SCREENING OF FIVE ELITE SWEET POTATO CULTIVARS AGAINST RENIFORM NEMATODE (*Rotylenchulus reniformis*) IN THE GREEN HOUSE

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ABSTRACT

Five elite sweet potato cultivars/lines were screened in the greenhouse for resistance against Reniform Nematode (Rotilenchulus reniformis) *through artificial inoculation. The cultivars showed varying degrees of above and underground symptoms of nematode infection. The longest vine length of 122.28 cm was observed in RAB36 and the shortest in L676 being 72.85 cm. The RAB36 had significantly lowest number of nematode per gram of soil compared to other cultivars and B11 had the highest. The RAB36 had the highest mean tuber weight of 112.3 g compared to other cultivars and the lowest being 56.2 g in case of B11. The dry weight of the cultivars closely followed the pattern in fresh weight. Ninety per cent nematode mortality was observed in case of RAB36 compared to B11 with only 10 % in phenolic test. The mortality percentage in RAB32, DOY2 and L676 were 85, 50 and 25 respectively. The highest tuber crack of 10% was observed on B11 and only six per cent on L676. No tuber cracking was observed on the cultivars.*

Key words: *Sweet potato, reniform nematode, phenolic extract, tuber crack.*

INTRODUCTION

The sweet potato (*Ipomoea batatas*) is one of the most important food crops of Papua New Guinea (PNG) with an estimated annual production of 1,223,800 tonnes worth K150 million (Bourke 1982 a & b). It is predominantly a subsistence crop, more recently; however, sweet potato has emerged as a significant cash crop (Bourke 1982 a & b). It is the staple food in most highland areas and is increasingly important in parts of lowlands due to its agronomic superiority over the other crops, such as taro and yams. Sweet potato provides between 65-90% of people's energy intake (Bourke 1982a & b; Kimber 1972) and up to 2.5-7.5% (dry weight) of protein intake in some areas (Goodbody 1984).

In recent years, decline in sweet potato yield in Papua New Guinea is a major concern in sweet potato cultivation (Hartemink *et al.* 2000). Diseases are a major constraint to production of sweet potato in many parts of the world, particularly in the temperate regions (Clark and Moyer 1988). Only a few of the most serious diseases of sweet potato are present in PNG, and appear to cause significant crop damage in certain parts of the country (Kokoa 2004).

Plant parasitic nematodes are recognized world wide, as potentially serious constrains to crop productivity. The reniform nematode (*Rotylenchulus* spp.), primarily occurs not only in tropical and sub-tropical areas but also in some temperate areas. It is one of the destructive pathogens of sweet potato affecting both yield and quality of the crop (Clark and Moyer 1988). Twenty-two different genera of plant parasitic nematodes have been reported to be associated with sweet potato in PNG (Bridge and Page 1982; Kokoa 1986 a & b; Levett *et al.* 1987; Kokoa 1991 a) and plant parasitic nematodes can be a major problem in sweet potato production.

Reniform nematode is an important semi-endoparasitic pest of many crops, especially in warmer climates. It is the principal nematodes damaging cotton in Egypt and in parts of the United States. This nematode also attacks tomato, soybean, and pineapple among others. It is also found in other tropical and subtropical areas of the world where sweet potato is grown (Birchfield and Martin 1965). Host range of *Rotylenchulus* species includes more than 300 species in 77 plant families (Robinson *et al.* 1997).

The population of *Rotylenchulus reniformis* can rise to very high levels, up to almost 10,000/100 cm3 of

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soil and the mineral balance of the host is disturbed. The reniform nematode also cause severe cracks on the roots (Clark and Wright 1983) affecting the quality and making it unsuitable for selling and human consumption. The amount and type of damage incurred by *Rotylenchulus reniformis* often depends on the host species and/or cultivars as well as the nematode population. In sweet potato, they may cause surface cracking of tubers (Gaur and Perry 1991).

The *Rotylenchulus reniformis* is present in several Pacific Island nations, including Papua New Guinea and has been rated as an important nematode of sweet potato (Bridge 1988). In Papua New Guinea, there is inadequate information on this pathogen infecting sweet potato.

