Improvement of Provitamin A in Maize Varieties Using Arbuscular Mycorrhizal Fungus, *Glomus clarum*

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Abstract

Arbuscular mycorrhizal fungus (AMF, *Glomus clarum*) has been used widely as a bio-amendment and bio-control agent in several biotechnological studies. In this study, biofortification of maize with provitamin A using AMF was investigated. Five maize varieties (V1 = white drought-resistant maize, V2= yellow provitamin A maize, V3= white drought-tolerant maize, V4= yellow striga-resistant maize and V5= white striga-resistant maize) were evaluated in a screen house experiment laid out in a completely randomized design with three treatments: T1 = maize + AMF before planting, T2 = maize + AMF, inoculated two weeks after planting and T3 (control) = maize only, and four replications. The result showed that AMF significantly ($p<0.05$, $p = 0.0029$) increased the provitamin A level of the maize varieties. White drought-tolerant maize (V3) had the highest provitamin A content (581.57 µg) after harvest, while the least (288.33 µg) was found in white drought-resistant maize (V1). Also, the effect of the treatments on the growth traits (plant height, leaf length, number of leaves per plant) of the maize varieties was highly significant. Therefore, AMF could be considered in breeding maize with high provitamin A content and improved morphological characters.

Keywords: Bio-fortification, Pro-vitamin A, *Glomus clarum*, *Zea mays*, Carotenoids.

Introduction

Maize (*Zea mays* L.) is a very popular cereal crop and a member of the popular grass family Poaceae, commonly known as corn in many countries (Scott and Emery, 2016). It is a stout, monoecious annual plant with overlapping sheath and well extended distichous blades (OECD, 2006). The seed endosperm contains natural provitamin A carotenoid pigments, which are responsible for the diversity of its kernel colours, which may be banded, spotted or stripped (OECD, 2006; Cuttriss *et al.* 2011). While the two most common varieties, the white and yellow maize, derived their colours from lutein and zeaxanthin, the red variety is due to anthocyanins and phlopaphenes, whereas, chrysanthemum is responsible for the kernels in purple corns (Kuhnen *et al.*, 2011).

Maize is an economically important crop of numerous domestic and industrial uses. Different parts of and products of the plants are used for various purposes (Olawuyi *et al.*, 2010; 2013). The grain is eaten fresh or dried, and are processed into human foods and livestock feeds, while other parts of the plant, known as corn stover are utilized as fodder, bedding for litters and soil enrichers (Olakojo *et al.*, 2007; Heuze *et al.*, 2017). In chemical industries, starch from maize is used to manufacture adhesives, fabrics, plastics and some other products. Biochemical laboratories produce corn steep liquor (a complex culture medium used for growing microbes for research purposes) through maize milling (Salam and Ishaq, 2019).

The arbuscular mycorrhizal (AM) association formed between the roots of higher plants and fungi belonging to the phylum Glomeromycota is the most prevalent ecosystem mycorrhizal symbiosis formed by the majority of the vascular flowering plants all over the world (Harrier, 2001). It dates as far back as the time of the first land plants, approximately 400 million years ago, and has improved sustainably in terms of physiological, nutritional and ecological importance as a consequence of co-evolution (Rodrigues and Rodrigues, 2014).This interaction accounts for around 80% of symbiotic associations found in land plants including crops of agricultural and horticultural importance and is a critical factor for determining the productivity and diversity of natural terrestrial ecosystems (Smith and Read, 1997; Jeffries *et al.*, 2003; Rodrigues and Rodrigues, 2014).

