Internet Journal of Food Safety, Vol.10, 2009, p. 85-91

Direct and Residual Influence of Inoculation with *Glomus mosseae* and *Bradyrhizobium japonicum* on Proximate and Nutrient Element Content of Cowpea Seeds.

*OMOMOWO, I.O[1], OLA, I.O[1], AKINTOKUN, A.K[1], BANKOLE, M.O[2] AND BABALOLA, O.A[3].

1,2,: Department of Microbiology, University of Agriculture, Abeokuta, Nigeria.
1: Department of Pure and Applied Biology, Lautech, Ogbomoso, Oyo - State.
3: Department of Soil Science and Land Management, University of Agriculture,Abeokuta, Nigeria.

Abstract : A pot experiment was conducted in a screen house in the University of Agriculture, Abeokuta, to investigate both the direct and residual effect of inoculation with *Glomus mosseae* and / or *Bradyrhizobium japonicum* on seed proximate and mineral nutient composition of cowpea seeds.

The residual response was assessed by repeating the experiment without fresh application of the microbial inoculant to the soil. Four treatments consisting, inoculation with *Glomus mosseae, Bradyrhizobium japonicum*, combination of *Glomus mosseae* and *Bradyrhizobium japonicum*, with a non-inoculated treatment which served as the control were used. The experimental design was completely randomised and treatments were replicated four times. The direct and residual influence of inoculation treatments, led to a significant increase in the seed proximate contents of cowpea at ($P<0.05$). Also, the seed mineral nutrients concentrations were significantly enhanced over that of the un-inoculated control.

Key words: *Glomus mosseae*, *Bradyrhizobium japonicum*, Proximate Composition, Nutrient Element contents.

* Corresponding author,
address; Department of Microbiology,University of Agriculture, Abeokuta, Nigeria.
Tel: +2348033331678, E-mail:wale_easy@yahoo.com

Introduction

Positive effects of the colonization of legumes by arbuscular mycorrhizal fungi (AMF) and rhizobia are subject of research in recent years {Thiagarajan *et al*., 1992, Daniels-Hylton and Ahmad, 1994}. Synergistic effects of dual colonization of roots with AM fungi and *Rhizobia* on growth, nutrient uptake and nitrogen fixation in soybean- {Bethlenfalvay *et al*., 1990, Chickpea -Champawat, 1990 and cowpea- Islam *et al*., 1990} have been reported.

Legumes enter into one type of symbiotic association with bacteria of the genus *Bradyrhizobium*, and into another with arbuscular mycorrhizal (AM) fungi which improve nutrition of plants {Manjunath and Bagyaraj, 1984}.

Leguminous crops are essential component of cropping systems throughout the world, because they helps to improve soil by natural nitrogen fixation and protect ground water from nitrate contamination resulting from excessive application of N-fertilizer. For the continued success of legumes in agricultural systems, the legumes should have effective root rhizosphere associations {Slattery et al., 2001}.

Cowpea (*Vigna unguiculata*) also called Crowder pea, black-eyed pea or southern pea is a protein rich legume with very high potential to improve the standard of living of peasant farmers in Africa, where the cost of animal protein is high –(Babaleye, 1993).

Cowpea is grown in most of the tropical and subtropical regions of the world. It is an important food legumes and an essential component of cropping system in the drier region of the tropics, covering parts of Asia and Oceania, the Middle East, Southern Europe, Southern U.S.A. and Central and South America –(Duke, 1990).

All the plant parts that are used for food are nutritious, providing protein, vitamins and minerals. Cowpea grain contains an average of 23-25% protein and 50-67% starch –(Quin, 1997).

Cowpea- *Vigna unguiculata*, is important in Nigeria as source of inexpensive protein –(IITA, 1989) for meeting human protein requirements.

Increasing population and the consequent increased demand for food production and food quality in Nigeria, require that proposed agronomic strategies for improvement should, in general, avoid high input costs. Also, chemical fertilizers are becoming increasingly difficult to obtain in most African countries like Nigeria, where delivery systems for farm inputs are ineffective and solely import dependent, hence expensive. Moreover, seed quality and nutrient composition of seeds, are traits of commercial interest to farmers. The presence of the various mineral nutrients such as Ca, Mg, K, N, Na, Zn, Cu, Fe and Mn are of biochemical importance to the physiology of the seeds. Nitrogen is a common constituent of protein synthesis, nucleic acid, RNA and DNA. Phosphorus is a constituent of co-enzyme NADH and NAD. Which are important energy producing units in biomembranes as in (ATP) Adenosine triphosphate. Calcium is important in cell-wall formation, and in the formation of cell membranes, lipid structures. Calcium is involved in normal mitosis thus, ensuring non-occurrence of abnormalities in seeds and plants [Delving, 1975]. Magnesium is an important constituent of chlorophyll molecule which ensures non-discolouration of young seedlings. (Webster and Varnera, 1954), reported that potassium is essential as an activator for enzymes involved in the synthesis of certain peptide bonds. Iron functions in the synthesis of chloroplastic protein and may impair the machinery for chlorophyll synthesis (Gauch, 1957, Alabi, 1987). Manganese plays an important role in respiration and nitrogen metabolism, while copper acts as a component of phenolases, laccase and Ascorbic acid oxidase [Delving, 1975].

