Relationship Between Mycorrhizal Infection Ratings and Chlorophyll A Content of Root Segments of Cassava Clones (TMS 30572 and 20555) Grown in Bentex T-Treated Soil.

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ABSTRACT

Two clones of cassava (TMS 30572 and 30555) were grown in varying levels/titres of Bentex T treated soil. Mycorrhizal infection rates and chlorophyll **a** content of the cassava clones were studied. The level of root colonization by vesicular arbuscular mycorrhizal (VAM) fungi had effect on the chlorophyll content of the test plant. The un-amended soil (control) had the highest VAM fungi root colonization (84%). Chlorophyll content was least at this treatment (91.1 µg/mm ^{2}) and highest at the 50µg/g soil treatment (133.9µg/g). Relationship between mycorrhizal infection ratings and chlorophyll content of the plant was correlated (r=0.425) in TMS 30572 and in TMS 30555(r=0.547). The test parameters had minimal variation (P>0.05) between treatments in both clones of the plant. However, clonal differences were observed in the level of root colonization by VAM fungi. The relevance of these results in the management and growth of the crop is discussed.

(Keywords: cassava, VAM fungi infection, chlorophyll content, Bentex T, soil treatment)

INTRODUCTION

Cassava, *Manihot esculenta* Crantz, is a dicotyledonous plant belonging to Euphorbiaceae. The plant originated in Northeast Brazil and has spread to various parts of the world. Cassava is grown in the area between latitude 30˚N and 30˚S (Cock, 1985; Nweke, 1994). In Nigeria it exhibits great potential in alleviating food shortage problems due to its high yielding ability, wide adaptability and low input requirements (Onwueme, 1978).

The crop has a coarse root system similar to plants which depend largely on vesicular arbuscular mycorrhizal (VAM) fungi infection for good growth (Barley and Rovira, 1970). Indeed, ability of cassava to yield reasonably well in soils low in phosphorus is reported to be due to the crops responsiveness to VAM fungi associated with its roots (Kang *et al*., 1980). Clonal differences in the levels of roots colonization and growth responses to infection by VAM fungi are known for cassava (Hahn *et al*., 1981).

Cassava leaves are the main source of assimilates through photosynthesis. Photosynthetic rates in cassava vary according to cultivar, ploidy level, soil-water status, relative humidity, as well as temperature. Typical photosynthetic rates under optimum growing conditions are 15-35µmolCO_2/m^2 between 20 and 40˚C (Ekanayeke *et al*., 1998). Chlorophylls are light harvesting pigments integral to the photosynthetic process. Chlorophyll concentration data will provide information on a plant photosynthetic potential (Arnon, 1949).

Mycorrhizal, meaning fungus, roots are symbiotic association between plant and fungi that colonize the surfaces and cortical tissue of roots during period of active plant growth. The fungus receives carbohydrate (sugar) and growth factors from the plant which in turn receives many benefits, including increased nutrient absorption. This provides a critical linkage between the plant root hairs and acts as an extension of the root system. For some plant species such as corn, carrots, onions, grapes, and coffee, the association with mycorrhizal fungi is indispensable (Hayman,1980).

The degree of dependence varies with plant species, particularly the root morphology, and

conditions of soil and climate. Plant with thick roots, poorly branched and with few root hairs is usually more dependent on mycorrhizae for normal growth and development.

The use of fungicide to control soil–borne pathogens is a common practice. An ideal experimental system for the study of VAM fungi would involve fungicide that could be used specifically to eliminate VAM fungi with little or no effect on the remaining biota (Schreiner and Bethlenfalvay, 1996). One of the most widely used of such fungicide is Benomyl (methyl 1-butyl carbamoyl benzimidazole-2-yl carbamate) which is marketed as Bentex T.

The effect of biocides targeted on VAM fungi is of interest to agriculture, since inhibition of these beneficial organisms may counteract benefits derived from them (Schreiner and Bethlenfalvay, 1996).

This research aims at investigating the mycorrhizal infection rates of root segment of cassava clones grown in Bentex T –treated soil in relation to the photosynthetic ability of the plant.