Although mycorrhizal associations are diverse structurally and functionally, various studies conducted on mycorrhizal physiology and ecology in the last four decades had helped to unravel its roles such as plant nutrient acquisition through the formation of mycelial network in the rhizosphere, protecting host species from abiotic (drought, salinity, heavy metals and deficiency of certain nutrients such as nitrogen and phosphorus), biotic stresses and modulating interactions between competitors in terrestrial ecosystems (Smith and Read, 2007; Abiala *et al.*, 2013; Olawuyi *et al.*, 2013, 2014; Rodrigues and Rodrigues, 2014). Maize has low vitamin A content; meanwhile deficiency of vitamin A is a general health issue in many developing countries, which has necessitated the biofortification of plant-based foods with provitamin A carotenoids. Since the method was successfully inoculated in the production of 'golden rice', many other biofortified crops have been produced (Giuliano, 2017). Biofortification involves the enhancement of the bioavailability and levels of constituent nutrients in crops through conventional breeding and genetic engineering (White and Broadly, 2005; Uchendu, 2013). It is usually employed in developing countries to enrich commonly consumed staple crops that are deficient in certain micronutrients so as to reduce health issues (caused by micronutrient malnutrition), increase food availability and life quality (Bious and Saltzman, 2017). Biofortification technique has been used to increase the amount of various micronutrients such as vitamin A, in cassava, maize, orange sweet potato, banana, plantain; zinc in sorghum, rice, wheat, beans, millet, lentils, Irish potato; iron in rice, wheat, beans, millet, lentil, Irish potato and cowpea (HarvestPlus, 2014). The utilization of AMF as bio-agent for plant improvement had been widely demonstrated by researchers. Li *et al.* (2006) reported the improvement of phosphorus uptake in wheat by AMF. Liu *et al.* (2000) documented the role of AMF root colonization in the improvement of above soil biomass in maize. In cucumber, AMF increases the survival rate of seedlings, fruit yield and concentration of zinc and phosphorus in shoots (Ortas, 2010). However, despite the popularity of biofortification and the use of AMF for boosting nutritional qualities, there are limited information on fortification of maize with pro-vitamin A using arbuscular mycorrhiza fungus. Therefore, this study aimed at evaluating the performance of *Glomus clarum* in fortification of provitamin A in maize.

MATERIALS AND METHODS

Experimental Locations, Sources of Soil, AMF (*Glomus clarum*) and Plant Materials

The experiment was carried out at the nursery farm of the Department of Botany, University of Ibadan, Ibadan, Nigeria (Longitude 7.4417N and Latitude 3.900 E) from November 2019 to January 2020. Top soil was obtained from the nursery, sterilized with electric soil sterilizer and cooled down before filling up 7kg of soil in polythene bags used for planting.

Arbuscular Mycorrhizal Fungi (AMF) used (*Glomus clarum*) were obtained from the Molecular Biology and Genetics Research Laboratory, Department of Botany, University of Ibadan, Ibadan. while the five varieties of maize seeds used (V1= white drought-resistant maize, V2= yellow provitamin A maize, V3 = white drought-tolerant maize, V4= yellow striga-resistant maize and V5= white striga-resistant maize) were sourced from International Institute of Tropical Agriculture, IITA, Ibadan.

Experimental Design and Treatments

The field experiment was carried out in complete randomized design (CRD) using four replicates. The already prepared *Glomus clarum* inocula in a mixture of macerated root, spores and soil were used. The inoculation was done following the procedure described by Olawuyi *et al.* 2014 and Olowe *et al.* 2020 with slight modifications. The three treatments were: Treatment 1 (T1) = Maize + AMF (inoculated before planting); Treatment 2 (T2) = Maize + AMF (inoculated two weeks after planting) and Treatment 3 (T3) = Maize only (control). Prior to planting, the potted soils designated for T1 were drenched with 15g (48 spores) of AMF while the same quantity of AMF was inoculated into soils for T2 close to the root of the maize plants 28 days after planting. A total of 60 maize plants were cultivated.

In vitro Propagation of Maize

Prior to field experiment, *in vitro* germination was carried out in order to obtain leaves for provitamin A analysis. Equal number of seeds for each variety were washed with water and detergent (Tween 20), disinfected with 70% ethanol for 5 minutes and rinsed three times with distilled water in order to prevent microbial contamination. The seeds from each variety were placed between two Whatman number 1 filter papers laid on Petridish and 10ml of water was dispensed from a syringe in order to moisten them. Germination started after 5days, but the set ups were left for some days to have enough leaves for provitamin A analysis.

Provitamin A Analyses

The Provitamin A analyses of leaves of the maize varieties were carried out before and after the field experiment at Kappa Biotechnology Laboratory, Ibadan, Oyo State. The amount of Provitamin A (beta carotene) was determined according to the procedure described by Aremu and Nweze (2017).

Planting and Agronomic Practices

Two seeds each of five maize varieties were planted per polythene bag containing sterile soil. Thinning was done two weeks after planting. Daily watering of each experimental pot was ensured with 30 ml of distilled water, while weeding was carried out from time to time.

