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Full Length Research Paper

Evaluation of the sensitivity of two plantain varieties essong and big ebanga to the nematode *Radopholus similis*

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Plantains are among the crops that enhance food security in Cameroon. However, plantain yields remain low because of several constraints of parasites such as *Radopholus similis*, a nematode that can cause losses up to 75% in fields when no protection measures are taken. In most cases, the sensitivity of local plantain varieties to *R. similis* is unknown; which generally results to low yields despite the control technique used. This limits their up scaling in the country. This work was undertaken to study the sensitivity of two varieties, Essong and Big Ebanga, to *R. similis*. To this effect, the parasitic activity of *R. similis* on the varieties Essong and Big Ebanga was assessed *in vivo*. The variety Grande naine known to be very sensitive to *R. similis* was used as the control. According to the results obtained, local varieties Essong and Big Ebanga were found to be sensitive to *R. similis* with the variety Essong being the most sensitive.

Key words: *Musa* spp., banana plantain, nematode, *Radopholus similis*, sensitivity.

INTRODUCTION

In Cameroon, national production of banana plantain (*Musa* spp.) is estimated at 1.45 million tons (Anonyme, 2007). It is a staple food for many people, and annual consumption varies between 109 and 128 kg per person (Dury et al., 2002). In general, plantain is consumed as a cooked or fried vegetable or is used to make chips, fries and donuts (Ngoh et al., 2005; Honfo et al., 2007). It is also a major source of income for many people and actors in the supply chain in the rural and urban sector (Nkendah and Akyeampong, 2003; Jacobsen et al., 2004). A wide range of varieties of plantain is grown in Cameroon. Local varieties such as Big Ebanga (*Musa*, AAB) and Essong (*Musa*, AAB) are among the most consumed varieties (Mengue et al., 2003; Ngoh et al., 2005; Okolle et al., 2009). Botanically, the varieties Essong and Big Ebanga belong to the banana plantain groups of French and Faux corne, respectively. These varieties which are abundant in most farms are characterized in terms of their tastes, being rich in vitamin A, carotenoids and other essential minerals like zinc and potassium (Anonyme, 2002; Ngoh et al., 2005; Lusty et al., 2006). In addition, early production associated with physical features of banana regime such as the size, number of hands and fingers are the basic criteria for the selection of cultivars by farmers (Anonyme, 2002;

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Mengue et al., 2003).

Although agronomic characteristics are oftentimes not included in the list of criteria considered by farmers for their selection of planting materials, the fact remains that the incorporation of certain agronomic parameters such as parasite sensitivity could significantly aid producers. Indeed, one of the factors that limit the sustainable production of plantain in most production areas is the high sensitivity of local varieties to nematode attacks (Gowen and Quénéhervé, 1990; Quénéhervé, 2008). Although the varieties Essong and Big Ebanga, are dominant in Cameroonian farms, no information regarding their level of susceptibility to plant parasitic nematodes is available. Knowing the level of sensitivity of these varieties to *R. similis* could be vital information for their vulgarization at the national level. Plant parasitic nematodes are microscopic worms, measuring less than 1 mm in length (Quénéhervé, 2008). They are obligate parasites that feed mostly on plant roots causing symptoms such as necrosis, lesions and galls on the roots (Sarah et al., 1996; Bridge et al., 1997; De Waele and Romulo, 1998). Attacked roots become unable to provide water and minerals to the plant and are often unable to anchor the plant, hence the toppling of plantain plants when heavily attacked (Sarah et al., 1996; Bridge et al., 1997; De Waele and Romulo, 1998). The immediate consequences of such attacks are a substantial decline in yields.