Moreover, the measurement of some proximate profiles such as protein contents, carbohydrate, lipids, moisture contents and ash percentage is often necessary to ensure that they meet the requirements of food regulators.

This research is therefore aimed at investigating both the direct and residual response of inoculation with *Glomus mosseae* and / or *Bradyrhizobium japonicum* on proximate and nutrient element composition of cowpea seeds. This is with the intention of studying the ability of this biofertilizers to improve the nutritional quality of this legume.

Materials and Methods

Experimental design and statistics

A completely randomized design was used. The main effects were:

1) Inoculation with *Bradyrhizobium japonicum.*
2) Inoculation with VAM fungi – *Glomus mosseae*.
3) Inoculation with both *Glomus mosseae* and *Bradyrhizobium japonicum*.

A non-inoculated treatment served as the control.

The resulting four treatments were replicated four times. Data were evaluated by analysis of variance. Probabilities of significance was used to test significance among treatments and LSD values ($P < 0.05$) were used to compare means.

Biological Materials

AM fungi, *Glomus mosseae* and *Bradyrhizobium japonicum* were collected from the International Institute of Tropical Agriculture, IITA, Ibadan. The seeds of cowpea – *Vigna unguiculata* - Tvu14476, that mature at 70 days after planting, were also collected from IITA, Ibadan.

Microbial Culture

Bradyrhizobium japonicum collected were confirmed by gram stain test, and monitoring the morphological characteristics and time of growth on yeast extract mannitol agar, containing congo red. The colony forming unit (CFU) was determined by serial dilution and counted using a colony counter. The number of colonies counted were 8.1×10^{6}cfu/ml, and represented the inoculum density inoculated into the soil.

Glomus mosseae spore inoculum density, containing the spores as well as root fragments and sand was determined using the method of (Daniel and Skipper 1982). The spore density was found to be 289 spores per 100g of soil.

SOIL AND GROWTH CONDITIONS

The soil used for planting was collected from the farm of University of Agriculture, Abeokuta, Nigeria. The sandy loam topsoil was sieved to remove extraneous particles. The soil was then sterilized in the oven for two hours at 180^{o}C and kept for two weeks to allow it to revitalize. The following physico-chemical analyses of the soil sample collected were carried out before planting; soil pH, particle size analysis, organic carbon, organic matter, exchangeable bases, available phosphorus and total nitrogen in soil.

Pot experiment

Soil was collected from the University of Agriculture farm, in Abeokuta (UNAAB). The soil was sieved using pore size of 2mm and sterilized for two hours at 180℃. Six and half kilograms of soil were weighed into each pot. The pots were arranged randomly and was used in planting cowpea in a screen house in UNAAB, Nigeria. The treatment pots received inoculation with *Glomus mosseae* and *Bradyrhizobium japonicum* as described by (Mahdi and Atabani, 1992, Wadisirisuk, *et al*., 1989).

Fifty grammes (most weight) of the *Glomus mosseae* inoculum, was placed in holes, 3cm deep and 10cm in diameter, in the centre of the pot before planting. The *Glomus mosseae* consists of soil and root fragments and spores. Ten seeds of soybean cowpea –*Vigna unguiculata* was planted in each pot respectively. Also, 1ml of 8.1×10^{6}cfu/ml *Bradyrhizobium japonicum* inoculum was inoculated in the pot experiment directly into the soil of the germinated seedlings, one week after planting (Bayne and Bethlenfalvay, 1987). The pots were watered at least once daily.

Harvest and Assays

At harvest, which was 70 days after planting (70 DAP) for cowpea, the number of pods and seeds were carefully cleaned and counted. The seeds were weighed, then grinded to pass through a 0.4mm screen for determination of mineral nutrient content of the legume, on a wet weight basis, using (AOAC method 1990). Atomic absorption spectrophotometer (AAS) was used in reading calcium and magnesium, flame photometer was used to determine sodium and potassium, while spectrophotometer was used for phosphorus and iron determination. The nitrogen content of the seeds were determined using Macro-Kjeldahl apparatus.

