

EFFECT OF INFECTION OF RENIFORM NEMATODE ON POLYPHENOL OXIDASE, PEROXIDASE ACTIVITIES AND TOTAL PHENOL CONTENT IN LEAVES OF BIDI TOBACCO FROM ANAND, GUJARAT

AARTI BAIRWA AND H.R. PATEL

Bidi Tobacco Research Station, AAU, Anand 388110 Email: drhrpatel10@gmail.com

(Received on 25th August, 2023 and accepted on 14th November, 2023)

Estimation of polyphenol oxidase, peroxidase activities and total phenol content in leaves of bidi tobacco due to infection of reniform nematode under pot conditions revealed higher polyphenol and peroxidase activity as well as reduced total phenol content in A 119 than ABT 10. Inoculation of 2000 J₄ of reniform nematode can reduce the polyphenol and peroxidase activity as well as total phenol content than no inoculation. The interaction effect indicated that inoculation of reniform nematode @ 2000 J₄ per plant reduced the peroxidase activity and total phenol content in A 119 and increased the same in ABT 10 compared to control.

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.), is an important non-food narcotic commercial crop, occupies less than 0.27 per cent of the net cultivated area and earns sizable amount of foreign exchange (Rs. 9,731 crores), central excise (Rs. 55,000 crores) to the national exchequer besides providing direct and indirect employment opportunities to 45.7 million people including 6 million farmers and workers. Plant parasitic nematodes, being a polyphagous pest and having wide range of host can cause severe damage to agricultural and horticultural crops. Root-knot (*Meloidogyne incognita*; *M. javanica*), reniform (*Rotylenchulus reniformis*) and stunt (*Tylenchorhynchus vulgaris*) nematodes are found predominantly attacking bidi tobacco in Gujarat. Worldwide loss in tobacco yield due to them was estimated to the tune of 14.7 per cent (Sasser and Freckman, 1987). Estimation of losses in production of transplants and cured leaf yield of bidi tobacco due to root-knot nematodes revealed 51 and 31 to 50 per cent loss, respectively (Shah *et al.* 1983; Markose and Patel, 1977; Patel *et al.*, 1986). Patel *et al.* (2002) reported avoidable loss to the tune of 33 per cent in root-knot

susceptible bidi tobacco cultivar Anand 119 (A 119) and up to 23 per cent in tolerant variety Gujarat Tobacco (GT 5) due to stunt, root-knot and reniform nematodes. Patel and Patel (2009) estimated 22.1 per cent avoidable loss due to infection of reniform nematode at first pulling and overall loss to the tune of 5.51 per cent in production of transplants in bidi tobacco nursery. Avoidable loss due to stunt nematodes in bidi tobacco nursery has been estimated to the tune of 14 per cent in production of transplants (Patel and Joshi, 2011). In the present study, attempts were made to estimate the polyphenol oxidase, peroxidase activities and total phenol content in the leaves of root-knot resistant and susceptible bidi tobacco cv. ABT 10 and A 119, respectively against infection of reniform nematode under pot conditions.

MATERIALS AND METHODS

An experiment was conducted, during the year 2011 and 2012, to estimate the polyphenol oxidase, peroxidase activities and total phenol content in leaves of root-knot resistant and susceptible bidi tobacco cv. ABT 10 and A 119, respectively due to infection of reniform nematode under pot conditions. There were four treatments comprising of two varieties viz. ABT 10 (V₁), and A 119 (V₂) and two inoculum levels viz. 0 {Control (I₁)} and 2000 J₄ (I₂) per pot. Each treatment was repeated four times in Completely Randomized Design. Thus, sixteen disinfested pots of 15 cm diameter were filled with 500 cc sterilized soil and FYM. Each pot was transplanted with healthy seedling of bidi tobacco according to treatments keeping one seedling per pot. Second stage juveniles (J₂) of reniform nematode were extracted from well mixed soil collected from the pure culture micro plot using Petri dish Assembly Method (Chawla

and Prasad, 1974). After 24 hours, nematode suspension was carefully collected, concentrated and kept for 15 days at room temperature till juveniles convert into pre-adult stages (J_4). The nematode count (pre-adult female) per ml in water suspension was estimated using stereoscopic binocular microscope for inoculation purpose. Required quantity (20 ml) of nematode suspension was inoculated per pot as per the treatment in the respective pot, while in control only 20 ml water without nematode was poured. The pots were kept on the bench in net house. The seedlings were watered regularly and protected from the insect damage using appropriate management measures. All agronomic practices in vogue were followed. Fresh leaves were collected from each treatment 60 days after inoculation (60 DAI) and immediately processed. Required quantity of the fresh leaf were weighed and analyzed for Polyphenol oxidase: Colorimetric method (Sheen, 1971), Peroxidase activity: Worthington's method (Guilbault, 1976) and Total phenol content: (Simson and Ross, 1971) in each treatment at the Department of Bio-Chemistry, BACA, AAU, Anand. Finally, the data were tabulated and analyzed by using standard statistical model at the Department of Agricultural Statistics, BACA, AAU, Anand.

