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Developing and Phenotypic Evaluation of Nematode Resistance in K326 X DDV 23 Tobacco Population

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Abstract: ***Resistance to the root - knot nematode (Meloidogyne spp) was identified in the nicotiana tabacum. The phenotypic evaluation was between F_2 population obtained from a cross between DDV23 (Resistant) and K326 (Susceptible) parents respectively. The experiment was conducted in 2018/2019 growing season in the greenhouse at TORITA (Tabora region) to determine nematode resistance from different tobacco genotypes.90F_2 plants with parents 90DDV23 and 90K326 were inoculated with nematode eggs at the rate of 1050 eggs/plant. Results showed that among 90 F_2 tobacco plants inoculated 68 were found resistants' and 22 susceptible. Chi - square analysis (X^2) showed that, the number of individual plant segregate at 3: 1 ratio of resistant to susceptible. This indicates that, the gene for resistance from DDV 23 was transferred to F_2 individuals and the trait was controlled by one or closely linked genes. Also data on disease incidence showed that larger number of F_2 progenies (25.5%) were resistant, followed by moderately resistant parent (21.75%) showing a scale of 2.5 and 2.4 respectively while the remaining susceptible parent showed a scale of 5 infection levels. Most of F_2 genotype exhibited resistance; these results confirmed the presence of dominant gene for resistance to root knot nematodes. Phenotypic evaluation and characterization is limited regarding the knowledge of the specific genes responsible for resistance therefore molecular genetic characterization has to be performed to identify the markers linked to nematode resistance***

Keywords: Tobacco, Root knot nematodes, *melodogyne spp*, resistance and susceptibility to nematodes

1. Introduction

Plant - parasitic nematodes are of great economic importance and one of the limiting factor in crop production. Most of the nematodes live in the soil, they represent one of the most difficult pest problems to identify, demonstrate and control (Nicole *et al*., 2011). Globally, nematodes have been reported to reduce agricultural production by approximately 11% (Viljoen *et al.*, 2016). Crop losses due to nematodes are difficult to estimate accurately, with global estimates varying extensively from $US80 billion (Nicole, 2011) to $US157 billion per year (Abad *et al*., 2003). Using reliable data from the United States, nematodes were estimated to cause annual crop losses of $US10 billion, compared with $US6.6 billion for insect pest losses (Coyne *et al*., 2018). Farmers, agronomists and pest management consultants, commonly underestimate their effects but it has been estimated that some 10 percent of world crop production is lost because of plant nematode damage (Nicole *et al*., 2011).

Root knot nematode species are amongst the most widely destructive plant parasitic nematodes infecting almost all cultivated plants. The parasite is responsible for yield loss amounting to billions of dollars (Bhanu *et al*., 2011). Root knot nematodes attack the roots of host plant in the nurseries and set up feeding location where it deforms the normal root cells and establishes giant cells leading to nodule or gall formation (Jimmy and Robert 2005). The disease is characterized by the presence of characteristic swellings called galls or root - knots on the roots of infected plants (Agrios, 2005). Symptoms include stunted growth, wilting and susceptibility to other pathogen leading to poor yields.

The development of a resistance may include a range of physiological outcomes including minor or complete absence of galling, differences in the degree of necrosis, the inability of the nematode to establish a permanent feeding site, and a decrease in female fecundity or egg output. The objective of this study was to develop a resistant variety to nematodes (*melodogyne spp*) from a cross between K326 and DDV 23).

2. Material and Methods

2.1 Location

The study was carried out in the screen house at Tobacco Research Institute of Tanzania (TORITA) in Tabora - Tanzania, during the cropping season 2018/2019. The site is located in western part on the central plateau between latitude 4° - 7° South and longitude 31º - 34º East at an altitude of 1 160m above sea level.

2.2 Plant and Fertilizer Management

Tobacco seeds were sown in a seed bed of 1.5 mx 5m. After 46 days, seedlings were planted in 10 liter pots containing 10 kg of sandy loam soil. Individual tobacco plant where planted in one pot. The tobacco plant was maintained in the screen house by watering every day to meet the crop

requirements however in cases where there was enough moisture, application of water would be skipped to guard against water logging.

Fertilizer was applied per pot at the rate of 2.5g of NPK (10: 18: 24) as basal application seven day after transplanting and top dressed at 4g of CAN (27%) twenty - one days after transplanting. Plants were watered daily as required.

2.3. Experimental Design and Treatment Application

2.3.1 Developing tobacco population

F_2 Tobacco plants for studying resistance to root knot nematodes (*melodogyne spp*) was developed from the crosses between the susceptible parent of K326 used as female parent and the resistant variety DDV 23 used as a male parent. These families were selfed to develop F_2 generation. The F_2 populations along with the parents were screened for nematode resistance.