MATERIALS AND METHODS

Screened house experiments were conducted at Benin City, Nigeria in July, 2008 using two clones of cassava plant.

Plant Materials and Experimental Design

Ten day old seedlings from two-bud node stem cuttings of two clones of cassava (*Manihot esculenta* Crantz), TMS 30572 and TMS 30555, obtained from a farmstead in Benin City, Nigeria were transplanted from moist sawdust into fungicide-treated soil in polyethylene bags at the Department of Crop science, University of Benin, Benin City on the 17th and 31st of July 2008, respectively.

A completely randomized experimental design (CRD) in which five different treatments (Bentex-T concentration) were replicated four times and duplicated for each clone giving a total of 40 samples per clone was used. Each polyethylene bag contained 5kg of sandy-loam soil taken from a field plot 100metres from the screened house were the experiment was carried out. The textural and chemical characteristics of the soil used for the experiment was determined by the Benin-Owena Laboratory Benin City, Nigeria.

The fungicide used for the study Bentex-T also known as Benlate contained 20% Benomyl (methyl1-butyl carbomyl benzimidazole 2-yl carbamate) and 20% Thiram (Tetramethyl thiram disulfide) as active ingredients. Fungicide was added to soil at the rate of zero (0), 50,100,500 and 1000µg/g soil. These doses constituted the five treatments, with the zero titre as control.

Data Collection and Analyses

Estimation of Endophytes Infection in Cassava Rootlets: The method of Hayman (1970) was used for data collection. Root segments of cassava cut 1cm each and fixed in F.A.A. (13ml formalin, 5ml glacial acetic acid, 200ml 50%ethanol) were heated at 90˚c for about 1hour in 10%KOH. This removed the host cytoplasm and most of the nuclei and the roots became clear with the vascular cylinder distinctly visible. The roots were then rinsed in water and acidified with dilute HCl. They were stained by simmering for 5 minutes in 0.05% trypan blue in lactophenol. Root segments were mounted on slides temporarily in lactophenol. Slight pressure on the cover slip flattened the KOH-treated roots for observation on the light microscope within a limited range of focus(x10). Quantitative estimates of root infection were made on 1cm segments by recording the number of segments with any infection and the amount of infection per unit length of root (Hayman, 1970). Analysis of variance (ANOVA) was done on the root infection data at (P> 0.05) level of significance.

Determination of Plant Chlorophyll: Leaf tissue samples larger than the tissue needed for analysis were cut from the plant, placed in aluminum foil and frozen on dry ice in a cooler. Samples were kept in a environment to prevent chlorophyll degradation (Arnon, 1949). Using a template (10x20mm rectangle), a given area was cut from each leaf tissue sample. The samples were then cut into small pieces using a scissors and placed in a mortar. The sample in a given volume of extraction solution(0.1normal NH_4OH solution with reagent grade acetone in a ratio of 1:9 (V:V) was ground, adding a little acid-washed quartz sand, with a pestle for approximately 30 seconds(until tissue was a fine slurry); extraction solution was used to wash any sample material adhering to the pestle. This was done by pouring

a known volume of extraction solution (Arnon, 1949). The extract was then centrifuged for 20 minutes at 500 gravity. The supernatant solution was decanted into a graduated cylinder and the volume brought to 6ml with 80% aqueous acetone. All samples obtained were read at 645nm using a medi-test combi 9 spectrophotometer, then read again after resetting the wavelength to 663nm. Chlorophyll concentrations were expressed on an area basis ($\mu g/mm^2$). Differences in chlorophyll content between treatments were assessed for significance using analysis of variance test.

RESULTS

Results of endophytes infection ratings in cassava rootlets after 14 weeks of planting in two clones of cassava plant (TMS 30572 and 30555) revealed that the degree of infection by VAM fungi infection varied proportionally with the amount of Bentex T applied (Table 1).

The infection rates in both clones of the plant were highest among roots of the un-amended soil (control); 84 and 70% for TMS 30572 and 30555 respectively and lowest in roots of plants with soil amended with 100µg/g Bentex T; 58 and 48% for TMS 30572 and 30555 respectively. Endophytes infection ratings for plants grown in soil amended with 500 and 1000µg/g Bentex T could not be determined because of the phytotoxic effect Bentex T had on the test plant at those titres.

