fruit production instead of relying on mineral fertilizer. Moreover the fungi help to maintain and preserve soil and water resources for future generations. By far the most important and widely distributed type of mycorrhiza is Arbuscular Mycorrhiza fungi (AMF) (Supaporn and Sompetch, 2006). In recent years, biofertilizers have emerged as a promising component of integrating nutrient supply system in agriculture. Thus, biofertilizers are organic products containing specific microorganisms in concentrated forms, derived from the soil root zone (rhizosphere) (Mishra and Dadhich, 2010). Arbuscular Mycorrhizal Fungi (AMF) are obligatory symbionts that colonize the roots of approximately 80% of terrestrial plants, improving their nutrition, growth and disease tolerance (Smith and Read, 2008; Elsen *et al*., 2008).

Arbuscular mycorrhizas (AMs) are characterized by the formation of unique structures, arbuscules and vesicles by fungi of the phylum Glomeromycota (AM fungi). AM fungi (AMF) help plants to capture nutrients such as phosphorus, sulfur, nitrogen and micronutrients from the soil. It is believed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonisation of land by plants and in the evolution of the vascular plants (Brundrette, 2002). The benefits of AMF are greatest in systems where inputs are low. Heavy usage of phosphorus fertilizer can inhibit mycorrhizal colonization and growth. As the soil's phosphorus levels available to the plants increases, the amount of phosphorus also increases in the plant's tissues and carbon drain on the plant by the AM fungi symbiosis become non-beneficial to the plant (Grant 2005). Arbuscular mycorrhizal fungi (AMF) are bio-control agents (Fapohunda *et al*., 2011) which improve the growth and yield performance, disease and drought resistance, fertilizer utilization and rooting depth of plants (Olawuyi *et al*., 2011; Olawuyi *et al*., 2012).

Cowpea (*Vigna unguiculata* (L) walp are widely grown crops with great economic value in countries like Nigeria. They are staple crops that constitute the main diet of many people and livestock fodder in tropical and subtropical Africa especially in the Northern and Southern Guinea Savanna of Nigeria (Ogunkanmi *et al*., 2006). Cowpea is of major importance to the livelihoods of millions of relatively poor people in less developed countries of the tropics (FAO, 2002). Islam *et al*. (2006) emphasized that all parts of the plant used as food are nutritious providing protein and vitamins, immature pods and peas are used as vegetables while several snacks and main dishes are prepared from the grains. Egho (2009) reported that Nigeria is the 2nd greatest consumer of cowpea in the whole world. Among the legumes, cowpea is the most extensively grown, distributed and traded food crop consumed, more than 50% (Ogbo, 2009; Agbogidi, 2010a). This is because the crop is of considerable nutritional and health value to man and livestock (Agbogidi, 2010b).

Efforts have been made to boost the production of cowpea due to extremely high demand of the crop as a result of several uses to which the crop is being put into; however, production have generally been low due to incessant degradation in soil nutrients partly due to constant uses of mineral fertilizers and seasonal dependence of the crop. It is against this background that this experiment was designed to access the impact of *G. clarum* on root biomass and root nodles of four different varieties of cowpea (*V. unguiculata* L)

MATERIALS AND METHODS

Sources of Experimental Materials

Four varieties of cowpea were used in this study, these are B301 obtained from IITA Kano substation; SAMPEA 7, SAMPLE 10 and IAR-1074 which were obtained from the Department of Plant Science, Institute of Agricultural Research (IAR), Zaria. The arbuscular mycorrhizal fungus, *Glomus clarum*, culture was obtained from IITA, Ibadan and Department of Agronomy, University of Ibadan, Ibadan. Top sandy loamy soil was collected from the Botanical Garden, Ahmadu Bello University, Zaria.

Study Area

The study was conducted in the Experimental Garden, Department of Biological Sciences, Ahmadu Bello University, Samaru, Zaria. It is located in the northern Guinea Savanna ecological zone of Nigeria (11^{o} 11'N and 7^{o}39'); it is 686m above sea level.

