Screening of Crossandra *(Crossandra infundibuliformis (L.) (Nees.)* genotypes for root lesion nematode

Abstract

An experiment was conducted with ten crossandra genotypes collected from various institutions and local sources at Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Coimbatore. The experiment was laid out using FCRD design with three replications with genotypes uninoculated and inoculated with nematodes. Observations were taken after 60 days of inoculation. The results revealed that Nilakottai local and Arka Shravya recorded the least percentage of reduction of shoot weight (13.73 & 14.55%) and root weight (18.83 & 16.93%) under inoculated condition. The increased percentage of phenol, polyphenol oxidase, peroxidase activity and phenylalanine ammonia lyase was higher in Nilakottai local and Arka Shravya and the least was noticed in Dharmapuri local under inoculated conditions. The root necrosis percentage ranged from 7.05 to 21.17. The lowest root lesion percentage was recorded in genotype Nilakottai local (7.05) followed by Arka Shravya (8.67) and the highest necrosis percentage was observed in genotype Dharmapuri local (21.17). Resistant reaction was noticed in genotype Nilakottai local and Arka Shravya. Tolerant reaction was observed in genotype Delhi crossandra, Arka Ambara, Arka Chenna, Arka Shreeya and Arka Kanaka. The susceptible reaction was found in genotype Villupuram local, Neyveli local and Dharmapuri local.

Introduction

Root lesion nematode (*Pratylenchus delattrei*) is an important plant parasitic nematode in Tamil Nadu, particularly on crossandra (Vadivelu and Muthukrishnan, 1981b). It is a migratory endoparasitic nematode feeding on roots. The nematode infested plant shows stunted growth with pale yellow and puckering of leaves, internodal length will be reduced and gives a rosette appearance. The roots showed brownish necrotic lesions initially and later stages, the whole roots get decay. *P. delattrei* causes serious damage to the crop (Jonathan *et al.*, 2001). A resistance mechanism to nematodes is considered as an excellent way which restricts or prevents nematode infestation. Apart from morphological characters, biochemical resistance plays a vital role in biotic resistance. While the plants affected with the nematodes by wounding, the plants will produce different defense enzymes. Hence, it is important to understand the resistance/tolerance through morphological and biochemical basis is a great importance. So, it is necessary to screen the genotypes for their reaction to nematodes. Hence, a study was conducted to screen crossandra genotypes for root lesion nematode (*Pratylenchus delattrei)* under pot culture conditions at the Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Coimbatore.

Materials and Methods

A random survey was conducted at Sempatti block of Dindugul district to identify the *Pratylenchus delattrei* infested sick field. The soil and root sample were collected at the depth of 25-30 cm in the nematode sick field. The collected soil samples were processed by using Cobb's decanting and sieving method (Cobb, 1918) followed by modified baermann's funnel technique (Schindler, 1961). The collected root samples were washed in running tap water to remove the adhering soil particles. Then, the roots are cut into small pieces. Later stained and destaining of root was done in boiling of acid fuschin lactophenol and in plain lactophenol for 48 hours. The nematode in soil and root was observed under stereozoom microscope (1-5x) and compound microscope to identify the nematode species by morphological characters at Department of Nematology, TNAU, Coimbatore. Then, the lesion nematode culture was maintained in crossandra under pot culture conditions for further studies (Mallaiah *et al.*, 2014).

Screening of crossandra genotypes

Ten crossandra genotype cuttings were collected from various institutions and local allowed for rooting under mist chamber at Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Coimbatore. The potting mixture consists of red soil, sand, farmyard manure in the ratio of 2:1:1 and sterilized using autoclave at 121°C for 30 minutes at 15 lbs. The rooted cuttings were transplanted in earthern pots @2 cuttings/pot. After 15 days of transplanting, two rooted cuttings thinned into one and the collected nematode suspension inoculated @ 2J2 /g soil in the rhizosphere region. The experiment was laid out using FCRD design with the genotypes of uninoculated and inoculated with nematodes and three replications for each genotypes were maintained. The plants were watered frequently and packages of practices were followed as per the crop production guide. Observations viz., shoot length,root length, shoot weight ,root weight, soil nematode population and root nematode population were taken after 60 days of inoculation.

Root lesion index (%) was recorded using root necrosis percentage by following the method described by Pinochet (1988).

