GAU Res. J., **34** (2) : 92-97 (2009)

Relationship between resistance to root-knot nematode *(Meloidogyne* spp.) and cortex layer in tobacco *(Nicotiana tabacum* L.)

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Abstract

Bidi tobacco *(Nictoiana tabacum* L.) is an important cash crop of India not only for farmers but also for the national exchequer and for generating employment. Root-knot is a major disease of tobacco, causing enormous loss in production. In India the disease was reported on all tobacco types grown in light soils of Gujarat, Karnataka and Andhra Pradesh. Gujarat is one of the major states producing bidi tobacco in India. Assuming that the nematode resistant/tolerant lines of tobacco possess some anatomical peculiarity in roots, which might be deterring the larvae from entering the inner layers. The deterrents could be either a thick hypodermis cell layer or number of cortical cell layers or combinations of both. In this context, a study to determine the mechanism of nematode resistance and to establish the relationship between number of cortex cell layers, which provides the thickness strength to the roots for resistance were initiated. The present findings revealed that there was highly significant negative correlation (-0.52 and -0.37) between number of hypodermis layers and root-knot index as well as total cortex layers and root-knot index, respectively. This suggests that number of hypodermis layers in roots increases the root-knot nematode resistance in tobacco.

Keywords : Tobacco, root-knot nemetode, resistance, cortex layer

Introduction

Bidi tobacco *(Nicotiana tabacum* L.) is an important cash crop of India not only for farmers but also the national exchequer and for generating employment. Gujarat is one of the major states producing bidi tobacco in India. Tobacco crop suffers from several biotic and abiotic stresses. In case of nematode disease, root-knot *(Meloidogyne* spp.) causes severe damage to the crop qualitatively as well as quantitatively. World wide loss in tobacco yield due to plant parasitic nematodes was estimated to the tune of 14.7 per cent (1). Shah *et al.* (2) estimated 51 per cent loss in transplantable seedlings in bidi tobacco nursery, while Markose and Patel (3) recorded 31 % loss of cured leaf yield; 33 % and 23 % cured leaf yield loss was recorded in cvs. A-119 and GT-5 respectively (4). The symptoms of root-knot nematodes infection can be clearly demarcated through its peculiar knot forming habit on roots, and hence screening of plants can be done after uprooting the plants. The infective stage, i.e., second stage juvenile larvae penetrate in to the root and establish a feeding site usually within the pericycle and vascular tissues of the roots (5). The thick and starchy cortical layer of Cv. GT-5 may play a role in prevention of feeding by root-knot nematodes (6). Looking to this, it can be imagined that rigid and thick cortical layers might be responsible for nematode resistance. The cortex consist of mainly parenchyma. The inner most cortical layer of roots of seed plants growing in soil differentiates as outer cortex or hypodermis or exodermits, inner cortex and endodennis. The subepidermal cortical layers (hypodermis or excderrnis) of root are often differentiated as protective tissue containing suberin in its wall (7). The present study was carried out with three varieties of tobacco viz. A-119 (susceptible to root-knot nematode), GT 5 (tolerant to root-knot nematode) and Bhavya (resistant to root-knot nematode) with their F_1, F_2 and back cross material.

Materials and methods

The research work was carried out at Bidi Tobacco Research Station (BTRS), Gujarat Agricultural

University, Anand during 1995-97. The experimental material consists of VFC tobacco variety Bhavya and Bidi Tobacco varieties GT-5 and A - 119 and its six generations viz. P_1, P_2, F_1, F_2, BC_1 and BC_2. The experiment was laid out in randomized block design with three replications. Each plot of P_1, P_2 and F_1 generations consisted of one row while two rows were kept for BC_1 and BC_2 and four rows for F_2 generations. Each row had 10 plants with inter and intra-row spacing of 90 cm x 75 ern. The agronomic practices in vogue were followed. At maturity plants were uprooted and roots were examined for root-knot index using 0-5 scale (8). The roots were also preserved in acetic 16 acid formalin- alcohol preservative for anatomical study. Acetic acid- formalin-alcohol preservative was prepared by mixing 90 cc of 50% alcohol, 5 cc of glacial acetic acid and 5 cc of formalin. The hand cross sections were taken from preserved roots and stained with safranin. Semi-permanent slides were prepared with the help of glycerin and nail polish, examined under microscope for recording number of hypodermis, inner cortex and total cortical cell layers present.