Various species of nematodes have been identified in Central and West Africa in the banana and plantain industry (Loubana et al., 2007). The most important are: *Radopholus similis*, *Helicotylenchus multicinctus*, *Meloidogyne incognita*, *Meloidogyne javanica*, *Hoplolaimus pararobustus*, *Pratylenchus coffeae* and *Pratylenchus goodeyi* (Loubana et al., 2007). However, the most common and economically damaging nematode is *R. similis* (Sarah et al., 1996; Fogain et al., 1998; Gowen et al., 2005; Loubana et al., 2007). *Radopholus similis* is a migratory endoparasite found in the roots of banana plantain and generally in areas of low altitude (Bridge et al., 1995; Sarah et al., 1996; Fogain et al., 1998). The annual loss of yield due to *R. similis* in Cameroon can reach 20 to 50% (Bridge et al., 1995; Fogain, 2000). The main objective of this study was to evaluate the susceptibility of local varieties of banana plantain Essong and Big Ebanga to *R. similis*.

MATERIALS AND METHODS

Banana plantain samples

In this study, *in vitro* plants of banana plantain varieties Essong and Big Ebanga and Grande naine were provided by the International Institute of Tropical Agriculture (IITA) in Nkolbisson-Yaoundé. These were used for *in vivo* evaluation of their susceptibility to *R. similis*. The cultivar Grande naine, which is known to be very sensitive to *R. similis* (Speijer and De Waele, 1997), was used as a reference.

Nematodes

Radopholus similis was obtained from necrotic plantain roots. Roots of plantain varieties Essong and Big Ebanga were collected at a farm near Mbalmayo, Cameroon. The infected roots were carefully washed, then cut into 1 cm long pieces. Pieces were rinsed and incubated for 48 h. The nematodes were then isolated using a modified Baermann technique and Cobb's sieving and gravity method (Hooper, 1990; Speijer and De Waele, 1997). Necrotic plantain roots (200 g) were transferred to a kitchen blender containing 500 ml of sterile distilled water (SDW) and ground for 30 s. The suspension was passed through a 40 μm diameter sieve to get rid of water and fine plant debris. The remaining root tissue was put onto a wire mesh (1 mm diameter) covered with tissue paper and submerged in water in a trough. The active nematodes passed through the tissue paper into clean water and were concentrated by decantation in a bowl. Using the keys by Southey (1970) and Luc et al. (1990), nematodes were identified on the basis of morphological and biometrical characters using a light microscope at 40× magnification. Specimens of the burrowing nematode were also compared to reported descriptions of *R. similis* (Blake, 1961).

Experimental design

60 plants of the banana plantain varieties Essong, Big Ebanga and the reference cultivar Grande naine were used to assess their susceptibility to *R. similis*. The shoots were grown for four to five months in a greenhouse in large plug-trays containing sterilized soil, and were then transferred into plastic pots (20 cm in diamater, 25 cm deep) containing a mixture of sandy soil (60% sand, 20% loam and 20% clay) and peat (4:1) which was autoclaved prior to utilization. Plants were grown under ambient conditions with temperatures varying between 25 to 30°C. Shoots were watered when necessary throughout the experiment. The banana plantain plants were arranged into three groups of 3 × 20 = 60 plants. For each cultivar, a set of plants that were not inoculated served as the controls (T0) which was compared to a lot that was inoculated with *R. similis* (T1).

Inoculation

Suspensions containing about 500 adults of *R. similis* per ml were prepared. In total, 2 ml of the suspension (= 1000 adults of *R. similis*) was used to inoculate each plant. Plants were inoculated by injecting 0.4 ml of the suspension in five holes of 2 to 3 cm deep, and were evenly distributed in the potting mix of each plant near the stipe, using a pipette. The inoculated plants were kept in the greenhouse under the conditions described above.