The prevention of epidemic and ultimately the reduction of losses in terms of yield and quality have been of great concern. Using chemicals may control the diseases, but chemicals create hazards to human health, produce undesirable side effects on non-target organisms and the environment as a whole.

Under the existing circumstances, the use of resistant variety (-ies) is (are) one of the most attractive approaches for the management of plant diseases.

The natural genetic resistance of plants to pest and diseases has no doubt played a key role in crop production since the dawn of agriculture. Their use requires no particular action by the growers during the growth period, is environmentally friendly and sustainable, compatible to other management practices; and is sometimes singularly sufficient to suppress the disease to a tolerable level.

In this regard, it is utmost important to screen the existing cultivars/lines to find resistance sources with higher yield, quality and adaptability before being distributed to the farmers for widespread cultivation. If the growers know the resistance level of the cultivars well before planting, they would know what to expect during the growing season with respect to disease development and probable preventive measures to take.

Hence, a green house trial was conducted to screen five elite sweet potato varieties/lines for resistance against *Rotylenchulus reniformis*.

MATERIALS AND METHODS

Selection of cultivars/lines

Five elite sweet potato cultivars were selected based on tuber shape, tuber skin color, tuber flesh color, time of maturity, flesh of texture and taste after boiling (Table 1).

Table1. **Characteristics of five selected elite sweet potato cultivars used in the study**

Accession number, class and origin	Tuber shape	Tuber skin colour	Tuber flesh colour	Time to maturity (months)	Flesh texture after boiling	Taste after boiling**
B 11 1st Class PNG	Proximal end narrow, distal end broad	White	White	4.5	Firm	Not sweet
RAB 36 1st Class PNG	Proximal end narrow, distal end broad	Purple	Orange with white patches	5(best not later)	Firm	Slightly sweet
L 676 1st Class PNG	Fusiform	Purple	White	5	Intermediate	Slightly sweet
RAB 32 1st Class PNG	Proximal end broad, distal end narrow	Pink	Orange with white	4.5(best not later)	Soft	Slightly sweet
DOY 2 1st Class PNG	Fusiform	Purple	White	5	Intermediate	Slightly sweet

** = Showing peoples' preferences in relation to the characteristics.

Soil collection and sterilization

The humus soil was collected, placed and half filled in 44 gallon drum where the soil was sterilized using heat from the fire. It was left over night to cool down.

Pot preparation, vine planting

About two kilograms of sterilized soil was placed in a plastic bag within a plastic pot of 17.5cm diameter and 14.5cm deep. Two vines of about 15 cm in length per cultivar were planted at 5 cm deep in the soil in each of the pots.

Experimental design

The cultivars in the pots were organized in a randomized complete block design with each of the treatments (cultivars) replicated four times.

Nematode isolation and inoculation

The soil was collected from the old sweet potato garden at Wawin High School farm at the Markham Valley at a depth of 10 cm. The collected soil was cleaned and placed on the tissue paper with the strainer underneath and the nematodes were isolated using the Baermann's tray method. A thermometer was set to monitor the room temperature at 27-30°C and left for 72 hours. Later the nematodes were transferred into a beaker and filtered using a 20im strainer into 20-milliliter vials. From the vials, the nematodes were placed on a counting slide and observed through a microscope using a 40X power lenses. The *Rotylenchulus reniformis* adults and juveniles were selected using a fine point needle and placed into a 5 milliliter vial and kept in a cool room. These juveniles and adult reniform nematode population of 4,000-10,000 were inoculated into the soil in each pot.

Cultural practices

Watering of plants was done regularly to keep the soil at Field Capacity (FC). Over watering was avoided as this would drain out the nematodes from the pots. Weeds growing on the pots were removed manually from time to time. Hand picking of leaf miner was done without any chemical sprays as this would affect the nematodes.

Harvesting of sweet potato

Sweet potato was harvested 10 weeks after planting. Sweet potato plants from each of the pots were removed, the roots were thoroughly washed and placed on a clear plastic to dry so that further observations on the root gall formation and tuber cracks could be made.

