FUNGAL TERRITORY
2020, VOL. 3, NO. 3, 23-26

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REGULAR ARTICLE

EFFECTS OF COMBINED INOCULATION OF BAMBARA GROUNDNUT (*Vigna subterranean L. Verdc*.) WITH *GLOMUS MOSSEA* AND *BRADYHRHIZOBIUM JAPONICUM* ON NITROGEN AND PHOSPHOROUS UPTAKE IN SHOOT, PLANT BIOMASS, LEAF CHLOROPHYLL AND MYCORRHIZAL INOCULATION EFFICIENCY (MIE).

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https://doi.org/10.36547/ft.2020.3.3.23-26

ABSTRACT

The response of Bambara groundnut to co-inoculation with Abuscular mychorrhizal fungi (*G. mossea*) and *Bradhyrhizobium japonicum* (strain USDA110) with regard to leaf chlorophyll, percentage shoot nitrogen and phosphorus, nodule weight and plant biomass was studied. Bambara plants were grown under screen house conditions in pots. Plants were inoculated with 1ml of *B. japonicum* USDA 110 strain (10^9 cfu /ml), Mychorrizal was applied to the plants 10g, and 20g (90 spores/g) and water was applied at 10ml, 20 ml and 50ml every other day. The obtained results showed that dual inoculation activity was able to improve both nitrogen and phosphorus in plant shoot, MIE, but did not improve biomass and leaf chlorophyll when compared with plants subjected to single inoculation with only *G. mossea* and only *B. japonicum.* More Nitrogen and Phosphorus was retained in the shoot of plants co-inoculated with *B. japonicum* and 20g *G. mossea* when given 50ml of water and also had higher biomass. Leaf chlorophyll reduced in plants as flowering approached. *B. japonicum* was able to positively influence and establish symbiosis with *G.mossea* and synergistically effectively act as "mycorrhiza helper bacteria" (MHB) when both were co-inoculated in Bambara plant.

Keywords: *G. mossea, B. japonicum, Water stress,* Bambara groundnut

INTRODUCTION

Bambara nut (*Vigna subterranean* L. Verdc.) is a leguminous seed crop of African origin (Nwanna *et al*., 2005), that is highly underutilized and been found to have a high nutritive value and drought tolerance (Anchirinah *et al*., 2001 and Ocran *et al*., 1998). It is considered to be a famine culture crop probably because it associates with mycorrhiza. Synergy between mycorrhizal fungi and rhizobia micro symbionts (nitrogen fixers) in legumes has been studies by (Jesus *et al*., 2005; Kaschuk *et al*., 2010) and their association described as a tripartite (Vega *et al*., 2010), where the Mycorrhizal help to increase the absorption and solubilisation of phosphorus to rhizobia in plant nodules (Scotti; 1997), while Rhizobia fix nitrogen provide it as ammonia to the plant, which provides carbohydrate to micro-symbionts (Silveira *et al*., 2001; Gross *et al*., 2004). The benefit of these microorganisms to the host plant depends on the compatibility between the rhizobial strain and mycorrhizal fungi inoculated. Positive symbiosis formed with mycorrhizal fungi (Frey-Klett *et al*., 2007), when found to be synergistically effective they are called "mycorrhiza helper bacteria (MHB) (Garbaye; 1994). Fungal-rhizobial inoculant has been able to increase N2 fixation in soybean by 30 % as compared to conventional use of rhizobia (inoculant) (Jayasinghearachchi and Seneviratne, 2004). For legumes cultivation, the relationship between mycorrhiza and rhizobia and is highly importance because it affects the rate of infection and mineral nutrition as well as the chemical and physical conditions of the soil by adding organic waste and increasing the growth of these plants (Andrade *et al*., 2000). When there is deficiency of phosphorus, there is low nodulation and nitrogen fixation in legumes have except if their roots are colonized by mycorrhizas or an alternative source of phosphorus in soil is made available. Moreover, the mycorrhizal condition influences the efficient competition among strains of rhizobia to occupy the nodules in the roots of the host (Miranda and Miranda, 2002; Garg and Manchanda, 2008). Kaschuk *et al*., (2010) studied the AMF-rhizobia symbiosis in 12 legume species, and they reported an increase in the photosynthetic rate and grain yield of legumes. However, according to Scotti (1997), the benefit of these microorganisms to the host plant depends on the compatibility between the strain of rhizobia and mycorrhizal fungi inoculated. Experiments are needed to study the compactibility of symbiotic microorganisms (bacteria and mycorrhizal fungi while considering water availability) for Bambara groundnut which will enhance the understanding of symbiosis (Gueye; 1992) in the plant and provide knowledge about their role in enhancing the plant resistance to drought this is because increasing the use of BNF is a major way to increase or maintain the yield of legumes (Ngakou *et al*., 2012), reduce the footprint of agriculture on the environment and may be used to address the current challenge of meeting the fast-growing demand for agricultural products worldwide.