List 1 : Root lesion index

Necrotic lesion percentage	Reaction	Lesion index
No lesions	Immune	1
1-10%	Resistant	2
11-20%	Tolerant	3
21-40%	Susceptible	4
>40%	Highly susceptible	5

Results and Discussion

Root lesion nematode resistance on plant growth

The lowest percentage of reduction for shoot length and shoot weight was recorded in genotype Nilakottai local but the highest shoot length and shoot weight was observed in Arka Ambara and Nilakottai local, respectively. Resistance genotypes had the least shoot length and shoot weight reduction than the susceptible genotypes. It is an important factor and directly influences yield of the genotype. Lowest reduction in shoot length and weight is always used for selection of the genotype at initial stages. Similar reduction was reported earlier by Vadivelu and Muthukrishnan (1981b) in crossandra inoculated with *P.delattre* , Kavitha *et al.* (2016) and Das *et al.* (2013) in banana screened against for *P. coffeae*.

The highest root length and root weight was recorded in genotype Arka Shravya and Nilakottai local, respectively, but the lowest reduction percentage was observed in genotyope Nilakottai local. Resistance genotypes had the least root length and root weight reduction than the susceptible genotypes. This might be due to the low nematode reproduction on resistance genotypes. Good root development favours resistance to genotypes. This line was in accordance with the findings of Vadivelu and Muthukrishnan (1981b) in crossandra inoculated with *P.delattrei*,; Kavitha *et al.* (2016) and Das *et al.* (2013) in banana screened against *P.coffeae.*

Root lesion nematode resistance

Among the genotype screened, Nilakottai local was recorded the lowest population in both soil (112.33) and roots (76.33). The resistant genotypes had lesser population of nematode in soil and root. Hence the growth of the plant was not affected. In tolerant genotypes, nematode population was slightly higher than others and hence it entered into the plants and the plants did not support the nematode multiplication. Susceptible genotype had higher number of population in soil and root and favours the nematode multiplication. Similar results were reported by Kavitha *et al.* (2016); Janarthani (2002), Damodaran *et al.* (2007) and Krishnamoorthy and Kumar (2004) in banana screened against *P.coffeae*.

Genotype Nilakottai local was recoded the lowest root lesion index followed by Arka Shravya. So, it is considered as a resistance genotype. Genotype Dharmapuri local registered higher root lesion index followed by Neyveli local and Villupuram local. Among the ten genotypes, two genotypes showed resistance, five genotypes showed tolerance and three genotypes showed susceptible symptoms. Similar results were reported by Kavitha *et al.* (2016), Janarthani (2002) and Damodaran *et al.* (2007); in banana screened against *P.coffeae.*

Biochemical basis of resistance to root lesion nematode

Phenol, polyphenol oxidase, peroxidase and phenylalanine ammonia lyase were an important enzymes that plays a vital role in defence mechanism. Hypersensitive resistance mechanism was followed. Increased phenol content was noticed in nematode inoculated genotypes compared to uninoculated genotypes. Among the genotypes screened resistant and tolerance genotypes had the highest phenol content compared to susceptible genotypes. Increased phenol content in nematode inoculated plants might be due to excess production of hydrogen peroxide by increased respiration (Farkas and Kiraaly, 1962). In resistant genotypes active phenols may be released from glycosides by increased activities of β-glucosidases and later get oxidized. It leads to browning and necrotic tissue (Acedo and Rohde, 1971). Similar increased phenol content was reported by Nithyadevi *et al.* (2007) and Damodaran *et al.* (2007) in banana screened against *P. coffeae*. In contrary, decreased activity of phenol content in resistant genoypes reported by Sundararaju and Pandisuba (2006) in banana screened against *P.coffeae*.

Polyphenol oxidase oxidizes the phenols to toxic quinines. It will be helpful for conversion of phenols into polymers like lignin. Thereby, it induces resistance in nematode inoculated plants. Among the screened genotypes, inoculated and resistant genotypes recorded the highest polyphenol oxidase activity than uninoculated and susceptible genotypes. Similar increased polyphenol oxidase activity was reported by Damodaran *et al.* (2007), Nithyadevi *et* *al.* (2007); Sundararaju and Pandisuba (2006) and Das *et al.* (2013) in banana screened against *P.coffeae.*

Peroxidase induces the defense mechanism by condensing the phenols into lignin like insoluble polymers through phenylpropanoid pathway. High peroxidase activity was noticed in inoculated and resistance genotypes compared to uninoculated and susceptible genotypes. These results are in accordance with the results of Nithyadevi *et al.* (2007), Damodaran *et al.* (2007) and Das *et al.* (2013).

