Journal of Forestry Research and Management. Vol. 14 (2), 133-144; 2017, ISSN 0189-8418

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Evaluation of Resistance of Pigeon Pea, *Cajanus cajan* (L) Millisp. to a Population of Root-knot Nematode, *Meloidogyne javanica*

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ABSTRACT

Pigeon pea is an important legume crop grown around the world for food, feed and fuel. Its role in soil nitrogen fixation is of great benefits in agriculture and natural ecosystems. Plant parasitic nematodes are major constraints to the yield of this valuable crop worldwide. The study was conducted for 6 weeks, to test the abilities of two weeks old seedlings of *Cajanus cajan* raised from brown, opaque white, coffee and mottled black coloured seeds, to resist the infestation of *Meloidogne javanica* nematode. This *in vivo* trial was carried out in a screenhouse; nematode identification and inoculation were carried out using standard procedures. The experiment was laid in a Completely Randomized Design (CRD) with four replicates and data were analyzed using Analysis of Variance (ANOVA) and significantly (p=0.05) different means were separated using F-LSD. The results showed that shoot and root weights of inoculated seedlings of brown and opaque white seed types were markedly significantly higher (p=0.05) than those of similarly inoculated coffee and mottled black colours, whereas they did not differ from those of uninoculated (control) seedlings. The shoot weights of control seedlings of the mottled black seeds were significantly higher (p=0.05) than those of the other control seedlings. The control seedlings of all colour types were significantly higher (p=0.05) than those of the inoculated seedlings. There were no galls nor nodules formed on the roots of the inoculated seedlings. This result suggests some degree of resistance of pigeon pea to infestation of *M .javanica* especially by the seedlings of the brown and opaque coloured seeds. We therefore infer that selection of brown and opaque coloured seeds of *C. cajan* can similarly select for resistance to *M. javanica.* Thus this could be harnessed for biological control of root-knot nematodes and as a tool in agroforestry for reducing nematode infestation levels in rotations before introduction of nematode susceptible crops.

Keywords: inoculated seedlings, screenhouse, agroforestry, galls, nematode infestation

Journal of Forestry Research and Management. Vol. 14 (2), 133-144; 2017, ISSN 0189-8418

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Introduction

Pigeon pea is a high-protein grain legume of semi-arid tropics and sub-tropics. It is the only known cultivated food crop of the 32 species that fall under the *Cajaninae* sub-tribe. The crop represents about 5% of world legume production (Hillocks *et al.*, 2000). Global annual production of pigeon pea is about 3.6 million tonnes (Mt) valued at around US$ 1,600 million (FAOSTAT, 2007). It has about 25% crude protein with well balanced composition in amino acids except for methinonine and cystine which are too low. The plant is used for forage, green manure, windbreaks and as shade for young crops in forest and vegetable seedling nurseries. Pigeon pea provides green forage of outstanding value when other forages are not available (Sloan, *et al*., 2009). The pods may be consumed as green peas or as ripened grains for human and stock feeds. In Kenya, pigeon pea has a significant domestic demand and for other local communities who use it during special occasions (Jones *et al.*, 2002). It is also mostly exported dry to America and other Asian countries (Kunde, 2000; Rusike and Dimes, 2006).

The high nutritive value of pigeon pea is perhaps the most important reason why it should find an important place among the smallholder poor farmers in Africa. ICRISAT have developed high protein lines (HPL) with up to 32.5% protein content and significantly higher sulphur-containing amino acids (Saxena *et al.*, 2002). Pigeon pea is therefore a good source of amino acids (Elegbede, 1998).