Data collection

The effect of the treatments was evaluated 90 days after inoculation by comparing the number of functional leaves, plant heights, trunk diameters, root infection severity and nematode growth rate. Plant height (in cm) of the shoot was measured from the base of the pseudo-trunk to the axilla of the youngest banana leaf using a measuring tape. The diameter of the trunk was measured fortnightly using a caliper, starting on the day the shoots were transferred into the pots. Root infection severity was measured using primary, secondary and tertiary roots. Percentage necrosis of functional roots was determined as follows: five 10 cm long roots were chosen at random and were cut and split vertically into two halves. The necrosis index which indicates the percentage of the root cortex showing signs of necrosis was determined. A 0 to 4 scale of necrosis index was used with 0 = no necroses found; 1 = <25%; 2 =

Table 1. Effects of infection by *R. similis* on plantain growth.

Variety	Treatment	Number of leaf	Plant height (cm)	Trunk diameter (cm)	Mean		
					Number of leaf	Plants height (cm)	Trunk diameter (cm)
Essong	T0	7.5	30.5	16.3			
	T1	5.7	22.4	13.3	6.6[b]	26.5[b]	14.8[b]
Big Ebanga	T0	8.1	37.0	19.4			
	T1	6.0	30.8	15.9	7.1[a]	33.9[a]	17.7[a]
Grande naine	T0	7.1	7.1	7.1			
	T1	5.0	5.0	5.0	6.5[b]	28.8[b]	12.2[c]
Mean	T0	7.6[a]	34.6[a]	16.2[a]			
	T1	5.9[b]	24.8[b]	13.5[b]			

[a,b,c]Numbers followed by the same letter do not differ at P = 0.05 (Anova followed by SNK). T0, non infected plants; T1, infected plants. [a,b,c]Numbers followed by the same letter do not differ at P = 0.05 (Anova followed by SNK).

25 to 50%; 3 = 51 to 75% and 4 = >75% of the root cortex showing signs of necrosis (Bridge, 1988). The infection degree was obtained by dividing the total sum of the entire necroses indexes by the total number of observed roots. The growth rate of *R. similis* in each individual pot was determined as follows: from each pot, 100 g of soil substrate was used, and using the same method as described for the nematode root extraction, the number of nematodes per pot was determined. The number of nematodes found in the soil sample multiplied by 100 minus the 1000 nematodes from the initial inoculation, divided by 1000 and multiplied by 100% provided the nematode growth rate. Approximately, 0.5 g of dry matter (powder of banana roots) and 10 ml of acetone/water (70:30) were shaken for 10 min, then ground with an Ultra-Turax. The mixture was filtered and 150 µl of the raw extract (EB) was used for the determination of total phenols by the Folin method (Singleton et al., 1999).

Data analysis

Data were analyzed using two way analysis of variance (ANOVA) with varieties (Big Ebanga, Essong and Grande naine) and treatments (T0 and T1) as independent variables. Nematode death rate data were arcsin transformed. The numbers of nematodes were transformed using $\log_{10}$ (n + 1). Where results were significant ($P < 0.05$), means were separated using the Student Newman-Keuls (SNK) test.

RESULTS

Effects of infection by *Radopholus similis* on plantain growth

90 days after inoculation, the average leaf number, size and root collar diameter of plants inoculated with *R. similis* were significantly lower ($P < 0.05$) than those from uninoculated plants (controls) (Table 1). The average number of leaves and plant height obtained from the variety Big Ebanga were significantly higher ($P < 0.05$) than those obtained from varieties Essong and Grande naine, whose values were measured and which did not differ significantly ($P > 0.05$). However, the average measurements of root collar diameter (Table 1) obtained on the Big Ebanga variety was higher compared to the varieties Essong and Grande naine. The lowest average measurements of root collar diameter were recorded in the varieties Grande Naine**.** The interaction (treatment × cultivar) was not significant ($P < 0.051$) for any of the measured aerial parameters.