With a view to study the correlation in different generation (P_1 , P_2, F_1, F_2, BC_1, BC_2) of individual crosses for number of cortex layers and rootknot nematode resistance in tobacco plant, the correlation coefficients were also worked out at phenotypic level according to the formula suggested by Johnson *et al.* (9).

The observed values of 'r' were tested for their significance by companng them with tabulated 'r' values as suggested by Snedecor and Cochran (10) at respective degrees of freedom.

Results and discussion

In all 202 root transverse sections of vanous plants from different generations were observed under microscope. The average hypodermis, inner cortex and total cortex layers for cv. Bhavya were 5.6, 6.0 and 13.2 for OT-5, 3.8, 9.0 and 12.8 and for cv. A- 119 were 3.3,5.3 and 8.7 respectively. In cross I hypodermal cell layers ranged from 5 to 6 for P_1, 3 to 4 for P_2, 6 to 7 for F_1 , 3 to 5 for F_1, 3 to 5 for BC_1 and 3 to 7 for BC_2, while inner cortex layers ranged from 5 to 7 for P_1,

Table 1. Number of hypodermis layers, inner cortex layers and total cortex layers, their frequencies and average, number of cortex layers of different generation in cross I.

Name of the Generation	No. of hypodermis layers			No. of inner cortex layers			Total no. of cortex layers		
	Layer No.	Freq. of plant	Av. No. of layers	Layer No.	Freq. of plant	Av. No. of layers	Layer No.	Freq. of plant	Av. No. of layers
Bhavya	5	2	5.6	5	1	6.0	12	2	13.2
	6	3	6.0	3	14	3.0			
GT 5	3	1	3.8	7	1	9.0	12	1	12.8
	4	5		9	6		13	5	
(Bhavya x	6	1	6.8	5	1	6.4	12	2	13.2
GT-5) F_1	7	4		6	1		14	3	
				7	3				
(Bhavya x	3	1	4.3	4	1	9.0	5		
GT 5) F_2	4	4		5	4	5.3	10	2	9.5
	5	3		6	3		11	1	
(Bhavya x	3	3	3.9	3	1	4.4	7	1	8.3
GT 5 x	4	2	5.0	2	8	4.0			
Bhavya)	5	2		5	4		9	1	
							10	1	
(Bhavya	3	2	4.4	4	2	5.4	7	1	9.9
x GT 5)	4	5		5	4		8	2	
x GT 5	5	3		6	5		9	2	
	6	1		7	1		10	3	
	7	1					11	1	
							12	2	
							13	1	

Table 2. Number of hypodermis layers, inner cortex layers and total cortex layers, their frequencies and average number of cortex layers of different generation in cross II.

Name of the Generation	No. of hypodermis layers			No. of inner cortex layers			Total no. of cortex layers		
	Layer No.	Freq. of plant	Av. No. of layers	Layer No.	Freq. of plant	Av. No. of layers	Layer No.	Freq. of plant	Av. No. of layers
Bhavya	5	1	5.8	6	4	6.4	11	1	12.0
	6	4		8	1		12	3	
							13	1	
A119	3	3	3.3	4	2	4.5	7	1	7.8
	4	1		5	2		8	3	
(Bhavya x A119) F_1	5	2	5.6	4	1	5.6	9	1	11.2
	6	3		5	1		10	1	
				6	2		12	2	
				7	1		13	1	
(Bhavya x A 119) F_2	3	3	4.9	4	2	5.3	7	1	10.2
	4	4		5	10		8	2	
	5	5		6	5		9	3	
	6	3		7	1		10	4	
	7	3					11	4	
							12	2	
							13	2	
(Bhavya x A 119) x Bhavya)	3	1	5.2	4	2	6.5	7	1	11.6
	4	2		5	3		9	1	
	5	2		6	1		10	1	
	6	6		7	2		11	3	
				8	1		12	1	
				10	2		13	2	
							15	1	
							16	1	
(Bhavya x A 119) x A119	3	2	5.2	3	2	5.2	8	1	10.3
	4	2		5	6		9	5	
	5	1		6	2		10	1	
	6	6		7	2		11	2	
	7	1					12	1	
							13	1	
							14	1	

9 for P_2, 5 to 7 for F_1, 4 to 6 for F_2, 3 to 5 for BC_1 and 4 to 7 for BC_2 (Table I). The total cortex layers ranged from 12 to 14 for P_1, 12 to 13 for P_2, 12 to 14 for F_1, 9 to 11 for F_2, 7 to 10 for BC_1 and 7 to 13 for BC_2. The average number of hypodermis ayers for all generations were counted, which were 5.6, 3.8, 6.8,4.3,3.9 and 4.4 for P_1, P_2, F_1, F_2, BC_1 and BC_2, respectively. While inner cortex layers were 6.0, 9.0, 6.4, 5.3, 4.4 and 5.4 whereas total cortex layers were 13,2, 12.8, 13.2,9.5.8.3 and 9.9 for P_1, P_2,F_1, F_2 and BC_2.