MATERIALS AND METHODS

Prepation of broth for inoculation

Pure cultures of the *rhizobia* isolates was obtained and inoculated into 100ml Erlenmeyer flasks containing 50 ml of yeast-mannitol. The inoculated broth was incubated at 28°C on a Rotary shaker for 7 days after which the bacterial count when satisfactorily determined to be about 10^9 cfu/ ml, was then used to inoculate plants at 1 week of growth (Woomer *et al*., 2012).

Pot Experiment

Sea sand was washed repeatedly with water to remove debris and to reduce pH to between 6.6 - 6.8 which is most suitable for *rhizobia* growth. The crushed gravel and medium sized gravel were also washed till the water was clean. The sea sand, crushed gravel and peat were mixed in a ratio 6:6:1 and mixed until it was evenly distributed. The mixture was then sterilized at 121^{o}C and 1.05 kg cm^{-2} for 15 minutes. The medium sized gravel was also sterilized (Woomer *et al*., 2012). Sterilized 500 ml pots were filled with the sterile sand and sterilized seeds were planted in in them and allowed to germinate. One week after planting (WAP), the BG plants were thinned to one viable plant per pot.1 ml of the inoculums which were already prepared as described above, was introduced into the cowpea plants. Bambara groundnut seeds were sterilized and planted in sterilized soil in pot under screen house conditions and allowed to germinate. Dual inoculation of *B. japonicum* (USDA110 strain) (1ml) (Somasegaran, and Hoben, 2012)) using sterile pipette and 10 g and 20g of mychorrhiza (*G. mossea)* (Carine *et al*., 2017 and Gomoung *et al*., 2017) was applied to plants and limited amount of water (10ml, 20ml, 50ml) was also applied. A completely randomized block experimental design was used treatments (see Table 1) (including three controls, KNO_3 treatment to which nitrogen was applied, Rhizobial application alone and the un-inoculated control) were replicated in each of the 4 blocks.

Application of nutrient and water to Plants

Cowpea plants were allowed to grow for 8 week during which they were given 20 ml of nutrient solution consisting of both micro and macro nutrient. To prepare nutrient solution given to plant, the stock solutions were mixed using 100 ml of

macro- stock solution and 10 ml micro- stock solution made up to 10 liters using distilled water. The nutrient solution was sterilized at 121°C and1.05 kgcm^{-2} for 15 minutes and was aseptically given to the plants weekly.
The solution for the N+ treatment (control containing nitrogen) was prepared using 5% of N in KNO_3 this was sterilized at 121°c and 1.05 kg cm^{-2} for 15 mins after which 50 ml of the solution was added to the plant weekly. Water application was done by giving 10ml, 20ml and 50ml of water (according to the treatment) every other day to the plants (Table 1).

Harvesting

Plants were allowed to grow for 10 weeks, after which they were harvested by cutting at the base with a secateur. The shoots and roots were placed in labelled paper bags in an oven were they were dried at 68°C for 72 hours until constant weight was obtained. The shoot dry weights were recorded.

Determination of chlorophyll

Chlorophyll readings were taken from leaves using a Spad meter at the 2ND 4TH and 6TH weeks after planting.

Determination of %N and %P in plant shoots

The total % Nitrogen in the shoots of the plants were determined using the micro Kjeldahl method (Kjeldahl; 1883) while phosphorus was determined by the molybdenum blue method (Murphy and Rilley, 1962). The three control treatments plants were used as a reference plant.

Statistical Analysis

The data collected was analysed for correlation (Pearson's) using SPSS version 20 and Fisher's least significance was used to compare means at $p \leq 0.05$.

RESULTS

3.1 %Nitrogen

the %N in the shoot increased with increase in the amount of water in both 10g and 20g G. mosses applications. 10g/10ml treatment had a lower %N than the treatment that had only 10g with no rhizobia inoculation, but the 10g/20ml and 10g/50ml treatments had higher %N than the 10g only treatment. All 10g *G. mosseae* had lower %N than the USDA110 only treatment but were higher than the un-inoculated control and the KNO_3 control while the 20g/10ml, 20g/20ml, 50g/ml all had higher %N than the KNO3 and un-inoculated control but only 20g/10ml had a %N that was lower than that obtained in the USDA110 only treatment.