2.3.2 Inoculum preparation

Tobacco roots were collected from symptomatic plants. The roots were placed in plastic bags and transported to the nematodes laboratory at TARI Kibaha. Nematodes eggs were extracted from tobacco roots. The roots were washed to remove excess soil then blended, the blended roots were immersed in 0.5% sodium hypochlorite in a plastic jar of 200ml (Hussey *et al.,* 1973). The mixture was agitated for five minutes and sieved with sieves of different size started with 150µm, 45 µm, and 38 µm.

2.3.3 Inoculation of nematode on tobacco plants

Tobacco F_2 plants and their parents (DDV23 and K326) were inoculated 3 weeks after transplanting.2ml of egg suspensions were diluted with 20ml of distilled water to inoculate plants at a rate of 2 - ml egg suspension of nematode population equivalent to 1050 eggs/plant. The equal amount of mixture was pipetted and inserted in three holes/spots in the soil medium, equidistance from each other made surrounding each tobacco plants in a plastic pot. The holes were then covered with top soil. The inoculated plants were maintained in a greenhouse.

2.3.4 Phenotypic evaluation

Sixteen weeks after inoculation the plants were removed from the pots and nematodes infestation assessment were carried out on the roots of F_2 plants and their parents (DDV23 and K326), to determine the presence or absence of nematodes galls. Data on the number of plants identified with galls symptoms were recorded basing on root knot rating chart (Appendix 1). The rating scores from 0 - 3 were rated as resistant; those scoring from 4 and from 5 - 10 were rated as moderately resistant and susceptible respectively, using a scale of 0 - 10 proposed by Bridge and Page, 1980) (Table 1).

Table 1: Root knot gall rating scale showing description used for nematodes assessment (Bridge and Page 1980)

Nematode rating Scale	Description
0	No knots on roots
1	Few small knots difficult to find
2	Small knots only but clearly visible main root clean
3	Some larger knots visible main root clean
4	Larger knots predominate but main root clean
5	50% of roots infested
6	Knotting on main roots
7	Majority of main root knotted
8	All main roots knotted few clean roots visible
9	All roots severely knotted plants usually dying
10	All roots severely knotted, no root system plants usually died

2.3.5 Experimental Design

In this experiment a random complete block design (RCBD) with two replications was used. There were three treatments. (F_2, K 326 and DDV 23) in one block. F2 tobacco plants was raised in a bulk. Each tobacco genotype served as an individual treatment. Each treatment had ninety (90) number of plants.

2.4 Data collection and analysis

A screen house experiments involving three tobacco genotypes (F_2, K 326 and DDV 23) were laid out in a Random Complete Block Design (RCBD) with two replications in pots. The number of gall rated in each scale were assembled in Excel and subjected to analysis of variance, (ANOVA) using GENSTAT 14th Edition (Payne *et al*., 2011). Treatment means were separated using Duncan's Multiple Range Test (DMRT) procedure at the 5% probability level.

2.4.1 Chi - Square Analysis

Phenotypic data for nematode resistance among the tested tobacco varieties and the number of genes controlling resistance to nematodes was estimated using a chi - square analysis which was used to assess whether the observed proportions of resistant and susceptible progeny match the expected ratio in each population. The formula for

Calculating the χ^2 value is:

$\chi^2 = \Sigma$ (observed – expected) ^{2} / expected

Where,

O = Observed frequency

E = Expected frequency

Σ = Summation of the data

3. Results

3.1 Phenotyping of F2 population and their parents

The mean disease incidence on tobacco genotypes inoculated with nematode is shown in Table 2. The results showed that, disease severity and incidence on tobacco genotypes varied significantly ($\leq$0.05). High disease severity was recorded on K326 and low in DDV and F_2. Disease incidence had similar trend, DDV 23 and F_2 families were rated resistant with disease score on a scale of 2.5 and 2.4

respectively. The mean number of plants rated resistant were 22 and 20 respectively.

Table 2: Mean disease incidence among the inoculated tobacco genotypes

Genotype	Disease incidence	Disease reaction class	Mean disease incidence
DDV	2.489a	R	21.75
F2	2.411a	R	19.5
K326	4.911b	S	25.5
Lsd	1.2		
SE	0.4		
F - test	0.014		

1=Based on 0 - 10 scale (**Bridge and Page 1980)**

3.2 Segregation ratio among F_2 plants

A total of ninety of F_2 plants derived from a cross between DDV 23 and K 326 were inoculated with nematodes (*melodoigyne spp*). Resistance or susceptible reaction of each individual was significance making phenotypic identification more clear. Among 90 F_2 tobacco plants inoculated 68 were found resistants' and 22 susceptible (Table 3). Chi - square analysis (χ^2) showed that, the number of individual plant segregate at 3: 1 ratio of resistant to susceptible. This indicates that, the gene of resistance from DDV 23 was transferred to F_2 individual and the traits was controlled by one or closely linked gene.