Phenylalanine ammonia lyase is the first enzyme produced in phenylpropanoid pathway. Lignins and phenols are produced from phenylpropanoid pathway. Thereby it involved in defence mechanism. In this present investigation also increased phenylalanine ammonia lyase content was noticed in nematode inoculated plants compared to uninoculated plants. Similar increased PAL content was reported by Nithyadevi *et al.* (2007), Sundararaju and Pandisuba (2006), Damodaran *et al.* (2007) and Das *et al.* (2013) in banana screened against *P.coffeae*.

From the experiment the results revealed that Arka Shravya recorded highest yield among ten genotypes evaluated and also showed resistance reaction for the root lesion nematode resistance. Hence, Arka Shravya can be recommended to cultivate under nematode infested soil**.**

Conclusion :

Among the ten genotypes studied, resistant reaction for nematode were noticed in genotype Nilakottai local and Arka Shravya. Comparing the yield parameters the genotype Arka Shravya can be recommended to cultivate under nematode infested soil.

Table 1. Effect of *P. delattrei* inoculated and uninoculated crossandra genotypes on shoot weight and root weight

Genotypes	Shoot weight (g)				Root weight (g)			
	Uninoculated	Inoculated with P.delattrei	Mean	Percentage difference	Uninoculated	Inoculated with P.delattrei	Mean	Percentage difference
Arka Shravya	20.18	16.98	18.83	-15.86	12.23	10.16	11.20	-16.93
Arka Kanaka	11.40	8.97	10.18	-21.32	5.12	3.78	4.45	-26.17

Arka Shreeya	16.54	12.82	14.68	-22.49	4.68	3.56	4.12	-23.93
Arka Ambara	20.69	16.73	18.71	-19.14	13.65	10.28	11.97	-24.69
Arka Chenna	15.24	12.68	13.96	-16.80	7.24	5.63	6.44	-22.24
Nilakottai local	23.02	19.86	21.44	-13.73	12.78	10.92	11.99	-14.55
Dharmapuri local	15.25	9.75	12.5	-36.07	5.64	3.02	4.33	-46.45
Delhi crossandra	8.00	6.50	7.25	-18.75	1.16	0.96	1.06	-17.24
Viluppuram local	9.72	7.31	8.51	-24.79	4.46	3.10	3.78	-30.49
Neyveli local	9.52	7.02	8.27	-26.26	4.58	3.19	3.89	-30.35
S.Ed	0.51	0.23	0.73	-	0.25	0.11	0.36	-
CD (P=0.05)	1.04	0.46	1.47	-	0.51	0.23	0.72	-

Table 2.Effect of *P. delattrei* inoculated and uninoculated crossandra genotypes on shoot length and root length

Genotypes	**Shoot length (cm)**				**Root length (cm)**			
	Uninoculated	**Inoculated with P.delattrei**	**Mean**	**Percentage difference**	**Uninoculated**	**Inoculated with P.delattrei**	**Mean**	**Percentage difference**
Arka Shravya	42.32	36.94	39.63	-12.71	38.50	33.25	35.88	-13.64
Arka Kanaka	34.62	26.35	30.49	-23.89	34.26	28.00	31.13	-18.27
Arka Shreeya	35.00	28.00	31.50	-20.00	35.65	30.26	32.96	-15.12
Arka Ambara	50.00	42.33	46.17	-15.34	35.00	29.83	32.42	-14.77
Arka Chenna	33.00	27.00	30.00	-18.18	34.00	28.96	31.48	-14.82

Nilakottai Local	43.07	39.23	41.15	-8.92	27.00	23.58	25.29	-12.67
Dharmapuri local	36.00	23.56	29.78	-34.56	36.00	28.62	32.31	-20.50
Delhi crossandra	16.00	13.89	14.95	-13.19	27.00	23.26	25.13	-13.85
Viluppuram Local	28.13	20.67	24.40	-26.52	22.00	17.00	19.50	-22.73
Neyveli local	23.00	16.00	19.50	-30.43	31.00	21.00	26.00	-32.26
S.Ed	1.06	0.48	1.50	-	0.96	0.43	1.36	-
CD (P=0.05)	2.15	0.96	3.04	-	1.94	0.87	2.75	-