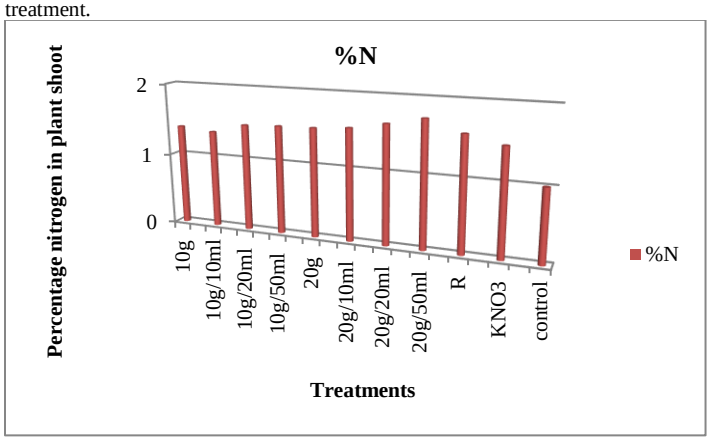

Figure 1 Uptake of Nitrogen in shoot of plants

3.2 %Phosphoru

%P increased with increase in the amount of water at both 10g and 20g *G. mosseae* applications. The %P of USDA110 only was lower than that of all other treatments except that of the un-inoculated control. While the 10g/50ml and 20g/50ml treatments had higher %P than their corresponding treatments that had only *G. mosseae* with no USDA110 applied in them.

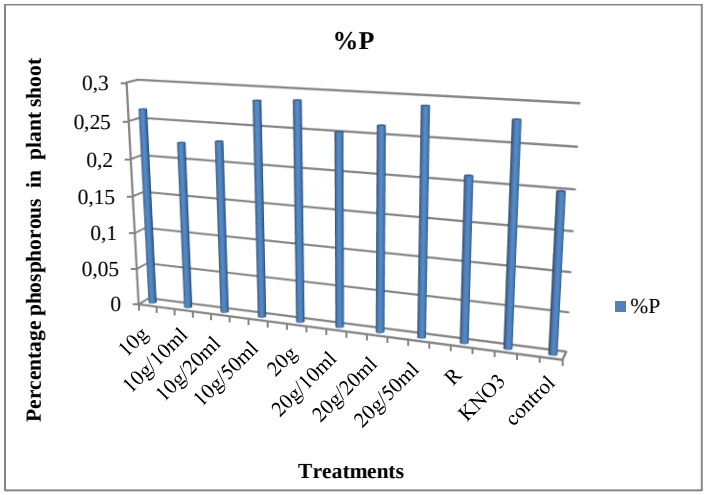

Figure 2 Uptake of Phosphorous in shoot of plants

Leaf chlorophyll

Leaf chlorophyll reduced steadily from the 2ND week to the 6TH week in all treatments and also decreased with increased application of water.

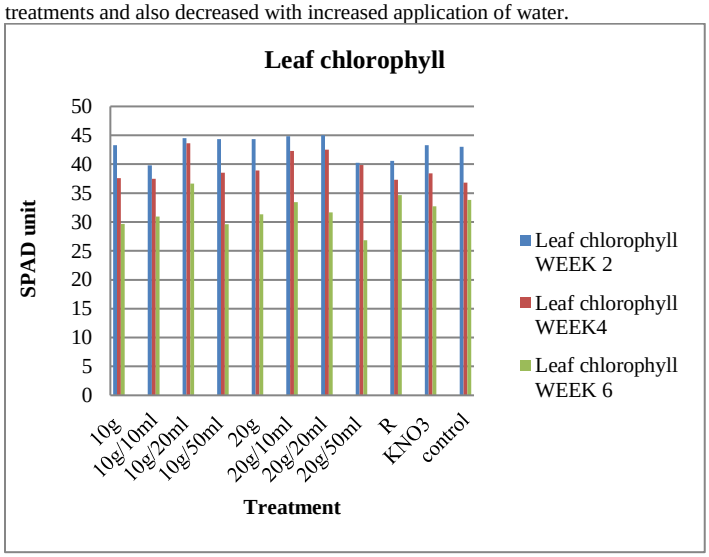

Figure 3 Leaf chlorophyll of experimental plants

Shoot weight

Shoot weight increased with increased application of water in both 10g and 20g *G. mosseae* treatments but plants with USDA110 inoculation had higher weights than their counterparts with no USDA110 inoculation.