The supplementation of cereals with protein rich legumes is considered as one of the best solutions to protein-calorie malnutrition in the developing world (Chitra *et al.*, 1996; Rampersad *et al.*, 2003). Pigeon pea is a rich source of carbohydrates, minerals and vitamins (Foodnet, 2002). Its flour is an excellent component in the snack industry and has been recommended as an ingredient to increase the nutritional value of pasta without affecting its sensory properties (Torres *et al.*, 2007). Pigeon pea seed has been recommended as an alternative to maize, soybean meal or groundnut cake in the diets of broilers (Amaefule and Obioha, 2001; Onu and Okongwu, 2006), pullet chicks (Amaefule and Obioha, 2005; Amaefule *et al.*, 2006) and layers (Agwunobi, 2000) in Nigeria.

Pigeon pea leaves have been used to treat malaria (Aiyeloja and Bello, 2006) in Nigeria, while in Southern Africa; it is currently one of the indigenous crops being promoted for potential medicinal use (Mander *et al.*, 1996). Pigeon pea is also widely used as fodder and feed for livestock (Rao *et al.*, 2002). Its foliage is an excellent fodder with high nutritional value (Onim *et al.*, 1985). The seeds are used as animal feed and fodder (Wallis *et al.*, 1986; Karachi and Zengo, 1998; Phatak *et al*., 1993; Chisowa, 2002). The plant is often used as live fences windbreaks and in soil conservation in Africa (Phatak *et al*., 1993). Ease of establishment and the simultaneous production of food make perennial pigeon pea a special agro-forestry option in several parts of Africa (Kwesiga *et al*., 2003). Drought resistance is a factor for plant survival and productivity and the most important environmental constraints to food security in the tropics (Speranza *et al*., 2007). Pigeon pea can withstand severe drought better than many legumes due to its deep rooting system (Flower and Ludlow, 1987) and osmotic adjustment (OA) in the leaves (Subbarao *et al*., 2000). It has the ability to fix up to 235 kg Nitrogen (N)/ha (Nene, 1987; Peoples *et al.,* 1995; Sakala *et al*., 2000) and produces more N per unit area from plant biomass than many other legumes. Nitrogen though one of the most abundant elements on earth yet is the most limiting nutrient for increasing crop productivity (Wani *et al*., 1995; Graham and Vance, 2003). The N-fixing ability of this legume plant is a potential for environmentally sustainable agricultural production (Myaka *et al*., 2006; Bodner *et al*., 2007). However, despite its various potentials, pests and pathogens are one of the major constraints to its yield (Srinivasan *et al*., 2004). Root-knot nematodes (*Meloidogyne* spp.) are major pests of agronomic crops, worldwide. They are obligate parasites of roots of thousands of plant species (Eisanback and Trianataphillou, 1991). Their damages result in root galling, shoot stunting and loss of yield (Starr *et al*., 2005 They cause approximately 5% of global crop loss as their larvae drain the plants photosynthate and nutrient resulting in decline in quality and reduced resistance to other stresses such as drought and pests (Sasser and Carter, 1985). Severe attacks can lead to total crop loss, as the damaged roots are unable to utilize water and nutrients effectively (Stirling *et al*., 1992). Ayoub (1980), reported that root-knot nematode cause damages to about 2000 plants. Though pigeon pea diseases have been reported to be of minor importance in Africa in the past, recent surveys has indicated that *Fusarium* wilt, sterility mosaic disease (SMD), leaf spot (*M. cajan*) and to a lesser extent powdery mildew (*Leveillula taurica*) are diseases of economic concern (Hillocks *et al.*,2000). Shanower *et al.* (1999); Minja *et al.* (2000) and Agona and Muyinza (2005) reported on the field and store insect pest infestation as one the factors limiting pigeon pea production. Although the host range of root-knot nematodes, *Meloidogyne spp*. is very wide, not much is known about the status of pigeon pea to root-knot infection in Nigeria, therefore the objective of this study is to determine the potentials of four different coloured seeds of pigeon pea at suppressing a population of (*M. javanica*) nematode *in vivo.*