Data Collection and Statistical Analysis

Four growth characters (Plant height (PH), Leaf length (LL), Leaf width (LW) and Number of Leaves (NL)) were observed and recorded on weekly basis. PH, LL and LL were measured with metre rule and ruler while NL was determined by physical counting. The data collected were subjected to Analysis of Variance (ANOVA) using SAS ver. 9.3 software. The differences in means were separated using Duncan Multiple Range Test at 95% level of probability ($p < 0.05$). Relationships among the growth characters were established using Pearson Correlation Coefficient and Principal Component Analysis (PCA).

RESULTS

Provitamin A analysis of Maize

The result in Table 1a shows the provitamin A analysis of maize leaves from *in vitro* propagation of maize seeds. Yellow provitamin A maize had the highest amount of provitamin A (121.53µg); this is followed by the amount present in white drought-tolerant maize (106.33 µg). Yellow striga-resistant maize and white drought-resistant maize had 99.73µg and 77.34µg respectively while the least value (68.47µg) was found in white

striga-resistant maize. The selection of result in Table 1b was based on the performance of the treated maize and control. There was no significant difference in the provitamin A content of the untreated maize (V1T2, V2T2, V4T2 and V5T2). Conversely, the amount of provitamin A present in the leaves of the five maize varieties after harvesting as shown in Table 1b indicates a roughly opposite trend. The highest amount of vitamin A (581.57µg) was present in V3T2, the second-highest (511.67µg) and the least (288.33µg) were found in V5T1 and V1T3 (control) respectively. The contents in yellow provitamin A treated with AMF before planting (418.33 µg) and untreated yellow provitamin A, control (392.67 µg) were relatively much closer.

Table 1a: Provitamin A analysis of maize leaves from *in vitro* propagation of maize seeds

Varieties	Description	Provitamin A content (ug)
V1	White drought-resistant maize	77.34
V2	Yellow provitamin A maize	121.53
V3	White drought-tolerant maize	106.33
V4	Yellow striga-resistant maize	99.73
V5	White striga-resistant maize	68.47

Table1b: Provitamin A analysis of maize leaves after harvesting

Varieties	Description	Provitamin A content (ug)
V1T1	White drought-resistant maize	336.67
V1T3	White drought-resistant maize	288.33
V2T1	Yellow provitamin A maize	418.33
V2T3	Yellow provitamin A maize	392.67
V3T2	White drought-tolerant maize	581.57
V4T1	Yellow striga-resistant maize	461.67
V5T1	White striga-resistant maize	511.67

T1: AMF inoculated before planting; T2: AMF inoculated two weeks after planting; T3: Control.

Interactive Effects of Varieties, Treatments, Weeks and Replicates

The mean square effects of varieties, treatment, weeks after planting and replicates of maize on growth and agronomic data are shown in Table 2. The result indicated that varieties and treatments had highly significant effect (p<0.01) on plant height, leaf length and number of leaves. The first order interaction for treatment and variety (T×V) produced highly significant effects in all the four morphological characters (leaf length, plant height, leaf width and number of leaves). In the case of treatment and week (T×W), all except leaf length (which is only significant at p<0.05) exhibited highly significant effect. This was similar to that observed with variety and week (V×W), and the second order interaction (T×W×V), except that the leaf lengths in both cases were not significant.

Effects of Treatments on Growth Characters of Maize

The effects of the treatment on the morphological characters (Table 2) revealed that, the leaf length, leaf width and number of leaves for maize plants inoculated two weeks after planting (T2) and control (T3) are not significantly different,

Table 2: Mean square interactive effects on number of varieties, treatment, weeks, and replicates

Source of variation	Df	Leaf length	Plant height	Leaf width	Number of leaves
Replicate	3	1780.57**	16129.69**	12.85**	24.12**
Treatment (T)	2	178.58**	2491.96**	1.57**	5.65**
Varieties (V)	4	88.85**	451.62**	0.66**	1.13**
Weeks (W)	6	27007.45**	25143.29**	190**	2448.03**
T × V	8	232.51**	313.23**	1.46**	0.60**
T × W	12	8.08*	21.91**	0.05**	5.65**
V × W	24	3.26	13.34**	0.04**	1.13**
T × V × W	48	2.44	12.285**	0.02**	0.60**