Effects of infection by *R. similis* on the total number of roots, fresh weight and dry weight

The infection by *R. similis* had a negative action on the total number of roots, fresh and dry weight of roots as evaluated three months after plantation (MAP) (Table 2). The statistical analysis of the influence of nematode infection by *R. similis* on the total number of roots, fresh and dry weight of roots showed a non significant ($P = 0.61$) interaction (treatment × cultivar). Analysis of variance showed that the average number of roots, fresh and dry weight of roots showed significant differences ($P < 0.05$) between plants inoculated with *R. similis* (T1) and non-inoculated plants (T0). With the exception of the values obtained for the fresh weight of roots, no significant difference ($P > 0.05$) was observed between the averages of total number and root dry weight of cultivars Essong and Big Ebanga. Compared with cultivar Grande naine, with the exception of root dry weight, significant differences ($P < 0.05$) were observed between the values of the total number and fresh weight of roots of cultivars Essong and Big Ebanga (Table 2).

Table 2. Effects of infection by *Radopholus similis* on the total number of roots, fresh weight and dry weight.

Variety	Treatment	Total number of root	Fresh weight	Dry weight	Mean		
					Total number of roots	Fresh weight	Dry weight
Essong	T0	79.6	231.8	20.6	72.7[a]	206.1[b]	18.6[a]
	T1	65.7	180.4	16.6			
Big Ebanga	T0	92.9	251.2	251.2	78.7[a]	231.4[a]	17.5[a]
	T1	64.5	211.6	211.6			
Grande naine	T0	73.2	217.8	21.0	63.8[b]	185.2[c]	18.9[a]
	T1	54.4	152.6	16.8			
Mean	T0	81.9[a]	233.6[a]	20.2[a]			
	T1	61.5[b]	181.5[b]	16.4[b]			

T0, Non infected plants; T1, infected plants. [a,b,c]Numbers followed by the same letter do not differ at P = 0.05 (Anova followed by SNK).

Table 3. Effects of infection by *Radopholus similis* on plantain root necrosis severity (RNS).

Variety	RNS (%)
Essong	34.5[a]
Big Ebanga	11.6[b]
Grande naine	36.7[a]

T0, Non infected plants; T1, infected plants. [a,b,c]Numbers followed by the same letter do not differ at P = 0.05 (Anova followed by SNK).

Table 4. Effects of infection by *Radopholus similis on* growth rate.

Cultivar	*R. similis* initially	*R. similis* finally	Growth rate
Essong	1000	2880[a]	+1.8[a]
Big Ebanga	1000	1007[b]	+0.01[b]
Grande naine	1000	2767[a]	+1.8[a]

T0, Non infected plants; T1, infected plants. [a,b,c]Numbers followed by the same letter do not differ at P = 0.05 (Anova followed by SNK).

Effects of infection by *Radopholus similis* on plantain root necrosis severity

In plants infected with *R. similis*, the mean values of root necrosis severity were high compared to those obtained in uninfected varieties. The average root necrosis severity obtained in the roots of the variety Big Ebanga was significantly lower ($P < 0.05$) than the varieties Essong and Grande naine, which showed no significant difference ($P > 0.05$) (Table 3).

Effects of infection by *R. similis* on growth rate

After infection with *R. similis*, the results show that the size of the initial population of nematodes increased in the pots with all the three varieties Big Ebanga, Essong and Grande naine (Table 4). The statistical analysis of the rate of multiplication showed a not significant ($P = 0.50$) interaction (treatment × cultivar). The reproduction rate of 0.01, 1.8 and 1.8, respectively were recorded invarieties Big Ebanga, Essong and Grande naine. Analysis of variance showed that the average population of *R. similis* per 100 g of roots and those of their rate of multiplication in the roots of plants of the variety Big Ebanga were significantly lower ($P < 0.05$) compared to those obtained in varieties Essong and Grande naine, which showed no significant difference ($P > 0.05$) (Table 4).

Table 5. Evaluation of the total phenols content of banana plantain varieties tested.