MATERIALS AND METHODS

Nsukka population of *Meloidogyne javanica* were isolated from infected three weeks old tomato seedlings raised at the nursery at the University of Nigeria, Nsukka vegetable garden. Four differently coloured seeds of *Cajanus cajan* (Pigeon pea) namely: brown, coffee, mottled-black and opaque-white were bought from the local Nsukka market. They were sorted according to uniform sizes and smoothness. Commercial bleach (10 % Sodium hypochlorite) was used to surface sterilize the seeds which also helped to soften the seed coats for quick germination. The seeds were rinsed four times with sterile distilled water. They were thereafter placed on sterile moistened blotter paper in Petri-dishes. The seeds germinated in eight to ten days. At 2 -weeks after germination, two seedlings each were pricked and transferred into nursery pots at two seedlings per pot. The seedlings were spaced at 10cm apart in the nursery pots.

To confirm the presence of *M. javanica*, this was done by observing the perineal patterns of mature females isolated from infested roots of 2 weeks old tomato seedlings as recommended by Franklin (1979). Ten galled roots of tomato from which the nematode culture were multiplied were carefully excised with a sterile surgical blade and blended to slurry using a warren blender. Sterile distilled water was added to the slurry to make it up to 500ml. The suspension was stirred vigorously with a stirring rod to disperse the nematode larvae evenly. Three 30ml beakers of the nematode suspension were counted using a dissecting microscope and a counter, an average of 500 to the nearest multiple of 100 was recorded. Two week old potted seedlings of *C. cajan*, from the different colours were each inoculated with 30 ml suspension of the *M. javanica* juveniles approximating 500 juveniles per pot. A hole of 1cm deep and 1 cm wide were made round the seedlings and the suspension was poured into the holes after stirring and then covered with top soil. The control plants were inoculated by pouring into the hole, sterile distilled water without nematode. The experimental units were 32 pots in all grouped at four per treatment. The nursery pots were labeled and placed randomly on cemented floor of the screenhouse at 1m x 1m spacing to avoid cross contamination. The plants were watered at two days interval to field capacity. At Six weeks after inoculation all the test plants were cut at the soil level and their fresh shoot weights were taken with a weighing balance. Roots were carefully removed from the poly pots by slicing the poly pots length wise with sharp razor. They were washed gently with running tap water, thereafter their weights were taken. The weights of the shoots and roots of the control plants were also taken. The roots were assessed for galling and nodulation. The experiment was laid in a Completely Randomized Design with four replicates. Data were analysed using analysis of variance (ANOVA) and significantly different means (p= 0.05) were separated using F-LSD.

RESULTS

The results are presented in tables 1 and 2. The shoot parts of all the inoculated parts did not look healthy. The stems were stunted and thin while the leaves were chlorotic and comparatively smaller than the control. Conversely the control plants were luxuriant with bigger stem, greener and larger leaves. These differences were markedly more pronounced between the inoculated and the control plants in the seedlings from the mottled-black and coffee coloured seeds. However, there were no differences in shoot weights between the control and the inoculated seedlings of brown and opaque white seeds (Table 1).

There were no galls nor nodules formed on the roots of the inoculated plants. Conversely the control plants produced light to heavy nodules on their roots except the opaque white seeds which did not nodulate (Table 2). The roots of the inoculated plants were less bunchy, brownish and had necrotic spots. There were significant differences (p = 0. 05) between the root weights of the inoculated versus the control plants (Table 3).

Table 1: Mean Fresh Shoot Weight (gm) of inoculated and uninoculated seedlings of coffee, mottled black, brown and opaque-white coloured seeds of *C. cajan*

Treatment	Shoot weight (gm)			
	Coffee	**Mottle black**	**Brown**	**Opaque white**
Uninoculated	16.74^b	18.71^b	16.5^b	19.52^b
Inoculated	8.21^a	10.18^a	9.65^a	10.28^a
F-LSD (p= 0.05)	1.50			

Means followed by the same letter are significantly the same at 5% level of probability for inoculated and uninoculated and between seed colours.