Soil Preparation and Inoculum Preparation

The top soil was mixed with the sand at 1:1 (V/V) ratio. This was then sieved and sterilized partially at 120^{o}C for two hours and the physic-chemical analysis of the sandy loan soil was carried out (Table 1).

The methods of Heckman and Angle (1987) were used to prepare AMF inoculum as follows; Sterilized planting bags were filled with 6kg of sterilized sandy loan soil per bag. About 50g of AM culture was mixed with the top 6cm of the soil in the planting bag and 20 grains of surface sterilized Sorghum seeds were planted per bag and then watered. These planting bags were kept in the screen house and watered every two days. At the end of two weeks after planting (WAP), the seedlings were thinned to 15 seedlings per bag. Watering continued, and then stopped at the end of 12 WAP. This was left for another ten days without watering. Then the shoots of the Sorghum plants in each bag were removed and the soil together with the root system in each bag was mashed together and mixed thoroughly to homogenize the soil and the colonized root parts to give the AM inoculums stock.

Preparation of the planting Bags and Inoculating the Soil

Perforated (at the bottom) planting bags of 15cm width were sterilized using 1% sodium hypochlorite (NaOC1) for 5 minutes then rinsed several times with distilled water. Each sterilized bag was then filled up with 2kg of the sterilized soil.

A hole of about 6cm deep was dug in the soil contained in each planting bag. The AM inoculum was applied in 4 rates (0g, 10g, 20g and 30g/bag). The inoculum was poured into the hole and Factorial design was adopted with complete randomized block design (CRD) arrangement. Both the treated and control were replicated three times and watered regularly.

Planting Procedure

Four cowpea seeds were planted per bag after the inoculum had been added to the soil. The seedlings were later thinned to two plants per bag at 2 WAP. The cowpea seedlings were sprayed fortnightly, from the age 4 WAP with Cabendazim 12% + mancozeb 63% (Ream) and 300g/l Dimethoate + 36g/l Cypermethrin (Balathoate). The mixture of 3g of Team, and 50ml of Bathoate to 10 litres of water was used to control fungal viral diseases and insect pests.

Data Collection

Three randomly selected plants from each treatment combination were used. The selected cowpea plants were cut into root and shoot with a knife. In other to determine the root biomass of the samples, samples were placed in labeled envelopes which were arranged in the oven set at 70°C and weighed at intervals until constant weight was obtained; then the weight of the dried samples was taken.

Data Analysis

The data were subjected to Analysis of variance (ANOVA) to determine the level of significance among the treatments and Duncan multiple range test (DMRT) was used to separate the means.

Results and Discussion

At the 4 and 6 WAP, the varieties SAMPLE 7 and IAR-1074 did not show significant response to *G. clarum*, but other varieties responded to the AM fungi at all the stages (Table 2). The B301 produced RB that was comparable with the least on each sampling date except at 6 WAP (10g and 30g/bag) and 8 WAP (Table 2). Varieties IAR-1074 and SAMPEA 7 at 0, 10 and 30g/bag produced the highest RB at 4 WAP (Table 2).

The general increase observed in the root biomass of the cowpea varieties inoculated with the *Glomus clarum* was probably due to increase in the nutrient uptake leading to increase in the root matter. This agrees with Pedraza-Santos *et al*. (2001) who reported that there was increase in the root of rice plants at early and later stages when these plants were inoculated with AM fungi. Similarly, Ruiz-lozano and Azcon (2001) and Saleh and Saleh (2006) observed that AMF stimulated more root formation in mycorrhized than non-mycorrhized plants.

In line with this, the higher root biomass values in mycorrhized cowpea varieties SAMPEA 7 and IAR 1074 compared to the mycorrhized SAMPEA10 and B301 varieties could be attributed to the preference of association between these plants roots and the AM fungi species. This is in agreement with the observation of Ibijbijen *et al*. (1996) that there were significant differences between the capacities of different AMF to colonize roots of different cowpea varieties.