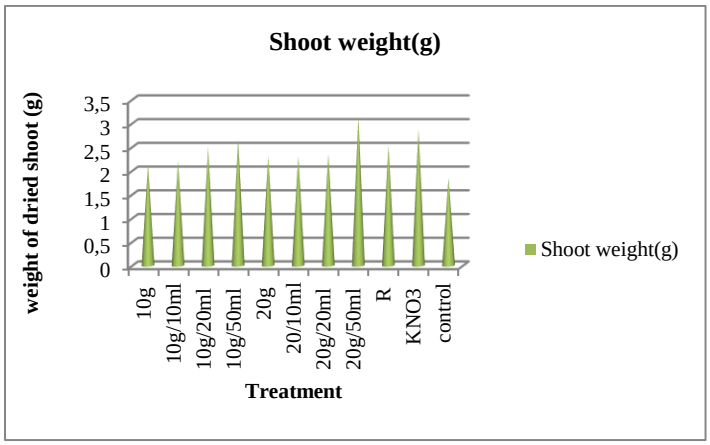

Figure 4 Dried weight of plant shoot

Mycorrhizal Inoculation Efficiency (MIE

MIE increased significantly with increase in the amount of water application when both 10g and 20g of *G. mosseae.* When 20g *G. mosseae* was applied there was no significant difference in the MIE on application of 10ml, 20ml and 50ml of water while there was significant difference for the 10g application.

Correlation

There was a positive correlation between leaf chlorophyll and shoot dry weight, %P and negative correlation between %N and MIE which were not significant. Significant negative correlation was found between the % N and shoot dried weight, and no significant correlation between the MIE phosphorous but n and other parameters although it had a negative correlation with the leaf chlorophyll at the 2nd and 6th week after planting.

Table 1 Mycorrhizal Inoculation Efficiency (MIE) in plants

Treatments	Water (ml)	Inoculum amt (ml)	*G. mossea* (g)	MIE(%)
10g	100	-	10	15.14
10g/10ml	10	1	10	18.9
10g/20ml	20	1	10	34.1
10g/50ml	50	1	10	43.78
20g	100	-	20	24.89
20g/10ml	10	1	20	24.89
20g/20ml	20	1	20	27.57
20g/50ml	50	1	20	69.73
R	100	1	0	0
KNO3	100	-	0	0
control	100	-	0	0

Table 2 Correlation between plant parameters

	leaf chlorophyll Week 2	leaf chlorophyll Week4	leaf chlorophyll Week 6	%Nitrogen	%Phosphorus	Shoot dried weight(g)	Mychorrhizal Inoculation Efficiency (%)
leaf chlorophyll Week 2	1	.110	.116	-.269	.393	-.112	-.146
leaf chlorophyll Week4	.110	1	.299	.411	.147	.177	.426
leaf chlorophyll Week 6	.116	.299	1	-.371	-.645*	-.382	-.593
%Nitrogen	-.269	.411	-.371	1	.582	.699*	.586
%Phosphorus	.393	.147	-.645*	.582	1	.643*	.569
Shoot dried weight(g)	-.112	.177	-.382	.699*	.643*	1	.600
Mychorrhizal Inoculation Efficiency (%)	-.146	.426	-.593	.586	.569	.600	1

*Correlation is significant at the 0.05 level

DISCUSSION

Availability of water had effect on the amount of Nitrogen and phosphorus uptake and shoot weight which all increased as the availability of water increased similar to the findings of Carine *et al*., 2017. Higher amounts of *G. mosseae* also had effects on the N, P, shoot weight and MIE (Fig 1, 2, 3 and Table 1) this is similar to the findings of Moila; 2018. The Mycorrhizal Inoculation Efficiency increased with the amount of water and amount of mycorrhizal when dual inoculation was used in plants and is similar to the report given by Esale *et al*., 2015 and Tsoata *et al*., 2015.

There was no significant increase in leaf chlorophyll when dual inoculation was applied differing from the result obtained by Kaschuk *et al*., 2010 were there was an increase in the photosynthetic rate. Although Bambara did not necessarily depend on mycorrhiza for satisfactory growth and nodulation with rhizobia as observed by Jesus *et al*., 2005, its presence enhanced the development, growth, %N, %P, and MIE especially under conditions of limited water.

The treatment with 20g/50ml of water had the highest value for all the parameters taken. The Mycorrhizal Inoculation Efficiency increased with the amount of water and amount of mycorrhizal when dual inoculation was used in plants and is similar to the report given by Esale *et al*., 2015, Tsoata *et al*., 2015.

CONCLUSION

Although Bambara groundnut did not necessarily depend on mycorrhiza for satisfactory growth and nodulation with rhizobia, its presence enhanced the development, growth, %N, %P, and MIE especially under conditions of limited water. In addition, *B. japonicum* was able to positively influence and establish symbiosis with G.mossea and synergistically effectively act as "mycorrhiza helper bacteria" (MHB) when both were co-inoculated in Bambara plant.

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