Table 2: Percentage nodules on roots of inoculated and uninoculated seedling raised from differently coloured seeds of *C. cajan.*

Treatment	Percentage noduling (%)			
	Coffee	**Mottle black**	**Brown**	**Opaque white**
Uninoculated	36	44	30	0.00
Inoculated	0.00c	0.00c	0.00c	0.00

Table 3: Mean Fresh Shoot Weight (gm) of inoculated and uninoculated seedlings of coffee, mottle-black, brown and opaque-white coloured seeds of *C.cajan*

Treatment	**Shoot weight (gm)**			
	Coffee	**Mottle black**	**Brown**	**Opaque white**
Uninoculated	11.28[a]	18.14[a]	13.0[a]	15.55[a]
Inoculated	1.39[b]	2.01[b]	2.22[b]	1.19[b]
F-LSD (p= 0.05)	1.98			

Means followed by the same letter are not significantly different at 5% level of probability.

DISCUSSION

Nodulation in legumes plays a significant role in world agricultural productivity (Freiberg *et al.*, 1997; Herridge and Rose, 2000). The disruption of nodulation by the nematode is therefore a major constraint to the potential of pigeon pea. Roots of the inoculated plants when teased and re-examined under the microscope showed that there were no nematode larvae found in the roots signifying no active colonization by the pathogen though there were initial infections after inoculation. The absence of nodules on the inoculated plants roots, suggests that *M. javanica* must have inhibited the symbiosis between the root and the bacteria. Barker *et al.* (1998) had earlier observed that root-knot nematode have inhibitory effects on nodulation or nitrogen-fixation in roots of leguminous plants. Mathesius (2003); Davis *et al.* (2008) studied the signaling and interaction involved in legume–nematode patho-system. It was observed that the J2 of the root-knot nematodes migrate between root cortical cells (intercellularly), to reach the root vascular cylinder. They synthesize and mobilize secretions and enzymes that cause dissolution of cell walls and alter the biochemical processes of their host (Goellner *et al.*, 2001). The nodules found in the roots of the control plants may not be the only reason for such significant differences in weights in only six weeks of assessment, rather the mechanisms employed by the roots of the inoculated seedlings for resisting the nematode entrance was the release of toxic metabolites which led to injurious reaction and loss of tender roots (Richard *et al.*, 1994). Many mechanisms are employed by plants to resist pest and disease attack (Ehlers, *et al.*, 2000). It has been noted that that in short term responses of plants to pathogens, the plant deploys reactive oxygen such as superoxide and hydrogen peroxide to kill the invading cells in the pathogen and becomes hypersensitive in which cells surrounding the site of infection are signaled to undergo apoptosis, or programmed cell death, in order to prevent the spread of the pathogen to the rest plant (Timperio and Rogers, 2012). This could be attributed to the poor growth, browning and necrosis on the inoculated roots as a result of hypersensitive effect. This could explain why the root number was fewer, brownish and necrotic in the inoculated seedlings. This is in conformity with an earlier observation by (Ogbuji, 2000), that at the entry point of a nematode, the adjoining cells collapse resulting in significant losses of plants fragile roots. The browning of the roots had been ascribed to the accumulation of phenols in the invaded roots and their oxidations into melanin. The host/parasite interaction which results in the phenol accumulation and their oxidation to toxic quinines has been reported to be injurious to nematodes (Sugumaran *et al*., 2000; Davies, *et al*., 2004; Davis, *et al*., 2008) and that may explain why a pre-trial inoculation experiment showed that there were nematode larvae in the roots of the pigeon pea seedlings up to the tenth day, but at six weeks after inoculation, they were no longer nematodes observed.

CONCLUSION

It is therefore concluded that pigeon pea, though a short perennial crop could resist the initial disease stress caused by nematode and still grow and yield well. This resistance mechanism established by pigeon pea could be harnessed as a tool in integrated pest and disease management in reducing nematode population in a soil environment.

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