Assessment of parameters

At the time of harvest, data on the following parameters like length of vines, above ground biomass (fresh and dry weights), degree of gall formation, nematode population was recorded.

Phenloic extract

Ten grams of fresh roots from each of the cultivars was taken and ddH20 added, crushed on the pestle and mortar and centrifuged at 10,000 rpm for 15 minutes. Five milliliters of the supernatant was placed in a plastic vial separately for each of the cultivars. Twenty live larvae (juveniles) of nematodes were selected and placed in the vial containing the root extract. The mortality of the nematodes in the phenolic extract from the roots was observed under the microscope after 30 minutes and the data was recorded. The second reading was taken after one hour, but there was no change in the mortality rate.

Estimation of tuber cracks (%)

The tubers from each cultivar were washed, placed on a clear plastic and examined for any tuber cracks. The percentage of tuber cracks was calculated as equal to the number of cracking tubers divided by the total number of tubers, multiplied by one hundred.

$$\text{Tuber cracks (\%)} = \frac{\text{Number of tuber cracks}}{\text{Total number of tubers}} \times 100$$

Data Analysis

The data was analyzed using the Software Minitab Student Release 12 Version. Data on nematode population, vine length and biomass were subjected to analysis of variance and mean separation to determine the statistical differences among the treatment means.

RESULTS AND DISCUSSION

The general observations from the pot plants showed symptoms of infections by the reniform nematodes (*Rotylenchulus reniformis*) with varying degree of severity on the five elite (Plate 1) sweet potato cultivars in the green house. The cultivar DOY 2 was growing healthy with a couple of dead vines, B 11 cultivar had a few dead vines, stunted growth, leaves were smaller and turning yellow and leaf miner

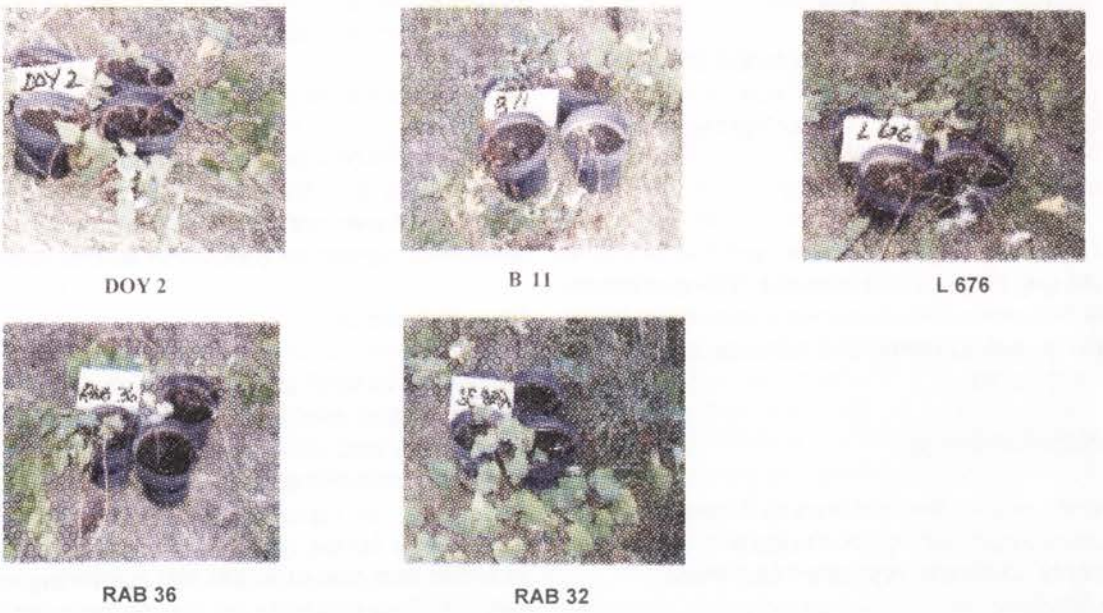

Plate 1. **Cultivars showing above ground symptoms of *Rotylenchulus reniformis* infection under Green house condition.**

