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Influence of Arbuscular mycorrhiza fungi (AMF) on drought tolerance and charcoal rot disease of cowpea

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ABSTRACT

The influence of Arbuscular mycorrhiza fungi (AMF) (*Glomus deserticola* and *Gigaspora gigantea)* were evaluated on drought tolerance and charcoal rot disease of cowpea genotypes: IT90K-277-2, IT84S-2246-4 and IT06K123-1. IT90K-277-2 and IT84S-2246-4 were sown in 3 kg of sterilized soil for drought experiment with five treatments. Treatment was established thirty days after germination with inoculation of *G. deserticola*, the mycorrhizal treated cowpea withstand the water stress and produced high yield. Biocontrol experiment had 2 kg sterilized soil potted into bags with cultivars IT90K-277-2 and IT06K123-1, fourteen treatments were established with soil drenched before planting and simultaneous inoculation. Soil drenched with AMF before planting and inoculation of *M. phaseolina* after 10 days of germination recorded higher growth parameters, while the simultaneous inoculated plant was the most effective in reducing disease severity. However, simultaneous treatment of *G. deserticola*, *G. gigantea* and *M. phaseolina* were most effective for both growth parameters and reduction of disease severity.

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is one of the most important drought-resistant leguminous food crops grown for its foliage and grain in tropical and sub topical Saharan Africa regions of the world particularly in Nigeria [1,2]. There are variations among cowpea genotypes in their drought tolerating potentials [3,4]. Turk et al. [5] cited in Ahmed and Suliaman [6] affirmed that cowpea is highly sensitive to water stress during the flowering and pod-filling stages. Though, there are limited information on the response of the crop to drought at different stages of growth. According to Ajibade and Amusa [7], its cultivation in humid agroecologies of South West Nigeria is also faced with several pests and diseases such as brown blotch, anthracnose , *cercospora* leaf spot, *choaniphora* pod rot, false smut and web blight, charcoal rot and *sclerotium* stem blight of which *Macrophomina phaseolina* is included. The effect of field diseases has led to reduction in yield of cowpea.

Macrophomina phaseolina (Tassi) Goidanich, is one of the most destructive plant pathogens in the tropics and subtropics causing diseases in a wide range of host plant [8,9]. The pathogen was detected in Chile in 1983 in *Pinus radiata* D., Don nurseries in the Bío-Bío Region In the last few years, dissemination of the pathogen had been detected from the nurseries to the plantations through asymptomatic plants. Mortality of plant would be observed in the first years of the plantation when they are predisposed to conditions such as hydraulic stress and high soil temperatures [10]. *M. phaseolina* is a saprophyte that survives in the soil due to micro-sclerotinia formation which is pseudoparenchymal tissue masses resistant to adverse environmental conditions [11].

The most successful control strategy for charcoal root rot in forest nurseries was soil fumigated with methyl bromide [10]. Although, some of the problems associated with the application of chemicals include high cost, environmental pollution, breaking up the ecological balance of the soil, as well as the destruction of the ozone layer [12,13]. Biological control has been considered as an alternative selective method to control this disease [14]. Several researchers had focused on antagonist microbes such as *Bacillus, Pseudomonas, Streptomyces, Trichoderma, Penicillium, Rhizopus, Aspergillus* but there are limited studies on the adoption of mycorrhiza fungi biotechnology as control strategy Olawuyi et al., 2014a,b.

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Mycorrhiza association is a symbiotic non-pathogenic relationship between plant roots and fungal hyphal [15,16]. In this relationship, the fungi obtain carbon compounds and other nutritional requirements from the symbiotic plant roots, and in return, supply the plant with most of the immobile mineral elements such as Nitrogen (N), Phosphorus (P), Potassium (K), Calcuim (Ca), Copper (Cu) and Zinc (Zn) from the soil solution. The importance of mycorrhiza association in both agricultural and ecological systems had earlier been widely recognised [17,15]. The increased plant growth by VAM association is usually due to increased mineral elements uptake by the hyphae from the soil [18], Olawuyi et al., 2012), improved water relations and pest resistance of host plants [19,20], plants tolerance to a variety of abiotic stresses [21], increased resistance to soil pathogens [22,23,24], 2013). Mycorrhizae can also resist drought in many plants under stress conditions therefore the plants infected with VA mycorrhizae are less likely to wilt under drought affected conditions [25,26,27].

Therefore, the study aimed at investigating the influence of AMF (*Glomus deserticola*) on water stress in cowpea and established the effect of AMF (*Glomus deserticola and Gigaspora gigantea*) on *Macrophomina phaseolina* causing charcoal rot in cowpea.