RESULTS AND DISCUSSION

Polyphenol oxidase: The results (Table 1) on polyphenol oxidase activity revealed significant difference for variety (V) and inoculum level (I), while it was not significant for the year, interactions YXV, VXI, YXI, YXVXI.

Significant differences were observed during 2011 and the pooled results indicated that, higher polyphenol oxidase activity in A 119 (V_2) compared to ABT 10 (V_1). Inoculation of 2000 J_4 (I_2) of reniform nematode significantly reduced the polyphenol oxidase activity compared to no inoculation (I_1) in individual years and in pooled analysis. Year effect and interaction effects were non-significant.

Peroxidase activity: Differences were found significant for variety (V) during 2011 and 2012 as well as interaction VXI in individual years and in pooled results (Table 1).

Inverse trend for peroxidase activity was observed, during 2011 and 2012, between two varieties indicating increase and decrease peroxidase activity in A 119 compared to ABT 10 during 2011 and 2012, respectively.

Though the difference was not significant due to inoculation of reniform nematode @ 2000 J_4 (I_2) per plant, it was found to reduce peroxidase activity during 2012 and in pooled results.

Interaction VXI indicated that inoculation of reniform nematode @ 2000 J_4 per plant in A 119 (V_2I_2) significantly reduced the peroxidase activity compared to no inoculation (V_2I_1) in individual years and in pooled analysis; while, it was significantly increased during 2012 and in pooled analysis in inoculated ABT 10 (V_1I_2) compared to no inoculation (V_1I_1).

Significant year effect and interaction YXV indicated that there was depressing effect of environment on peroxidase activity during 2012 compared to 2011.

Total phenol content: Significant differences in total phenol for variety (V) during both the years, inoculum level during 2012 and in pooled and interaction VXI, YXV and YXVXI were observed in individual years and in pooled analysis (Table 2).

Significantly higher total phenol content was observed in ABT 10 (V_1) compared to A 119 (V_2) during 2011 and 2012.

Inoculation of 2000 J_4 (I_2) of reniform nematode significantly reduced total phenol compared to no inoculation (I_1) during the year 2012 and in pooled result.

Interaction VXI indicated that Inoculation of 2000 J_4 of reniform nematode in ABT 10 (V_1I_2) and A 119 (V_2I_2) significantly increased and decreased total phenol content compared to respective no inoculation (V_1I_1, V_2I_1), respectively in individual years and in pooled analysis.

The above result revealed that between two varieties A 119 recorded high polyphenol and peroxidase activities as well as reduced total

Table: 1. Effect of infection of reniform nematode on polyphenol oxidase, peroxidase activities and total phenol content in leaves of bidi tobacco.

Treatment	Activity					
	Polyphenol oxidase, O.D. at 460nm/min./g/ fresh tissue			Peroxidase, O.D.at 460nm/min./g/ fresh tissue		
	2011	2012	Pooled	2011	2012	Pooled
Variety (V)						
ABT10 (V ₁)	0.141	0.136	0.138	0.192	0.179	0.186
A119 (V ₂)	0.158	0.150	0.154	0.380	0.156	0.268
S.Em.±	0.003	0.007	0.003	0.017	0.003	0.047
CD 0.05	0.010	NS	0.008	0.051	0.010	NS
S.Em.± (YXV)		0.004			0.009	
CD 0.05 (YXV)		NS			0.025	
Inoculum level J ₄ (I)						
0 J ₄ (I ₁)	0.158	0.154	0.156	0.302	0.165	0.233
2000 J ₄ (I ₂)	0.141	0.131	0.136	0.270	0.170	0.220
S.Em. ±	0.003	0.007	0.003	0.017	0.003	0.006
CD 0.05	0.010	0.021	0.008	NS	NS	NS
VXI						
V ₁ I ₁	-	-	-	0.163	0.121	0.142
V ₁ I ₂	-	-	-	0.221	0.238	0.229
V ₂ I ₁	-	-	-	0.441	0.209	0.325
V ₂ I ₂	-	-	-	0.320	0.102	0.211
S.Em.±	0.005	0.010	0.004	0.025	0.005	0.009
CD 0.05	NS	NS	NS	0.072	0.014	0.025
Year		NS			Sign.	
S.Em. ± (YXI)		0.004			0.009	
CD 0.05 (YXI)		NS			NS	
S.Em. ± (YXVXI)		0.006			0.013	
CD 0.05 (YxVxI)		NS			NS	
CV %	6.5	14.2	10.8	17.1	5.7	15.6

Table: 2. Effect of infection of reniform nematode on total phenol content in leaves of bidi tobacco.