*=significant at p<0.05, **= highly significant at p<0.01, DF: degree of freedom

Table 3: Effects of treatment on growth characters of maize

Treatment	Leaf length	Plant height	Leaf width	Number of leaves
T1	45.87[b]	46.51[c]	3.87[b]	4.13[b]
T2	47.98[a]	54.76[a]	4.07[a]	4.55[a]
T3	47.55[a]	52.18[b]	4.02[a]	4.87[a]

Treatment 1: AMF inoculated before planting, Treatment 2: AMF inoculated two weeks after planting and Treatment 3: Control. Means with the same letter in the same column are not significantly different at p≥0.05 using Duncan's Multiple Range Test (DMRT)

but are higher than maize plants inoculated before planting (T1). In the case of plant height, T2 is relatively higher and more significant.

Varietal Influence on Growth Characters of Maize

The result in Table 4 shows that plant height (54.02cm), leaf length (47.09cm) and leaf width (4.08cm) in yellow provitamin A maize (V2) is significantly higher (p<0.05), but not different from all other varieties. The leaf length, leaf width and number of leaves in V2, white drought-tolerant maize (V3) and white striga-resistant maize (V5) are not significantly different (p>0.05) from one another.

White drought-resistant maize (V1) was the least performed variety for leaf length (45.66cm) and leaf width (3.86cm), yellow striga-resistant maize (V4) for plant height (48.21cm) and V5 for number of leaves (4.17). White drought-resistant maize (V1), white drought-tolerant maize (V3) and white striga-resistant maize (V5) are significantly different (p<0.05) in terms of plant height with

values 52.26, 51.83 and 49.44cm respectively. The leaf length and leaf width of yellow provitamin A, yellow drought-tolerant maize and white striga-resistant maize are not significantly (p>0.05) different according to the separation of their means. V1 had the lowest value (45.66cm) for leaf length, while V2, V3 and V5 are not significantly different, but produced significant higher values (4.08, 4.05, and 4.02cm respectively) for leaf width. However, the number of leaves per plant had significant effect (p<0.05) on all the varieties.

Principal Component Analysis (PCA) On Growth Characters of Maize

The PCA result in Table 5 grouped the five maize varieties into four Principal axes, Prin 1, 2, 3 and 4, meanwhile, Prin 1 accounted for the highest percentage and Eigen value (62.72% and 2.51 respectively). It is obvious that leaf length and leaf width are closely related, having roughly similar Eigen values which ranged between -0.38 and 0.57 in the first three Principal axes. A similar

relationship is found in Prin 4 for plant height and number of leaves with 0.04 and 0.01 respectively.

Correlation Analysis of Growth Characters of Maize.

The result of the correlation analysis of growth characters of maize is shown in Table 6. Positive and very strong correlations ($p < 0.05$) were found between plant height/leaf length ($r = 0.93$), plant height/leaf width ($r = 0.93$), leaf length/leaf width ($r = 0.99$), plant height/number of leaves ($r = 0.95$), number of leaves/leaf length ($r = 0.95$), number of leaves and leaf width ($r = 0.95$). These are similar to those found in weeks and all the four morphological characters (where r lied between 0.89 and 0.97).

DISCUSSION

There were significant variations in the contents of provitamin A in the five varieties of maize investigated. The provitamin A contents in the maize varieties increased significantly with AMF biofortification. This finding is in accordance with the report by Hart *et al.* (2015) that AMF has the potential to boost the level of carotenoids present in crops. The enhancement of production of phytochemical compounds which has tendency to improve nutritional values of crops apart from provitamin A by AMF has also been reported by Sbrana *et al.* (2014) and Rouphael *et al.* (2015).

Table 4: Varietal influence on growth characters of maize

Varieties	Leaf length	Plant height	Leaf width	Number of leaves
V1	45.66[c]	52.26[b]	3.86[b]	4.71[a]
V2	47.90[a]	54.02[a]	4.08[a]	4.67[a]
V3	47.83[a]	51.83[b]	4.05[a]	4.46[a]
V4	46.56[b]	48.21[d]	3.93[b]	4.58[a]
V5	47.71[a]	49.44[c]	4.02[a]	4.17[a]

Means with the same letter in the same column are not significantly different at $p \geq 0.05$ using Duncan's Multiple Range Test (DMRT).

