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Screening of Cowpea Genotypes for Resistance to *Macrophomina phaseolina* Infection using Two Methods of Inoculation

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ABSTRACT

Five cowpea cultivars, ITO3K -316-1, ITO4K-217-5, IT06K-154-1, IT99K-216-44 and IT03K-369-3, obtained from the International Institute of Tropical Agriculture, Ibadan were screened for resistance to *Macrophomina phaseolin*a infection using two methods of inoculation viz, pouring of spore/mycelia suspension in the soil and wrapping of inoculum meal around wounded lower stem of the seedlings. Cowpea cultivar ITO4K-217-5 was resistant to the pathogen in both inoculation methods while the other four cowpea cultivars i.e., ITO3K-316-1, IT99K-216-44, ITO6K-154-1 and ITO3K-369-3 showed varying degree of susceptibility to the pathogen in both inoculation methods.

Key words: Resistance, cowpea genotypes, *Macrophomina phaseolina,* inoculation, cultivar

INTRODUCTION

Cowpea is an important grain legume widely consumed in Nigeria as a cheap source of high quality protein and other important nutrients (Phillips and Baker, 1987; Olajide and Olaoye, 1999; El-Jasser, 2010). Nigeria is the world's largest producer of the crop (Oparaeke *et al*., 2005; USAID, 2008). Greatest losses occur as a result of seed decay and seedling damping-off caused by a number of pathogens including *Macrophomina phaseolina* (Emechebe and Shoyinka, 1985). *Macrophomina phaseolina* (Tassi) Goid (Synonym *Rhizoctonia bataticola* (Taub) Butler) causes other diseases like charcoal rot, dry root rot, wilt, leaf blight and stem blight in a wide range of hosts including cowpea (Young, 1949; Ghaffar *et al*., 1964). Micro-sclerotia in the soil and plant waste is the major source of inoculum to cause new infections (Khan, 2007). Cultural, chemical or biological strategies for disease management are not adequate to control the disease efficiently or economically. Identification of germplasm with resistance to the pathogen appears promising for reducing damage and yield losses caused by the pathogen. This study was carried out to evaluate the reaction of five cowpea cultivars to infection by *Macrophomina phaseolina* using two inoculation procedures.

MATERIALS AND METHODS

The cowpea seeds used, IT0K-316-1, IT04K-217-5, IT06K-154-1, IT03K-369-3, IT99K-216-44, were obtained from the International Institute for Tropical Agriculture (IITA) Ibadan. Clean and healthy seeds were separated from physically damaged ones and were then kept in polythene bags before use. The inoculum used, *Macrophomina phaseolina* (Tassi) Goid, was also obtained from the

pathology laboratory of IITA Ibadan. The inoculum culture was stored in sterile Acidified Potato Dextrose Agar (APDA) slant in McCartney bottles until needed.

Two and half kilogram of sandy loam soil previously autoclaved at 121°C for 1 h was weighed into perforated plastic pots (30×25×12 cm). Three pot replicates was set-up for each of the five cowpea cultivars, two inoculation methods and a control giving a total of forty five treatment units. The inoculation methods used were: Pouring 5 mL of the inoculum mycelia/spore suspension around exposed feeder roots of the seedlings; wrapping of 0.5 g of inoculum meal around the seedling stem wounded by slightly rubbing it with sterile carborundum. The control units did not receive any inoculum. The mycelia/spore suspension was prepared by blending ten 5 mm mycelia disc from 10-15 day-old culture of the fungus in 100 mL of sterile distilled water in a warring blender. The inoculum meal was prepared by adding ten 5 mm mycelia disc from 10-15 day old culture of the fungus to 50 g of sterile ground wheat in 100 mL conical flask. The mixture was moistened to facilitate growth of the fungus. After 14 days of incubation period at room temperature, the mixture was air dried for 48 h.

Five seeds from each of the cowpea cultivars were sown in each of the pots. Thinning to three seedlings per pot was later carried out 7 days after planting and the seedlings were inoculated 10 days after planting. The plants were watered daily using sterile distilled water.

Disease incidence was recorded as percentage of seedlings with symptoms noting the number of days after inoculation to the first appearance of the symptoms. Seedlings were also scored for disease severity on 7th day after inoculation using modified Persson *et al.* (1997) scale as shown below:

- 0 = Healthy plant without any visible symptom
- 5 = Discolouration of less than 5 mm of the root system
- 10 = Discolouration of about 20 mm of the root system
- 25 = About 5% of the root system discoloured
- 50 = The whole root system discoloured but no symptom on the epicotyls or leaves
- 75 = The whole root system as well as epicotyls discoloured with the lower leaves wilted
- 100 = Dead plants

Information on height of the plants, fresh shoot and root weights were recorded 35 days after planting. Data analysis was carried out using COSTAT statistical package and comparison of means was done using Duncan Multiple Range Test at 0.5% level of probability.