Treatment	Total phenol content µg/g fresh tissue		
	2011	2012	Pooled
Variety (V)			
ABT10 (V ₁)	787.963	732.650	760.306
A119 (V ₂)	556.863	644.848	600.855
S.Em.±	21.642	25.683	32.042
CD 0.05	63.169	74.965	NS
S.Em.± (YXV)		16.793	
CD 0.05 (YXV)		47.498	
Inoculum level J ₄ (I)			
0 J ₄ (I ₁)	684.750	741.863	713.306
2000 J ₄ (I ₂)	660.075	635.635	647.855
S.Em. ±	21.642	25.683	11.874
CD 0.05	NS	74.965	33.586
VXI			
V ₁ I ₁	711.450	590.475	650.963
V ₁ I ₂	864.475	874.825	869.650
V ₂ I ₁	658.450	893.250	775.650
V ₂ I ₂	455.675	396.445	426.060
S.Em.±	30.607	36.222	16.793
CD 0.05	89.334	106.016	65.927
Year		NS	
S.Em. ± (YXI)		16.793	
CD 0.05 (YXI)		NS	
S.Em. ± (YXVXI)		23.749	
CD 0.05 (YXVXI)		67.172	
CV %	9.1	10.5	9.8

phenol content than ABT 10. Inoculation of 2000 J₄ of reniform nematode reduced polyphenol and peroxidase activities as well as total phenol content than no inoculation. Interaction VXI indicated that inoculation of reniform nematode @ 2000 J₄ per plant reduced peroxidase activity and total phenol content in A 119 and increased the same in ABT

10 compared to no inoculation. Our results are in agreement with the results of Sherif *et al.* (1980), Patel and Patel (1990 a, b) and Shukla and Haseeb (2004) with respect to polyphenol oxidase activity and total phenol. Thakar (1978) observed high phenol content in reniform nematode resistant pigeonpea variety 556-1 compared to susceptible

variety. In our case, higher amount of phenol was observed in root-knot nematode resistant cv. ABT 10 compared to susceptible cv. A 119.

REFERENCES

- Guilbault, G. G. 1976. Handbook of Enzymatic Methods of Analysis. Marcel Dekker, Inc. New York. pp: 147.
- Markose, V. T. and G. J. Patel. 1977. Effect of soil fumigation and nitrogen manuring on bidi tobacco. **Tob. Res.** 3: 7-10.
- Patel, H. R. and K. R. Joshi. 2011. Assessment of avoidable loss due to stunt nematodes in bidi tobacco nursery (Abst.). Paper presented at National Symposium on "Nematodes: A challenge under changing climate and agricultural practices" held at KJMM Animation Centre, Kovalam, Thiruvananthapuram organised by NSI, ICAR, New Delhi and Kerala Agril Univ., Vellayani during Nov. 16-18, 2011. Pp: 91-92.
- Patel, A. S. and H. R. Patel. 1990a. Biochemical changes brought out by the infection of stunt nematode in bidi tobacco. **Indian J. Mycol. Pl. Pathol.** 20: 174-175.
- Patel, R. G. and D. J. Patel. 1990b. Pathogenicity and biochemical changes induced by reniform nematode *Rotylenchulus reniformis* Linford and Olivera, 1940 in pigeon pea. **Current Nematology** 8: 113-115.
- Patel, H. R. and B. N. Patel. 2009. Assessment of avoidable loss due to reniform nematode in bidi tobacco nursery. **Indian J. Nematol.** 39: 111-113.
- Patel, H. R.; B. N. Patel and N. A. Bhatt. 2002. Comparative avoidable losses in yield of bidi tobacco A 119 and GT 5 due to nematodes. **Tob. Res.** 28: 60-64.
- Patel, S. K.; D. J. Patel and H. V. Patel. 1986. Estimation of losses due to root-knot nematodes in bidi tobacco field (Abst.). National conference on plant parasitic nematodes. India: Problems and Progress held at IARI, New Delhi. Dec. 17-20. p: 12.
- Sasser, J. N. and D. W. Freckman. 1987. A world perspective on Nematology: The role of society. In: *Vistas on Nematology: A Commemoration of the Twenty Fifth Anniversary of the Society of Nematologists* edited by J. A. Veech and D. W. Freckman. Pp: 1-4.
- Shah, H. M.; D. J. Patel and G. B. Valand. 1983. Assessment of losses due to root-knot disease in bidi tobacco nursery. **Tob. Res.** 9: 108-109.
- Sheen, S. J. 1971. The colorimetric determination of chlorogenic acid and rutin in tobacco leaves. **Tob Sci.** 15: 116-121.
- Sherif, M. A. ; M. H. Belal and T. Badra. 1980. Activity of polyphenol oxidase in tomato healthy and infected by *Rotylenchulus reniformis* and *Meloidogyne incognita*, *Anzeiger für Schöding-Skünde Pflanzenschutz Umweltschutz*. 53:10-13.
- Shukla, P. K. and A. Haseeb. 2004. Studies on occurrence, pathogenic potential and management of reniform nematode on spearmint. **Indian J. Nematol.** 34:140-146.
- Simson, J. T. and A. F. Ross. 1971. Changes in phenol metabolism associated with systemic resistance to tobacco mosaic virus in samsun NM tobacco. **Phytopathology** 61: 1261-65.
- Thakar, N. A. 1978. Studies on the reniform nematode, *Rotylenchulus reniformis* Linford and Olivera, 1940 infecting pigeon pea. Ph.D. Thesis submitted to RAU, Udaipur.