In different generations of cross II hypodermis layers ranged from 5 to 6 for P_1, 3 to 4 for P_2, 5 to 6 for F_1, 3 to 7 for F_2, 3 to 6 for BC_1 and 3 to 7 for BC_2 (Table 2). The inner cortex layers ranged from 6 to 8 for P_1,4 to 5 for P_2, 5 to 7 for F_1, 4 to 7 for F_2, 4 to 10 for BC_1 and 3 to 7 for BC_2, while total cortex layers ranged from 11 to 13 for P_1, 7 to 8 for P_2, 9 to 13 for F_1, 7 to 13 for F_2, 7 to 16 for BC_1 and 8, 10, 14 for BC_2. The average number of hypodermis layers, inner cortex and total cortex layers for different generations were 5.8, 6.4 and 12.0 for P_1, 3.3, 4.5 and 7.8 for P_2, 5.6, 5.6 and 11.2 for F_1, 4.9, 5.3 and 10.2 for F_2, 5.2, 6.5 and 11.6 for BC_1 and 5.2, 5.2 and 10.3 for BC_2, respectively.

For cross III hypodermis layers ranged from 3 to 4 for P_1, remained 4 for P_2, 4 to 5 for F_1, 3 to 5 for F_2, 3 to

Table 3. Number of hypodermis layers, inner cortex layers and total cortex layers their frequencies and average, number of cortex layers of different generation in cross III.

Name of the Generation	No. of hypodermis layers			No. of inner cortex layers			Total no. of cortex layers		
	Layer No.	Freq. of plant	Av. No. of layers	Layer No.	Freq. of plant	Av. No. of layers	Layer No.	Freq. of plant	Av. No. of layers
A 119	3	4	3.3	5	4	5.3	8	3	8.7
	4	2		6	2		9	2	
							10	1	
GT 5	4	9	4.0	9	9	9.0	13	9	13.0
(A 119 x	4	3	4.7	4	2	5.0	9	4	9.7
GT 5) F_1	5	7		5	6		10	5	
				6	2		11	1	
(A 119 x	3	7	4.0	3	2	5.5	6	1	9.5
GT 5)	4	9		4	2		7	1	
F_2	5	6		5	8		8	3	
				6	4		9	5	
				7	4		10	9	
				8	2		12	2	
							13	1	
(A 119 x	3	4	4.5	3	1	4.7	7	2	9.2
GT 5)x	4	3		4	6	8.0	2		
A 119	5	3		5	4		9	3	
	6	4		6	2		10	6	
				7	1		12	1	
(A 119	3	5	4.4	5	4	6.2	8	2	10.2
GT 5) x	4	7		6	6		9	4	
GT5	5	3		7	5		10	4	
	6	1		8	1		11	3	
							12	1	
							13	2	

6 for BC_1 and BC_2 (Table 3). Inner cortex layers ranged from 5 to 6 for P_1, remained 9 for P_2, 2 to 6 for F_1, 3 to 8 for F_2, 3 to 7 for BC_1 and 5 to 8 for BC_1, while total cortex layers ranged from 8 to 10 for P_1, remained 13 for P_2, 9 to 11 for F_1, 6 to 13 for F_2, 7 to 12 for BC_1 and 8 to 13 for BC_2. The average number of hypodermis layers, inner cortex layers and total cortex layers for P_1, were 3.3, 5.3 and 8.7 for P_2, 4, 9 and 13 for F_1, 4.7, 5.0 and 9.7 for F_2, 4.0, 5.5 and 9.5 for BC_1, 4.5, 4.7 and 9.2 for BC_2, 4.0, 6.2 and 10.2 respectively.

Though the number of plants observed were less in number, comparative numbers showed that this character may be governed by dominant gene, as F_1, invariably showed higher numbers of hypodermis. As there is no such type of information available in tobacco, this study may be useful in generating some basic information of this nature.

Hypodermis layers ranged from 3 to 7, inner cortex layers ranged from 3 to 10 and total cortex layers ranged from 6 to 16 (Table 4).