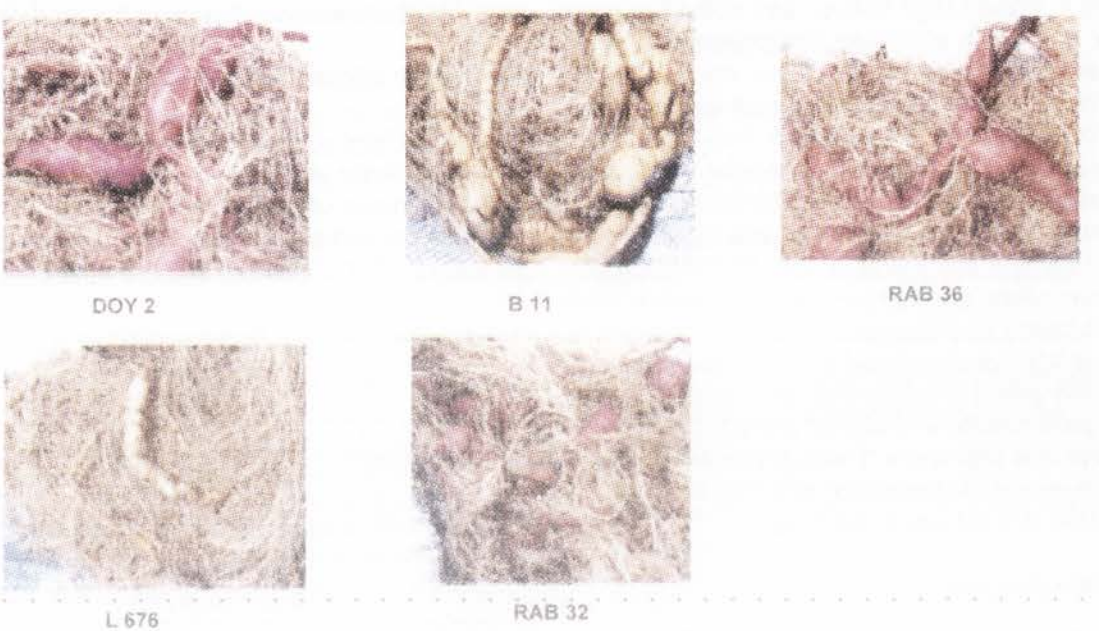

Plate 2: **Cultivars showing symptoms of root infection due to *Rotylenchulus reniformis* infection.**

attacking the leaves. The RAB 36 cultivar was growing healthier with long vines, L 676 cultivar had a few dead vines at the base with long vines; and RAB 32 cultivar was growing healthier without any visible symptoms of disease, however, there were a few leaf miner attacking the leaves.

The underground symptoms of *Rotylenchulus reniformis* are shown as varying degrees of deformities/abnormal growth on the tubers (Plate 2).

It is observed that moderate levels of abnormalities in the form of cracks were found in B 11 followed by L 676. The tubers of the other three cultivars looked fresh and smooth without any abnormalities.

The result on the nematode population isolated from each of the cultivars (Table 2) shows that the number of nematodes per kilogram of soil was highest in B11 (308.5) and the lowest in RAB 36 (164.5) and this difference was significant at pd"0.05 (LSD). The

Table 2: **The parameters used to assess resistance of five sweet potato cultivars to reniform nematode in the green house.**

Cultivars	Mean nematode population/kg of soil	Biomass			Phenolic test			Cracking defects (%)
		Length of vines (cm)	Fresh weight (g)	Dry weight (g)	Live (x/20)	Dead (x/20)	Mortality (%)	
DOY 2	186.5c	94.25b	82.4b	75.78b	10	10	50	0
B 11	308.5a	74.95c	56.2d	44.85d	18	2	10	10
RAB 36	164.5c	122.28a	112.13a	95.05a	2	18	90	0
L 676	291.25a	72.85c	62.93cd	52.40cd	15	5	25	6
RAB 32	217.5b	87.05bc	74.18bc	62.78bc	3	17	85	0

number of nematodes in L 676 was 291.25 per kilogram of soil and this was not statistically different from B 11 at pd"0.05 level, however, this was significantly (pd"0.05) higher than DOY 2, RAB 32 and RAB 36. Even though the nematode population was higher in DOY 2 (186.5) than RAB 36, this difference was not statistically significant at pd"0.05 level.