RESULTS AND DISCUSSION

Cowpea cultivar IT03K-316-1 was the first to show symptoms of infection by *Macrophomina phaseolina* (Fig. 1) with mycelia suspension inoculation method. The symptoms which ranged from chlorotic leaves to lower stem discoloration (Fig. 2, 3) appeared 7 days after seedling inoculation. Symptoms appearance however came earlier in all cowpea cultivars inoculated with wrapping of inoculum meal around the stem. The symptoms appeared 3, 4, 4, 4, 5 days after seedling inoculation for cultivars IT03K-369-3, IT03K-316-1, IT04K-217-5, IT99K-216-44 and IT06K-154-1, respectively (Table 1). The table also shows that the percentage incidence of the disease was more in the inoculum meal inoculation method than in the inoculum suspension method of inoculation. The percentage incidence in the former ranged from 66.6 to 100 while it ranged from 0 to 66.6 in the latter.

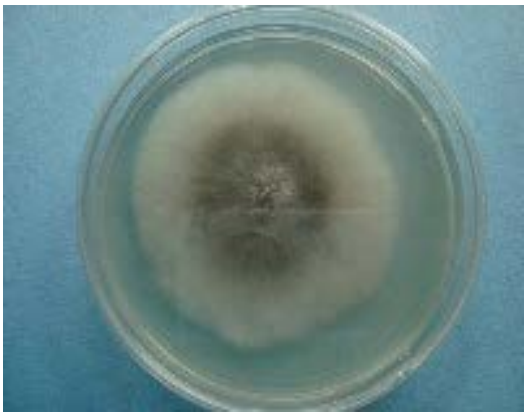

Fig. 1: Five day-old pure culture of *Macrophomina phaseolina* on acidified potato dextrose agar

Fig. 2: Seventeen day old seedling showing symptom of Charcoal rot disease

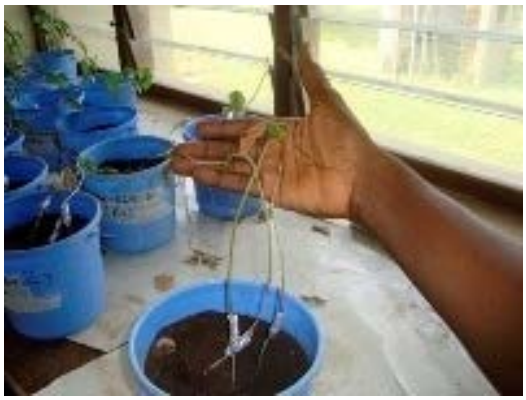

Fig. 3: Seedling dying of charcoal rot infection

Table 1: Incidence of charcoal rot disease in cowpea cultivars inoculated with *Macrophomina phaseolina*

Inoculation method	Cowpea cultivars	Days after inoculation to first appearance of symptoms	% incidence of disease 28 days after inoculation
Mycelial suspension	IT03K-316-1	7	66.6
	IT04K-217-5	20	33.3
	IT06K-154-1	18	66.6
	IT03K-369-3	15	66.6
	IT99K-216-44	26	0.00
Inoculum meal	IT03K-316-1	4	66.6
	IT04K-217-5	4	100
	IT06K-154-1	5	100
	IT03K-369-3	3	100
	IT99K-216-44	4	100

Table 2: Severity Index and susceptibility class of cowpea cultivars to charcoal rot disease

Inoculation methods	Cowpea cultivars	Disease severity index	Plant rating [a]	Susceptibility/resistance class
Spore/mycelial suspension	IT03K-316-1	0.0[a]	0	Highly resistant
	IT04K-217-5	0.0[a]	0	Highly resistant
	IT06K-154-1	4.66[b]	1	Moderately resistant
	IT03K-369-3	4.66[b]	1	Moderately resistant
	IT99K-216-44	6.66[b]	2	Moderately resistant
Inoculum meal	IT03K-316-1	70[e]	5	Highly susceptible
	IT04K-217-5	33.33[bc]	4	Moderately resistant
	IT06K-154-1	66.66[e]	5	Highly susceptible
	IT03K-369-3	91.66[e]	6	Highly susceptible
	IT99K-216-44	83.33[e]	6	Highly susceptible

Values are means of three replicates. Values with the same letter are not significantly different at 0.5% significant level by DMRT. A based on 0-6 scale in which 0 (highly resistant) = 0 on persson scale, 1(moderately resistant) = 1-5 on persson scale, 2 (moderately resistant) = 6-10 on persson scale, 3(moderately susceptible) = 11-25 on persson scale, 4 (moderately resistant) = 26-50 on persson scale, 5 (highly susceptible) = 51-75 on persson scale, 6 (Highly susceptible) = 76-100 on persson scale