Table 3: Segregation ratio and chi square test for nematodes resistance between F_2 population, DDV 23 and K326 genotypes after inoculation based on 1 - 10 scale)

Popn	No. plants	Observed no		Segregation. Ratio	Chi square Test	
		R	S		χ^2	P
F2	90	68	22	3: 1	3.84	0.05
DDV 23	90	69	21	3: 1	3.84	0.05
K326	90	20	69	3: 1	3.84	0.05

3.3 Proportion of plants infected by nematodes after inoculation among tobacco genotypes

The summary of extent of damage based on 0 - 10 scale as describe by Bridge and Page, (1980) among tobacco genotypes inoculated with nematode is shown on Table 4. The results showed that majority of F_2 individual plants were resistant to nematode infestation compared to the susceptible genotype K326. The scale number 1 - 3 infection levels showed that 83% of 90F_2 were observed to be resistant to nematodes compared to the susceptible parent K326 where only 20% of the 90 population showed resistance to nematodes infection. However, few plants were observed susceptible (with score scale 4 to 6 infection levels) in F_2 population compared to K326. Few individual plants from DDV 23 accounting to 2%, were observed susceptible (Scoring at 5 scale infection levels) while 74% of K326 were scored susceptible (score scale 5 to 8 infection level). To demonstrate the levels of nematode damage of tobacco genotypes after inoculation, Figures 1 - 7 are showing the extent of damage on each scale grade (1 - 10).

Table 4: The mean number and percentage of plants infected by nematodes after inoculation among tobacco based on 1 - 10 scale) genotypes

	The number of affected plants per genotypes			The average number of affected plants in percentage (%)		
Scale	DDV 23	F_2	K326	DDV 23	F_2	K326
1	16^{abcd}	12.5^{abcd}	1.5^{a}	17.78	13.89	1.67
2	26^{bcd}	32.5^{d}	3.5^{a}	28.89	36.11	3.89
3	29.5^{cd}	29.5^{cd}	13.5^{abcd}	32.78	32.78	15
4	16.5^{abcd}	9.5^{abc}	13^{abcd}	18.33	10.56	14.44
5	2^{a}	4.5^{ab}	29^{cd}	2.22	5	32.22
6	0	1.5^{a}	11	0	1.67	12.22
7	0	0	15	0	0	16.67
8	0	0	3.5	0	0	3.87
9	0	0	0	0	0	0
10	0	0	0	0	0	0

Figure 1: Nematode rating score 1 (Few knot difficult to find)

Figure 2: Nematode score rating 2 small knots observe

Figure 1: Image showing nematode rating score 3 some larger knots visible

Figure 5: Image showing nematode score rating 5most of 50% of roots infested

Figure 4: Image showing nematode rating score 4 larger knots predominate

Figure 6: Image showing nematode rating score 6 knotting of main roots

Figure 7: Image showing nematode rating score 7 majority of main knotted

4. Discussion

The phenotypic evaluation of a certain disease shows the true disease symptoms observed on a given pathogen. This has become a concern of vital importance in any disease management program. The development of a resistance response in Nematode may include a variety of physiological outcomes including minor or complete absence of galling, differences in the egree of necrosis, the inability of the nematode to establish a permanent feeding site and a decrease in female fecundity or egg output (Bernar *et al.*, 2017).

Results of this study showed that there were varied responses between F2 genotype, DDV 23 and K 326 after nematodes inoculation. K326 recorded to have higher gall

score among all genotypes. This indicate that K 326 responds to the invading nematode head by forming galls to serve as feeding sites and for growth and development of the nematode as it was reported by (Lambert and Bekal, 2002).

Most of F_2 progenies and the resistant parent DDV 23 had few gall scores indicating that these genotypes exhibited some levels of resistance. The availability of resistance on these genotypes may be due to the genetic context in which the resistant gene is located (Andersen *et al.,* 2018). Also few galls score indicate that there was reduced penetration of nematodes in the resistant variety by inhibiting penetration of the root knot juvenile. These results confirm the presence of dominant gene for resistance to root knot nematodes in tobacco (Zhang *et al*., 2008, Crowder *et al.,* 2003). The resistance gene present in tested genotypes may be *Rk1* which was reported by Schneider (1991) to reduce the population of *melodogyne* preventing establishing a feeding site. Reed *et al.* (2016) also reported that most commercial tobacco cultivar possess the *Rk1* gene, which provide resistance to races 1 and 3 of *Meloidogyne incognita* and race 1 of *M. arenaria*.

Nematode resistance gene has also been found in some crops such as tomato (Ernst *et al.,* 2002), potato (Paal *et al.,* 2004), pepper (Fazari *et al.,* 2012) and sugar beet (Cai *et al*., 1997). Ng'ambi *et al*. (1999b) and Fellers *et al. (*2002) reported diversity of resistance to root - knot nematode in tobacco genotypes which revealed that *N. tabacum* houses gene which provides resistance to root knot nematodes as confirmed by results from F_2 lines in this study.

5. Conclusion and Recommendation

The study has identified genotypes resistant to root knot in Tanzania. However, the molecular genetic characterization has to be performed to identify the markers linked to nematode resistance on these genotypes. Phenotypic evaluation and characterization is limited regarding the knowledge of the specific genes responsible for resistance. Molecular characterization will give the information about these genes.

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