Treatment	Variety			Mean
	Big Ebanga	Essong	Grande naine	
T1	465.0±42.6	454.8±42.6	441.6±42.6	453.8±42.6[a]
T0	340.3±42.6	320.6±42.6	301.0±42.6	320.6±42.6[b]
Mean	402.5±42.6[a]	387.7±42.6[a]	371.3±42.6[a]	

T0, Non infected plants; T1, infected plants. [a,b,c]Numbers followed by the same letter do not differ at P = 0.05 (Anova followed by SNK).

Evaluation of the total phenols content of banana plantain varieties tested

Analysis of variance performed on the concentration of total phenols showed significant differences ($P < 0.05$) between the measurements obtained in the roots of infected plants and those of uninfected plants. Although no significant difference ($P > 0.05$) was observed between the mean values of phenol content of the varieties tested, the amount of phenols in the Big Ebanga variety was higher compared to the varieties Essong and Grande naine. The lowest concentrations of phenols were recorded in the varieties Grande naine (Table 5).

DISCUSSION

This study reveals the sensitivity of two local varieties of plantain, Big Ebanga and Essong to *R. similis*. The impact of *R. similis* on the growth parameters of the aerial and underground parts is related to the destruction of the underground plant parts, the roots. These results agrees with those obtained by Sarah et al. (1996) and Van den Bergh et al. (2000), who observed that underground tissue destruction leads to reduced water and mineral nutrition, resulting in slower growth and development of plantain plants. The increase in the percentage of root necrosis, following infection of banana plants by *R. similis* can be explained by the formation of root necrosis characteristics of this nematode. These results agrees with observations by Sarah et al. (1996) in a similar test and Guedira et al. (2004) in a test for assessing nematode resistance *R. similis* and *Meloidogyne* spp. Furthermore, the development of necrosis in the roots resulting from infection of the plants tested led to the death of roots. Thus, the greatest percentage of dead roots obtained from the variety Grande naine, confirmed the findings of Sarah et al. (1996), Speijer and De Waele (1997), and Duong et al. (2002), who, respectively demonstrated that nematodes *R. similis*, necrosis appeared in the roots and depending on the severity of the attack, may cause rotting. Indeed, root death is related to root damage caused by *R. similis*, which, after penetrating the root apex, moves by creating damage in tissues, which may enlarge to form large necrotic areas. Moreover, according to Sarah et al. (1996), necrosis reached the central cylinder in a short time, resulting in the destruction and death of the whole root.

Globally, the results obtained show that the varieties of plantain Essong and Big Ebanga are also susceptible to *R. similis* although somewhat less than the reference variety Grande naine, since the number of nematodes found in the roots of plants infected by *R. similis* was relatively high, and it seemed to directly affect other measured parameters; it is likely that in this study, the local varieties of plantain Essong and Big Ebanga tested showed sensitivity with regard to *R. similis* as well as the reference variety Grande naine, for at least 90 days after inoculation. The high levels of total phenolics obtained in the roots of infected plants and relatively low in the roots of uninfected plants, certainly could be explained by an additional secretion of these compounds in the defense mechanisms of the plant after the infection. According to Wuyts (2006), the banana plantains varieties resistant to nematodes contain more phenolics than susceptible varieties.

Conclusion

The main objective of this study was to assess the sensitivity of two local varieties of banana plantain Essong and Big Ebanga to *R. similis*, the main pest of plantain in Cameroon. To do this, the pathogenicity of the nematode *R. similis* on the two varieties was evaluated *in vivo* (in pots). Considering the results obtained following infection of plants by nematodes *R. similis* and on the basis of an evaluation of the sensitivity of banana plantain varieties to *R. similis* as defined by Speijer and De Waele (1997), it appears that the two varieties of plantain Essong and Big Ebanga are sensitive to *R. similis*; although the two local varieties are also susceptible to nematode *R. similis* when compared to the reference variety Grande naine; variety Essong appeared more sensitive to *R. similis* than the variety Big Ebanga. These results are important especially for breeders and small-holder producers of banana plantain, given the economic importance of Essong and Big Ebanga. Farmers prefer them to other local varieties, despite low yields generally obtained.

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