Analysis of variance carried out on the infection data showed no significant difference at (P> 0.05) in the mean mycorrhizal infection between the treatments in both clones of the plant; VAM fungi root infections from the un amended soil was not significantly different from those of 50 and 100µg/g Bentex T-treated soils (Tables 2a and b). However there was significant clonal differences at (P<0.01) in the mycorrhizal infection rating (Table 3).

Table 1: Mycorrhizal Infection Ratings (%) of Root Segments from 14 weeks old Cassava (TMS 30572 and 30555) Grown in Soil Treated with Different titre of Bentex T.

Sample (Replicates)	Soil treatment(Bentex T titre µg g^{-1} soil)		
	0 (control)	50	100
	TMS 30572		
1	82[c]	70	62
2	84	72	56
3	80	68	58
4	88	76	54
Mean	**84**	**72**	**58**
	TMS 30555		
1	70	52	48
2	72	50	40
3	66	56	56
4	72	48	48
Mean	**70**	**52**	**48**

[c] *infected number out of 100 root segments examined (%)*

Table 2a: Analysis of Variance (ANOVA) for Mycorrhizal Infection Rate Data in TMS 30572.

	Sum of squares	df	Mean square	F	Significance
Between groups	20.667	2	10.333	1. 061	*0.243
Within groups	56.000	9	6.222		
Total	76. 667	11			

*P>0.05 = Not significant

Table 2b: Analysis of Variance (ANOVA) for Mycorrhizal Infection Rate Data in TMS 30555.

	Sum of squares	df	Mean square	F	Significance
Between groups	38.167	2	19.083	3.137	*0.093
Within groups	54.750	9	6.083		
Total	92.917	11			

**P>0.05 = Not significant*

Table 3: Analysis of Variance for Mycorrhizal Infection Rates in both TMS 30572 and 30555.

Source of variation	SS	df	MS	F	P-value [a]	F_{crit}
TMS	1231.667	1	1232.667	75.9863	**0.007068010	4.413863
Treatment	2360.333	2	1180.167	72.75	**0.000237571	3.554561
Interaction	112.3333	2	56.16667	3.462329	[NS]0.053430563	3.554561
Within	292	18	16.22222			
Total	3997.333	23				

*[a]** significance at P<0.01 ,F= frequency, P = calculated value, Fcrit = table value*
[NS] Not significant , SS = Sum of squares , df = degree of freedom, MS = mean square

Results of the chlorophyll a content of cassava plant grown in Bentex T treated soil samples show the mean value of chlorophyll a (chl a) obtained in leaf samples of the two clones (TMS 30572 and 30555) of cassava plant (Table 4). The chlorophyll content in both clones of the plant was highest at the 50µg/g Bentex T concentration; 133.9 and 78.2µg/mm2 for TMS 30572 and 30555, respectively.

Analysis of variance done on the data obtained showed that the treatments did not affect chl a differentially as there was no significant difference between treatments at (P> 0.05) in both clones of the plant (Table 5a and b). Relationship between mycorrhizal infection ratings and chlorophyll content was correlated(r=0.425) in TMS 30572 and also in TMS 30555(r=0.547). Correlation analysis is shown in Table 6.

Table 4: Chlorophyll a (Chl a) Content of Cassava Leaves, TMS 30572 and 30555 Grown in Soil Treated with Different Titres of Bentex T.

Sample (Replicates)	Soil treatment (Bentex T titre µg g^{-1} soil)		
	0 (control)	50	100
	TMS 30572		
1	77.5[a]	124.2	88.3
2	106.4	112.9	151.4
3	82.7	162.7	124.9
4	97.6	136.1	79.8
Mean	**91.1**	**133.9**	**111.1**
	TMS 30555		
1	70.4	63.8	88.4
2	79.2	76.4	80.7
3	63.0	82.0	71.1
4	61.4	90.5	61.3
Mean	**68.5**	**78.2**	**75.4**

[a]chl conc (µg/mm^2 leaf surface area)

Table 5a: Analysis of Variance for Chl a Data in TMS 30572.