The numbers in a column representing nematode population; vine lengths and biomass weight are the means of four replications.

Means followed by the same letters within a column are not significantly different at Pd"0.05 (LSD).

The mean vine lengths ranged from 72.85cm to 122.28cm (Table 2). The longest vine length of 122.28cm was observed in RAB 36 and the shortest in L 676 being 72.85cm. The vine length in RAB 36 was significantly longer than the rest of the cultivars. The vine length of DOY 2 (94.25cm) was significantly longer than B 11 (74.94cm) and L 676 (72.85) but the vine length difference between DOY 2 (94.25cm) and RAB 32(87.05cm) was non-significant. Similar was the case among B 11, L 676 and RAB 32.

The mean fresh weight (Table 2) was highest in RAB 36 (112.13g) and lowest in B 11(56.2g) and the difference between the two was significant at pd"0.05 (LSD). The fresh weight of RAB 36 was significantly higher than the rest of the cultivars. The fresh weight of DOY 2 (75.78g) was significantly higher than B 11(56.2g) and L 676 (62.93g) but the fresh weight difference between DOY 2 (82.4g) and RAB 32 (74.18g) was non-significant at pd"0.05 level (LSD). Similarly, the fresh weight difference between B 11 and L 676 cultivars are also non-significant. The dry weight of the cultivars closely follows the same pattern as in case of fresh weight.

Phenolic test (Table 2) shows that the highest mortality of 90% was observed in RAB 36 and the lowest mortality of 10% in B 11. The mortality of nematodes in case of RAB 36 (90%) was higher than RAB 32, DOY2 and L 676 that were 85, 50 and 25% respectively.

Table 2 further shows that only two of the cultivars had some tuber cracking. The highest tuber cracking of 10% was observed in case of B 11 followed by L 676 with 6% cracking. No tuber cracking was observed in RAB 32, RAB 36 and DOY2.

The use of varietal resistance is one of the most attractive, cheap, environmentally friendly and sustainable ways for the management of plant disease when highly resistant varieties are available. One of the most important requirements for the development of resistant variety is to screen/test the varieties against the pathogen concern.

Five elite sweet potato cultivars were tested in the greenhouse against the reniform nematodes (*Rotylenchulus reniformis*) through artificial inoculation.

The cultivars showed symptoms of leaf chlorosis, stunted growth (Plate 1) and tuber cracks (Plate 2) with varying level of severity because of the differences in the resistance/tolerance level of the cultivars. Most severe symptoms including stunting, leaf chlorosis, vine death and root deformation/crack was found in B11 and L676. The other cultivars showed very low to no infection at all. Similar results on symptoms development were also described by Thomas and Clark 1983; Robinson *et al.* 1997. The population of nematodes in different cultivars was affected by the resistance of the cultivars as demonstrated by the mortality of the nematodes in the phenolic test. These in turn affected the leaf size, vine length and vine death; leading to the reduction in photosynthetic areas that in turn led to significant yield reduction in susceptible cultivars. The concentration of phenolic compound was quite higher in RAB 36 and RAB 32 compared to the other three cultivars and that affected the nematode numbers on different cultivars.

The changes in leaf morphology are related to inadequate root function. The leaf tissues have compact cell resulting in leaves becoming smaller than normal and absorb less light than healthy leaves (Thomas and Clark 1983; Robinson *et al.* 1997) and this leads to reduced yield. Similar phenomenon might also have been responsible for stunted growth and significant yield reduction in case of B11 and L676 cultivars.

The evidence of root damage on B11 and L676 in the form of crack is quite clear affecting the quality and making unsuitable for selling and human consumption. Similar results were also suggested by Clark and Wright 1983.

Considering the assessment of the various important parameters in the greenhouse trial, RAB 36 could be categorized as resistant, RAB 32 and DOY as moderately resistant, L 676 as moderately susceptible and B 11 as highly susceptible.

The outcome of the greenhouse trial need to be tested in proper field experiments as the results of the greenhouse trials might be affected by the different levels of interaction in the filed.

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