Results

The physico-chemical parameters of the soil used for planting.

The analyses of the soil, showed that the textural class was a loamy sand. The pH of 5.9 indicates that it is acidic in nature. The results of the exchangeable bases, organic matter, organic carbon, total nitrogen and available phosphorus, show that the soil is degraded nutritionally (Table 1).

Direct effect of inoculation treatments on the Proximate Analysis of cowpea

Inoculation treatment significantly ($P<0.05$) affected the crude protein, ash content and the crude fat contents of cowpea. Inoculation with *Bradyrhizobium japonicum* gave the highest increase in fat content, followed by *Glomus mosseae* and the dual inoculation.

The carbohydrate content of cowpea was however, significantly ($P < 0.05$) higher in the un-inoculated control (Table 2).

Direct effect of inoculation treatments on the Mineral nutrients composition of the seeds of cowpea

Inoculation significantly ($P < 0.05$) affected the mineral nutrients composition of the seeds of cowpea.

G. mosseae inoculation significantly ($P < 0.05$) increased the seed contents of nitrogen, phosphorus, calcium, magnesium and iron, as compared to the uninoculated (control). Dual inoculation with both *G. mosseae* and *B. japonicum* significantly ($P < 0.05$) increased the seed calcium, magnesium and iron. In terms of contribution of seed mineral nutrients composition, all the inoculated treatments made the most consistent contributions to the seed biomass production in cowpea, except with respect to potassium and sodium, where control is higher than inoculated treatment (Table 3).

Residual influence of inoculation of *Glomus mosseae* and *Bradyrhizobium japonicum* on proximate analyses of cowpea.

The proximate composition of cowpea seeds were enhanced by the inoculation treatments when compared with the control. Inoculation significantly ($P < 0.05$) enhanced the ash, crude protein and the crude fat contents of cowpea seeds. However, the control had the highest carbohydrate content, compared to the inoculated treatments. There was also no significant difference ($P < 0.05$) in the crude fibre content of cowpea seeds (Table 4).

Residual effect of inoculation treatments on the mineral nutrients composition of the seeds of cowpea

Generally, inoculation significantly ($P < 0.05$) affected the mineral seed composition of the cowpea. Inoculation increased the seed nitrogen, phosphorus, calcium, magnesium and iron concentrations. Dual inoculation gave highest increase in seed calcium, magnesium and sodium. *Glomus mosseae* inoculated treatments improves best, the seed nitrogen and iron. *Bradyrhizobium japonicum* treatments, improves the seed nitrogen and phosphorus best. There was no significant difference in both the control and inoculated treatments seed potassium and sodium concentrations (Table 5).

Discussion

In this study, both the direct effect and residual response of arbuscular mycorrhiza (AM) fungi – *Glomus mosseae and/ or Bradyrhizobium japonicum* was investigated on the seed proximate and nutrient element composition of *Vigna unguiculata* seeds.

The result showed that inoculation treatments led to improved seed nutrient composition to a varying degree, over the uninoculated control.

The direct effect of inoculation with *Bradyrhizobium japonicum*, *Glomus mosseae* and the combination of both *Bradyrhizobium japonicum* and *Glomus mosseae* treatments during the first growth period on cowpea seed proximate and seed nutrient element composition, in this study might have resulted mainly from increased availability of phosphorus (Ames and Bethlenfalvay 1987), although hyphal translocation and assimilation of other plant nutrients (Barea *et al*., 1987) are possibilities.

Interactions between the microsymbionts are complex, especially under phosphorus stress (Bethlenfalvay *et al*., 1985). Nitrogen-fixation is impaired during phosphorus deficiency (Sa and Israel, 1995) and can be partially alleviated by mycorrhiza-mediated uptake of otherwise unavailable phosphorus.

The result showed that there were significant effects on the proximate contents of cowpea seeds due to inoculated treatments. This corroborates the findings of (Martins *et al*. 2003), which reported that biological nitrogen fixation associated with cowpea is able to improve plant growth and grain production.

The seed mineral nutrients of cowpea were also significantly different at ($P<0.05$). The seeds nitrogen, phosphorus, magnesium, sodium and iron were increased by inoculation. (Kawai and Yamamoto, 1986) reported that inoculation with AM fungi increased plant development through supply of some elements such as calcium and magnesium. Several minerals such as calcium, iron, potassium, sodium and phosphorus are essential for human and animal health.