2. Materials and methods

2.1. Experimental location and research design

Planting of cowpea seeds was done at the screen house, while the isolation and identification were carried out at the research farm and pathology laboratory respectively in the Department of Botany, University of Ibadan. complete randomized design was used for the two experiments with three replicates.

2.2. Source of seed samples and inocula

Cowpea seeds were collected from the germplasm unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Seeds varieties include: drought susceptible (IT90K-277-2 and IT84S-2246-4), and pathogen susceptible (IT06K-123-1 and IT90K-277-2). Rhizosphere soil of cowpea where the pathogenic organism was isolated, was also collected from IITA, while Arbuscular mycorhiza fungi (*Glomus deserticola* and *Gigaspora gigantea*) were obtained from the Department of Botany, University of Ibadan.

2.3. Media preparation, isolation of organism and slide preparation

20 g of PDA dissolved in 500mls of distilled water was prepared and autoclaved at 121 °C for 15mins.The prepared PDA was allowed to cool to 45 °C, while streptomycin was added to inhibit the growth of bacteria and the solution was gently swirled to obtain a homogeneous mixture. 2 g of soil sample was serially diluted using dilution factors of 10^{-1}, 10^{-2},10^{-3},10^{-4},$10^{-5}$and 10^{-6}, 1 ml is taken each from 10^{-1}, 10^{-3} and 10^{-6} inoculated into prepared Potato dextrose Agar media (PDA) in Petri dishes using pour plate method, Incubation was done at room temperature for 5–7 days. Mixed culture was sub-cultured to obtain pure culture of the pathogen. Stock culture was obtained from the pure culture of the organism which were prepared on PDA slant and stored at about 4 °C in the refrigerator. Slides of pure cultures obtained were prepared using sterilized needle to pick mycelia growth of the organism onto the slide surface. With the aid of the needle and addition of drop of sterile distilled water, the mycelia were properly dislodged. A drop of lacto phenol in cotton blue stain was then added to aid clarity when viewed under microscope.

2.4. Morphological identification and characterization of fungi isolates

The identification and classification of these structures were compared to that of Domsch et al. [28] in compendium of soil fungi.

The electronic microscope was used in viewing the prepared slides for the various morphological characters ranging from micro conidia, macro conidia, conidia shape and hyphal arrangement.

2.5. Soil sterilization and seed viability test

The soil (sandy loam soil) used in conducting the research work was collected from the Research farm of the Department of Botany, University of Ibadan. Sterilization of the soil was done using electric soil sterilizer and allowed to cool before packed into pottling bags.

The test for viability was done using the method of, in which 10 seeds were sown per bag. Each variety was sown in triplicates and the percentage germination was obtained from the formulae below;

$$\%\text{germination} = \frac{\text{No. of germinated seed}}{\text{Totalno of seed planted}} \times 100$$

2.6. Seed planting and pathogenicity test

A sterilized sandy loam soil weighing 2 kg and 3 kg potted into pottling bags were used for disease resistance and drought tolerance experiments in varieties of cowpea respectively. Ten seeds were planted per bag and then thinned to two per bag after three weeks of growth.

The pathogenicity test was carried out using modified method of Ahmed et al. [51]. The mycelia suspension of *Macrophomina phaseolina* isolate was prepared by blending ten 5 mm mycelia disc from 10 to 15 day-old culture of the fungus in 100 ml of sterile distilled water using warring blender. 5 ml of the inoculum mycelia suspension was injected round the wounded region of the lower stem of the seedlings after 10 days of germination.

Reactions of cowpea varieties to drought as influenced by Glomus deserticola

Ten seeds each of the drought susceptible cowpea varieties (IT90K-277-2 and IT84S-2246-4) were sown separately in 3 kg sterilized sandy loam soil. Germination of seeds was observed after 4 days of planting. The plants were thinned to two seedlings per plant after 14 days of emergence. Five treatments were established on the two cowpea varieties after 30 days of germination with inoculation of 5 ml mycelia suspension of *Macrophomina phaseolina* and 10 g (32spores) of *Glomus deserticola*. These treatments included;

T1- cowpea alone + watering
T2- cowpea alone + water stressed
T3- cowpea infested + AMF (*Glomus deserticola*) + water stressed
T4- cowpea infested + AMF *(Glomus deserticola*) + watering
T5- cowpea infested + AMF (*Glomus deserticola*) + *Macrophomina phaseolina* + water stressed

The rating scale for drought was from 1 to 5 according to the procedures of Auge [29], Al-Karaki et al. [30] and Olawuyi et al. Olawuyi et al. (2011b,c).

1. Excellent, normal plant growth, number of plant less than 10% water stress.
2. Good, slight drought but noticeable stunting, slight reduced leaf and number of leaf 11–25%