Cowpea varieties SAMPEA 10 and SAMPEA 7 showed increase in number of root nodules values at 0, 10,20g/bag and between 0 and 10g/bag respectively. But for IAR 1074, presence of *G clarum* tend to reduce the production of root nodules (Table 2). The variation observed in the increase in the number of root nodule values among the cowpea varieties used in all the sampling dates, could be due to varietal crop dependency on AMF mycorrhization. Also, the enhancement of nutrient uptake especially the non-mobile minerals being made available by increasing searching zone, converting the minerals into useable form might have aided nodule formation. On the other hand, these varieties (SAMPEA 7 and SAMPEA 10) also showed higher root biomass under this AMF application and could have also enhanced their nodulation. Ibijbijen *et al*. (1996) in their study observed a similar result that the roots of nodulating beans were well nodulated, but nodules were not found on the roots of the non-nodulating beans when inoculated with AMF and bacteria that aid nodule formation (BNF). Koide and Moses (2004) reported that normal nodulation of various legumes depend on mycorrhization, which increased nodulation and N-fixation as a result of improbed nutrition in plants. Johnson *et al*. (2004) also reported that, presence of AMF in mycorrhized legume plants enhances nodulation and that the symbiosis is dependent on phosphorus concentration which was made to be more available by the mycorhization.

Therefore, it is evident that certain concentration of *G. clarum* has varying effects on the RN and RB of different cowpea varieties. SAMPEA 7 and SAMPEA 10 responded better to certain concentration and can therefore be used as an alternative source of fertilizer for the varieties.

Table 1: Physicochemical parameters of soil

Soil parameters	
SAND	87.6%
SILT	11.2%
CLAY	1.0%
ORGANIC CARBON	1.5%
TOTAL	100.5%
pH	6.92
	Per Ash
N	(%)
P	10
Na	1.34
K	0.48
Ca	2.60
Mg	7.96
Fe	3.19
Cu	0.50
Zn	4.00
CEC	0.500
EA	12.06
% CARBON	0.17
	0.47

Table 2: effect of G. clarum on the Root Biomass (RB) and Root Nodules (RN) of Cowpea varieties.

AM conc. (g/pot)	Variety	4wap		6 wap		8wap	
		RB (g)	RN	RB (g)	RN	RB (g)	RN
0	B 301	0.2de	0.00 b	0.3 d	0.00f	0.3 ab	8.00 bcd
	SAMPEA 10	0.2cde	0.67 b	0.4 bcd	2.00 ef	0.2 ab	1.67 d
	SAMPEA 7	0.4 a	0.00 b	0.3 cd	9.67 a-f	0.1 b	4.00 cd
	IAR -1074	0.4 a	6.33 a	0.3 cd	15.67 ab	0.3 ab	32.33 a
10	B 301	0.1e	0.00 b	0.4 bcd	8.67 a-f	0.3 ab	8.00 bcd
	SAMPEA 10	0.2cde	0.00 b	0.3 cd	8.67 a-f	0.4 a	10.33 bcd
	SAMPEA 7	0.4 a	0.00 b	0.3cd	15.33 ab	0.2 ab	15.33 bcd
	IAR -1074	0.4 a	1.00 b	0.4bcd	3.00 def	0.2 ab	16.33 bcd
20	B 301	0.3abc	0.00 b	0.2 e	8.00 a-f	0.2 ab	6.33 bcd
	SAMPEA 10	0.3abc	0.00 b	0.3 cd	3.33 c-f	0. 2 ab	11.67 bcd
	SAMPEA 7	0.3abc	0.00 b	0.3 cd	14.00 a-d	0.2 ab	8.67 bcd
	IAR -1074	0.3abc	10.33 a	0.3 cd	17.33 a	0.2 ab	20.67 ab
30	B 301	0.3abc	0.00 b	0.5a	8.33 a-f	0.2 ab	14.67 bcd
	SAMPEA 10	0.3abc	0.00 b	0.4 bcd	4.67 b-f	0.2 ab	9.67 bcd
	SAMPEA 7	0.4 a	0.00 b	0.3 cd	15.00 abc	0.2 ab	14.67 bcd
	IAR -1074	0.4 a	3.33 ab	0.3 cd	12.67 a-e	0.2 ab	19.67 abc
SE±		0.04	1.69	1.71	3.46	0.01	4.72

Means followed by the same letter (s) in each column, are not significantly different (P>0.05).
WAP = weeks after planting.

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