A second planting season, without fresh application of inoculation treatments was used to determine the residual effect of inoculation of AM fungi and / or *Bradyrhizobium japonicum* on seed proximate and seed mineral nutrients composition of cowpea. This also showed that there were significant effects on the seed proximate and seed mineral nutrients composition of cowpea. Similar result was reported by (Vejsadova *et al.*, 1993).

Also, the mineral nutrients composition of cowpea seeds were also significantly affected. This result agrees with that reported by (Ames et al., 1991).

The residual benefits of inoculation with *Glomus mosseae* and / or *Bradyrhizobium japonicum* on growth response of cowpea is probably due to the fact that organophosphate bound ions and organic matter in the soil are broken down over time and made available for the plant usage by the mediating action of the microbial inoculants. This is corroborated by the work of (Mahdi and Atabani, 1992), that biofertilizers such as Mycorrhizal fungi and *Rhizobia* usually have a longer lasting effect in soil and do not pose ecological threats.

(Omomowo *et al* 2006), had reported a similar result of using inoculation treatments with *Glomus mosseae* and / or *Bradyrhizobium japonicum* to increase the seed weight and proximate composition of cowpea, and showed that they are promising microbial inoculants for the improvement of the quality of leguminous crops, especially in the tropics.

Conclusion

Inoculation treatments with *Glomus mosseae* and / or *Bradyrhizobium japonicum* led to both direct and residual significant benefits on the seed proximate and seed nutrient element composition of cowpea.

These results have shown that programmes, focusing on biofertilization can be implemented in Nigeria to improve the productivity of food legumes and also to improve the seed quality.

However, it is recommended that to achieve improved productivity; there is the need to do suitable strain selection of the *Rhizobium*, AM fungi and legume combination to obtain inoculants that are compatible and highly competitive.

References

Alabi, D. A., 1987. Proximate analysis of food and mineral contents of eleven cultivars of okra(*Abelmoschus esculentus* (L) Moench). *Proceed of Hort. Soc. Nig., 9: 75-83.*

Ames, R. N., Thiagarajan, T. R., Ahmad, M. H., Mclaughlin, W. A. Co-selection of compatible Rhizobia and VAM fungi for cowpea in sterilized and non-sterilized soils. *Biology Fertility Soils* 1991; 12: 112-116.

Ames, R. N. and Bethlenfalvay, G. J. Localized increase in nodule activity but no competitive interaction of cowpea rhizobia due to pre-establishment of vesicular-arbuscular Mycorrhiza. *New Phytologist* 1987; 95: 381-396.

AOAC. Official methods of analysis (14th ed.) Washington DC, USA: Association of Official Analytical Chemists.1990.

Babaleye, T. Hope rises on the development of pest free cowpeas. *IITA Public Information. 1993*

Barea, J. M., Azcon-Agular, C. and Azcon, R. Vesicular arbuscular Mycorrhiza improve both symbiotic N_2 fixation and N uptake from soils as assessed with a ^{15}N technique under field conditions. *New Phytologist* 1987; 106: 717-725.

Bayne, H. G. and Bethlenfalvay, G. J. The *Glycine – Glomus –Rhizobium* symbiosis. IV. Interactions between the mycorrhizal and nitrogen fixing endophytes. *Plant, Cell and Environment*, 1987; 10: 607-612.

Bethlenfalvey, G. J., Brown, M. S. and. Franson,. R. L. *Glycine-Glomus*
Bradyrhizobium symbiosis. Relationships between leaf gas exchange and plant
and soil water status in nodulated, mycorrhiza soybean under drought stress.
Plant Physiology 1990; 94: 723-728.

Bethlenfalvay, G. J., M. S. Brown and. Stafford, A. E. *Glycine-Glomus*
Rhizobium symbiosis ii. Antagonistic effects between mycorrhiza colonization
and nodulation. *Plant Physiology* 1985; 79: 1054-1058.

Champawat, R. S. Response of chickpea (Cicer arietinum) to *Rhizobium* and
VAM dual inoculation. *Acta Microbial Polonica* 1990; 39: 163-169.

Daniel, B. A. and Skipper, H. D. Methods for recovery and quantitative
estimation of propagules from soils. In: Schenk, N. C. (ed). Method and
Principles of Mycorrhiza research 1982; Pp. 29-35. The American
Phytopathological Society, St. Paul, Minnesota.

Daniels-Hylton, K. D. M. and Ahmad, M. H. Inoculation response in kidney
beans (*Phaseolus vulgaris L*.) to VAM fungi and Rhizobia in non-sterilized soil.
Biology Fertility Soils.1994; 18: 95-98.