	Sum of squares	df	mean square	F	Significance
Between groups	3690.432	2	1845.216	3.184	*0.090
Within groups	5215.258	9	579.473		
Total	8905.689	11			

**P> 0.05 = Not significant*

Table 5b: Analysis of Variance for Chl a Data in TMS 30555,

	Sum of squares	df	mean square	F	Significance
Between groups	198.282	2	99.141	0.902	*0.440
Within groups	989.475	9	101.942		
Total	1187.757	11			

**P>0.05 = Not significant*

Table 6: Correlation Analysis between Mycorrhizal Infection Ratings and Chlorophyll a Content of Cassava Clones (TMS30572 and 30555).

Clone	Correlation coefficient (r)	
TMS 30572	0.425	**
TMS 30555	0.547	**

*** Significant*
r >0.5 = significant

DISCUSSION

The growth response of cassava (*Manihot esculenta* Crantz) to fungicide treatment ranged from growth promotion to growth depression (Habte, 1994). Plant responses to colonization by mycorrhizal fungi have been observed to have a similar trend (Allen, 1992). The level of root colonization by VAM fungi affected the growth responses (photosynthetic ability) of the test cassava plant.

Vesicular arbuscular mycorrhiza VAM fungi contributed to the growth and development of the test cassava plant. However, the presence of VAM fungi lead to reduced photosynthetic ability in the plant as was observed in the study. The 50µg/g Bentex treated soil after about 8 weeks of cultivation thrived more than the un-amended test plant and had the highest chlorophyll content. This can be attributed to competition for carbon and other assimilates between plant and fungus (Harley and Smith, 1983).

Environmental factors which interfere with photosynthesis may also affect mycorrhizal root colonization and soil spore numbers; such factors include exposure to ozone (Trappe et al., 1984), shading and reduction in light intensity (Mosse, 1973; Hayman, 1980 and Harley and Smith, 1983). However, beneficial effects of VAM fungi on water relations and other plant physiological processes have been reported (Cock, 1985). In the study, chlorophyll concentration increased with subsequent increase in the rate of photosynthesis.

The test parameters examined had minimal variation at P > 0.05 between treatments in both clones of the test plant studied. The least value obtained for plant chlorophyll content was on the un amended soil (control). At P > 0.05 treatments levels did not affect chlorophyll a differentially in both clones of the cassava plant. However, clonal differences were observed in the level of root colonization by VAM fungi. Different cultivars of a plant species varying in symbiotic response to mycorrhizal fungi have been observed (Bagyaraj and Menge, 1978).

The mycorrhizal infection ratings in both clones of the plant were highest among roots with no Bentex T application (control) and lowest in roots of plants with the highest amount of Bentex T application. This is consistent with previous findings (Boatman *et al*., 1978).

Application of the systemic fungicide benomyl, has been shown to reduce development of VAM fungi in soil planted with a variety of crops such as cassava (Balota *et al*., 1997); onions and strawberries (Boatman *et al*., 1978) and potatoes (Ocampo and Hayman, 1980). In the last study cited, consistently fewer VAM spores were found in benomyl treated plots. The versatility of the cassava crop to adapt to low fertility soils has been related to the occurrence of VAM fungi (Balota *et al*., 1997). They showed that VAM inoculation increased cassava root yield by 42, 141 and 205% over un-inoculated plants for CV TMS 30572, 91934 and 4(2) 1425 respectively. Also, survival and development of cassava plantlets have been increased by modifying tissue culture protocols by mycorrhizal inoculation (Balota *et al*., 1997).

CONCLUSION

The presence of vesicular arbuscular mycorrhizal (VAM) fungi in roots of cassava plants promotes a reasonable performance of the crops especially in extremely infertile soil (Skogs and Lantbr, 1998). However, root colonization by VAM fungi may lead to growth depression as observed in the reduction of the photosynthetic ability of the un-amended test plant. Since agriculture is dependent on the biological processes of the soil, mycorrhiza a key factor in nutrient and carbon recycling is of utmost relevance. Mycorrhizal technology should be studied and researched on to boost cassava growth and development.

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