Table 3.Activity of defense enzyme of phenol and polyphenol oxidase in nematode inoculated and uninoculated crossandra genotypes

Genotypes	**Phenol (mg/g)**				**Polyphenol oxidase (ΔA/g/min)**			
	Uninoculated	**Inoculated with *P.delattrei***	**Mean**	**Percentage difference**	**Uninoculated**	**Inoculated with *P.delattrei***	**Mean**	**Percentage difference**
Arka Shravya	4.30	6.20	5.25	44.19	0.63	0.98	0.81	55.56
Arka Kanaka	3.10	4.06	3.58	30.97	0.43	0.57	0.50	32.56
Arka Shreeya	3.75	5.00	4.38	33.33	0.48	0.64	0.56	33.33
Arka Ambara	4.26	5.92	5.09	38.97	0.59	0.90	0.75	52.54
Arka Chenna	3.90	5.22	4.56	33.85	0.51	0.73	0.62	43.14
Nilakottai local	4.50	6.80	5.65	51.11	0.70	1.20	0.95	71.43
Dharmapuri local	2.50	2.68	2.59	7.20	0.32	0.35	0.34	9.37
Delhi crossandra	4.10	5.90	5.00	43.90	0.62	0.96	0.79	54.84

Viluppuram local	2.60	2.95	2.78	13.46	0.39	0.47	0.43	20.51
Neyveli local	2.75	3.09	2.92	12.36	0.34	0.39	0.37	14.71
S.Ed	0.15	0.07	0.21	-	0.03	0.01	0.04	-
CD (*P*=0.05)	0.30	0.14	0.43	-	0.05	0.02	0.07	-

Table 4. Activity of defense enzyme of peroxidase and phenylalanine ammonia lyase in nematode inoculated and uninoculated crossandra genotypes

Genotypes	**Peroxidase (ΔA/g/min)**				**Phenylalanine ammonia lyase (ΔA/g/min)**			
	Uninoculated	**Inoculated with *P.delattrei***	**Mean**	**Percentage difference**	**Uninoculated**	**Inoculated with *P.delattrei***	**Mean**	**Percentage difference**
Arka Shravya	1.40	2.16	1.78	54.29	1.36	2.45	1.91	80.15
Arka Kanaka	1.29	1.72	1.51	33.33	1.20	1.88	1.54	56.67
Arka Shreeya	1.32	1.80	1.56	36.36	1.25	2.05	1.65	64.00
Arka Ambara	1.40	1.98	1.69	41.43	1.33	2.35	1.84	76.69
Arka Chenna	1.32	1.88	1.60	42.42	1.26	2.16	1.71	71.43
Nilakottai local	1.60	2.70	2.15	68.75	1.40	2.60	2.00	85.71
Dharmapuri local	1.10	1.18	1.14	7.27	0.98	1.12	1.05	14.29
Delhi crossandra	1.42	2.08	1.75	46.48	1.35	2.39	1.87	77.04
Viluppuram local	1.10	1.27	1.19	15.45	1.15	1.32	1.24	14.78
Neyveli local	0.88	1.00	0.94	13.64	1.02	1.18	1.10	15.69
S.Ed	0.05	0.02	0.08	-	0.06	0.03	0.08	-

CD (*P*=0.05)	0.11	0.05	0.16	-	0.12	0.05	0.17	-

Table 5. Nematode population, lesion index and reaction of crossandra genotypes against *P. delattrei*

Genotype	Final nematode population in soil (200cc)	Nematode population in root (5g)	Root lesion index	Reaction level
Arka Shravya	136.33	87.23	8.67	Resistant
Arka Kanaka	180.00	172.66	13.67	Tolerant
Arka Shreeya	176.33	164.33	11.33	Tolerant
Arka Ambara	165.67	141.55	10.50	Tolerant
Arka Chenna	169.00	156.32	11.00	Tolerant
Nilakottai Local	112.33	76.33	7.05	Resistant
Dharmapuri local	323.67	243.78	21.17	Susceptible
Delhi crossandra	153.03	128.37	10.16	Tolerant
Viluppuram Local	297.33	212.65	20.17	Susceptible
Neyveli local	280.30	225.98	20.50	Susceptible
S.Ed	0.11	0.10	-	-
CD (*P*=0.05%)	0.23	0.22	-	-

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