Disease severity was significantly different ($p<0.05$) for the different inoculation methods. The disease was more severe in seedlings of all susceptible cultivars inoculated with the inoculum meal than those inoculated with the mycelia suspension (Table 2). This parameter placed the different cowpea cultivars under different susceptibility and resistance class. Cultivars IT03K-316-1, IT06K-154-1, IT03K-369-3 and IT99K-216-44 were all resistant to the pathogen in the spore/mycelia suspension inoculation method but susceptible in the inoculation by the use of inoculum meal. IT04K-217-5 was resistant to the pathogen in both inoculation methods.

Information on the mean height and biomass of the plant recorded at 35 days after planting is presented in Table 3a-c. The results showed a significant difference ($p<0.05$) in the recorded values for the different inoculation methods. In all cases, the mean values for the un-inoculated seedlings were consistently higher than for the inoculated seedlings and the spore/mycelia suspension inoculation showed higher values than the inoculum meal inoculation method. This observation shows that the cowpea cultivars were more severely affected by inoculation with inoculum meal than inoculation with spore/mycelia suspension.

The methods of inoculation used were chosen among those described in literatures for *Macrophomina phaseolina* (Haque and Mukhopadhyaya, 1979; De la Pena-Devesa *et al*., 2009) on the basis of the way the pathogen inoculate host tissues and were adapted to the available

Table 3a: Effect of *Macrophomina phaseolina* on average plant height

Cowpea cultivars	Inoculum meal	Spore suspension	Blank
IT03K-316-1	0.0[a]	31.6[b]	38.0[ab]
IT04K-217-5	32.6[b]	45.3[b]	47.0[b]
IT99K-216-44	31.4[b]	40.1[b]	45.3[b]
IT03K-369-3	21.3[c]	24.4[a]	28.0[a]
IT06K-154-1	21.5[c]	25.3[a]	26.6[a]

Values are means of three replicates. Values with the same letter are not significantly different from one another at 0.5% significant level by DMRT

Table 3b: Effect of *Macrophomina phaseolina* on fresh shoot weight

Cowpea cultivars	Inoculum meal	Spore suspension	Blank
IT03K-316-1	0.00[a]	1.50[a]	2.91[a]
IT04K-217-5	3.70[b]	4.76[b]	7.05[b]
IT99K-216-44	2.36[b]	4.55[b]	4.81[ab]
IT03K-369-3	0.51[ab]	2.16[ab]	4.28[ab]
IT06K-154-1	2.68[b]	3.50[b]	5.15[b]

Values are means of three replicate. Values with the same letter are not significantly different from one another at 0.5% significant level by DMRT

Table 3c: Effect of *Macrophomina phaseolina* on fresh root weight

Cowpea cultivars	Inoculum meal	Spore suspension	Blank
IT03K-316-1	0.00[a]	0.19[b]	0.26[a]
IT04K-217-5	0.33[a]	0.43[b]	0.50[b]
IT99K-216-44	0.18[a]	0.33[b]	0.43[a]
IT03K-369-3	0.00[a]	0.13[a]	0.31[a]
IT06K-154-1	0.25[a]	0.26[b]	0.43[a]

Values are means of three replicates. Values with the same letter are not significantly different from one another at 0.5% significant level by DMRT

resources of our laboratory. The two inoculation methods resulted in successful development of disease symptoms in the plants. The pathogen is soil-borne with wide host range and worldwide distribution (Olaya *et al*., 1996; Aboshosha *et al*., 2007). It attacks and infects the vascular system of the host roots thereby obstructing water and nutrient movement to the above ground parts of the plant.

The symptoms observed varied and severity of infection also differed as observed in the different cowpea cultivars. Symptoms appeared earlier in some cultivars than in others as shown in the results. This could be as a result of difference in the genetic makeup of host plants and the inoculation methods used thereby causing more rapid build-up of the pathogen in the susceptible cultivars showing the symptoms earlier, than in those showing the symptoms much later. The pathogen is known to exhibit high degree of variation in morphological, physiological and pathological characteristics. Dhingra and Sinclair (1973) reported variation in the above characteristics of the pathogen isolated from different part of the same plant.

CONCLUSION

This study represents only a limited evaluation of two inoculation techniques and five cowpea cultivars. Further testing of these inoculation methods as well as comparison with others on more cowpea cultivars will be necessary to identify cowpea cultivars with resistance to

Macrohomina phaseolina. Inoculum meal inoculation method gave the highest disease severity index, it may therefore be recommended for pathogenicity/virulence studies of *Macrophomina* sp., on cowpea.

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