Delving, A. M., 1975. Detection, Occurrence and Availability of the Essential

Table 1. The physicochemical parameters of the soil analyses before planting.

Parameters	
Sand(%)	87.2
Silt(%)	7.6
Clay(%)	5.2
Textural Class	Loamy Sand
Soil pH	5.9
Exchangeable bases (cmol/kg)	
Ca	4.3
Na	0.3
K	0.5
Mg	0.8
Organic matter(%)	2.4
Organic carbon (%)	1.4
Total Nitrogen (mg/kg)	0.1
Available phosphorus (mg/kg)	2.8

Table 2: Direct effect of AM fungi, and or *Bradyrhizobium* inoculation on Proximate analyses of cowpea at harvest (70 DAP).

Treatments	**Moisture content %**	**Dry matter %**	**FAT content %**	**Crude protein %**	**Crude fibre %**	**Ash content %**	**Carbohydrate %**
Control	18.2b	81.1a	5.3d	35.5b	2.2a	3.4d	34.6a
Glomus	21.2a	78.2b	6.1b	36.4a,b	2.2a	3.8c	29.6b
Bradyrhizobium	18.0b	81.6a	8.6a	38.2a	2.2a	4.4b	27.1c
Glo + Brady	18.1b	80.5a,b	5.5c	38.2a	2.3a	5.1a	29.7d
LSD	**1.7**	**2.6**	**0.2**	**1.8**	**0.2**	**0.2**	**0.5**

Means not sharing a common letter in a column are significantly different at P≤0.05

Table 3: Direct effect of AM fungi, and or *Bradyrhizobium* inoculation on Mineral nutrients of cowpeaseeds at harvest (70 DAP).

Treatment	**Nitrogen (mg/g)**	**Phosphorus (mg/g)**	**Potassium (mg/g)**	**Calcium (mg/g)**	**Magnesium (mg/g)**	**Sodium (mg/g)**	**Iron (mg/g)**
Control	7.2b	1.1b	12.5a	2.3d	4.3d	2.8a	4.3d
Glomus	7.5a	1.3a	10.6b	2.5c	5.1c	2.4c	6.7c
Bradyrhizobium	7.4a,b	1.2a,b	9.8c	2.8b	6.8b	2.3c	9.2b
Glo + Brady	7.4a	0.8c	9.4c	3.7a	8.2a	2.5b	11.2a
LSD	**0.3**	**0.2**	**0.5**	**0.2**	**0.1**	**0.1**	**0.3**

Means not sharing a common letter in a column are significantly different at P≤0.05

Table 4: Residual effect of AM fungi, and or *Bradyrhizobium* inoculation on Proximate analyses of cowpea plants at harvest (70 DAP).

Treatments	**Moisture content%**	**Dry matter%**	**FAT content%**	**Crude protein%**	**Crude fibre%**	**Ash content%**	**Carbohydrate %**
Control	50.8b	47.8a	3.2c	21.9a,b	1.4a	3.5c	18.8a
Glomus	58.9a	40.6b	3.4c	20.7b	1.4a	3.7a	10.7c
Bradyrhizobium	53.4b	42.2b	3.7b	22.3a	5.1a	3.4b	11.3c
Glo + Brady	51.2b	48.3a	4.7a	22.9a	1.5a	3.5b	13.3b
LSD	**4.5**	**2.7**	**0.3**	**1.4**	**7.9**	**0.2**	**1.8**

Mean not sharing a common letter in a column are significantly different at P≤0.05

Table 5: Residual effect of AM fungi, and or Bradyrhizobium inoculation on Mineral nutrient of cowpea seeds at harvest (70 DAP).

Treatment	Nitrogen (mg/g)	Phosphorus (mg/g)	Potassium (mg/g)	Calcium (mg/g)	Magnesium (mg/g)	Sodium (mg/g)	Iron (mg/g)
Control	7.1c	0.5c	7.3a	1.6b	3.2d	2.5b	8.7c
Glomus	8.2a	0.6b	7.4a	1.8b	4.7c	2.5b	22.6a
Bradyrhizobium	8.4a	0.6a	7.7a	1.8b	5.5b	2.3c	21.8b
Glo + Brady	7.8b	0.5d	7.3a	2.1a	7.7a	2.6a	8.9c
LSD	**0.2**	**0.04**	**0.6**	**0.2**	**0.2**	**0.2**	**0.4**

Means not sharing a common letter in a column are significantly different at $P \leq 0.05$