Table 5: Principal component analysis (PCA) on growth characters of maize

	Prin 1	Prin 2	Prin 3	Prin 4
Leaf length	0.57	-0.38	0.12	-0.72
Plant height	0.49	0.40	-0.78	0.04
Leaf width	0.56	-0.42	0.16	0.70
No of leaves	0.36	0.71	0.60	0.01
Eigen value	2.51	1.03	0.40	0.06
Proportion %	62.72	25.85	10.03	1.40

Table 6: Correlation of growth characters of maize.

Correlation	LL	PH	LW	NL	T	V	W	R
LL								
PH	0.93							
LW	0.99	0.93						
NL	0.95	0.95	0.95					
T	0.04	0.11	0.05	0.04				
V	0.01	-0.06	0.01	-0.04	0			
W	0.96	0.89	0.97	0.95	0	0		
R	-0.11	-0.32	0.11	-0.16	0	0	0	

LL: Leaf Length, PH: Plant Height, LW: Leaf Width, NL: No of Leaves, T: Treatment, V: Varieties, W: Weeks and R: Replicate; **=highly significant at $p<0.05$

The morphological characters of the candidate varieties are significantly different from one another, and this situation is the same with the first and second order interactions of the traits. Thus, this result is in agreement with the studies of Olawuyi *et al.* (2012) and Olawuyi and Onuoah (2017) who reported significant variances among some growth and yield traits of *Abelmoschus esculentus* and *Amaranthus* genotypes respectively, treated with *Glomus clarum*. This study also established that *G. clarum* has more tendencies to improve growth characters of maize, especially plant height, in the early growth. Plant growth is promoted when the root surface area increases through the hyphal network formed by the AMF (Ahanger *et al.,* 2014; Salam *et al.*, 2017). This observation is in agreement with earlier report by Nakmee *et al.* (2016) where plant height and other growth characters of sorghum was enhanced with AMF. The use of AMF as plant growth enhancer and biocontrol agent had been widely investigated. Mycorrhiza-plant interaction facilitates the release of essential organic acids, enzymes and siderophores capable of increasing mineral concentrations in many crops through degradation of organic compounds (Smith and Read, 2008; Monika *et al.*, 2018**).** Olawuyi *et al.* (2011) reported the suppression of pathogenic effects of striga disease which includes inhibition of plant growth, and improvement of soil fertility by AMF in maize.

The best growth performance was observed in yellow provitamin A maize treated with AM. This could be as a result of AM enhancing the growth of the host under unfavourable conditions by modulating a series of complex reactions between the plant and the fungus thereby bringing about improvements of traits connected with photosynthesis and gaseous exchange (Birhane *et al.,* 2012). The host benefits from a mycorrhizal relationship are not only determined by species of the fungal partner, the host genetic constitution, available nutrient, stress and environmental factors also play an integral role (Trouvelot *et al.*, 2015; Chen *et al.*, 2018). Therefore, other maize varieties may perform better with appropriate mycorrhizal species (Olawuyi *et al.*, 2014).

The highest percentage and magnitude of Eigen vector revealed in Prin 1 showed that the maize varieties exhibit significant morphological variations that could be explored in maize breeding and improvement. Correlations among the growth parameters are strong; this reveals that the genetic constitution of the varieties of maize had huge and significant impact on the expression of the traits (Olawuyi *et al.*, 2015).

CONCLUSION AND RECOMMENDATIONS

Putting into consideration the performances of the candidate maize varieties with respect to their responses to AM biofortification, white drought-tolerant maize had the best potential, and could be useful in alleviating deficiency of vitamin A in many vulnerable citizens in underdeveloped countries where maize is an indispensable staple food. The application of the beneficial micro-organism, AMF (*Glomus clarum)* as a bio-amendment significantly enhanced provitamin A in all the five maize varieties. This enhancement also brought about significant improvement in the morphological characters of maize studied. It is therefore recommended that, for agronomic improvement of growth and yield characters of maize and other related crops, AMF could be utilized as an organic amendment and further improvement of the maize varieties with higher performances through selective breeding should be encouraged. More maize varieties performed better when AMF was applied before planting. Therefore, AMF is an efficient soil amendment for maize production that could be considered in breeding maize with high provitamin A content and improved morphological characters. Further experiments that will consider other important factors, such as environment and soil conditions, concentration and time of application should be conducted to help understand more about fortification of provitamin A content in maize using AMF.

Competing interests

The authors declare that they have no competing interests.

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