The average root-knot nematode index for 3, 4, 5, 6 and 7 hypodermis layers were 3.46, 2.56, 1.95, 1.37 and 1.33, respectively. The average root-knot nematode index for 3, 4, 5, 6, 7, 8, 9 and 10 inner cortex layers were 2.25, 2.12, 2.50, 2.20, 1.78, 1.83,

Table 4. Number of cortex layers their respective plant frequency, root knot nematodes index and its average root-knot rematode index and its average root-knot nematode index in 202 plants of tobacco.

No. of corlex layers	Total plants	N frequency							Av. No. of layers
		0	1	2	3	4	5	Cumulative frequency	
				Hypodermis layers					
3	39	1	2	4	10	15	7	135	3.46
4	61	5	6	22	18	8	4	156	2.56
5	41	2	9	18	9	2	–	80	1.95
6	43	5	19	14	4	-	–	59	1.37
7	18	3	8	5	2	-	–	24	1.33
				Inner cortex layers					
3	8	1	1	3	2	-	1	18	2.25
4	25	2	6	7	4	4	1	53	2.12
5	69	4	12	22	16	11	5	173	2.50
6	50	6	11	13	10	7	5	110	2.20
7	27	3	9	9	4	1	1	48	1.78
8	6	-	2	3	1	-	-	11	1.83
9	15	-	2	6	5	2	-	37	2.47
10	2	-	1	-	1	-	-	4	2.00
				Total cortex layers					
6	1	-	-	-	-	-	1	5	5.00
7	8	-	2	3	1	1	1	20	2.50
8	22	2	2	-	5	9	4	73	3.32
9	42	3	4	11	17	6	1	106	2.52
10	45	3	8	19	8	3	4	102	2.27
11	20	2	6	7	3	2	-	37	1.85
12	25	5	8	9	2	1	-	36	1.44
13	26	8	10	7	1	-	-	27	1.04
14	11	2	6	2	1	-	-	13	1.18
15	1	-	-	1	-	-	-	2	2.00
16	1	1	-	-	-	-	-	0	0.00

2.47 and 2.00 respectively, while root-knot index for 6,7 ,8,9, 10, 11, 12, 13, 14, 15 and 16 total cortex layers were 5.0, 2.5, 3.32, 2.52, 2.27, 1.85, 1.44, 1.04, 1.18, 2.00 and 0.00 respectively.

There was highly significant positive correlation between 20 number of hypodermis layers and total cortical layers (Table 5). This suggested that as number of hypodermis layers increases total cortex layers also increases. Similarly, numbers of inner cortex and total cortex layers also showed positive correlation. Significant negative correlation was observed between hypodermis layers and root-knot index, total cortical cell layers and root-knot index.

This was clearly indicated that hypodermis layer and root-knot index relationship is important. The negative correlation indicated that as number of hypodermis layers increases root-knot index decreases and same is true for total cortex layers, root-knot nematode index. Moreover, as there was no significant correlation observed between number of inner cortex and root-knot index. It may be stated that the correlation observed between total cortex layers and root-knot index is due to increase of hypodermis cell layers.

From the foregoing discussion it is clear that as number of hypodermis layers increases, root-knot

Table 5. Correlation among hypodermis, inner cortex, total cortex layers and root-knot nematode index in roots of tobacco plants.

	Hypodermis layers	Inner cortex layers	Total cortex layers	Root-knot nematode index
Hypodermis layers	1.0000			
Inner cortex layers	0.01029	1.0000		
Total cortex layers	0.64141**	0.77040**	1.00000	
Root-knot nematode index	-0.52048**	-0.05437	-0.36504**	1.0000

* significant at 5 %   ** significant at 1 %

nematode resistance of plant also increases. This may be due to the difficulty in penetration of root by nematodes as the cell wall of hypodermis cell is more suberized or thicker in comparison to inner cortex cells. It is known that when juveniles of nematode penetrate in to the tissues of the host plant the stylet is withdrawn after penetration of cell and another hole, adjacent to the first is made in the cell wall. Repetition of this process is continued till the hole is made. After this cutting cycle, the larva passes through the hole in the cell wall and repeat the attack on deeper cell in the plant to locate the site of feeding. This process should be completed within a month. For nematode resistance two factors were likely to work simultaneously viz., cell wall hardiness and number of hypodermis layers. As the hypodermis layers increase, nematode face more trouble in entering and thus may leave the plant or die due to starvation.

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