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Screening of Turmeric Germplasm for Resistance to Rhizome Rot

M. Lakshmi Naga Nandini[1*], C.H. Ruth[1] and K. Gopal[2]

[1]Department of Plant Pathology, College of Horticulture, Dr. YSRHU, Anantharajupeta- 516105, Andhra Pradesh, India
[2]Department of Plant Pathology, Dr. YSRHU, Venkataramannagudem-534101, Andhra Pradesh, India
**Corresponding author*

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A B S T R A C T

Thirty varieties of turmeric *Curcuma longa* L. were screened in the field against *Pythium aphanidermatum* for one year during 2016-2017 at College of Horticulture, Anantharajupeta, Y.S.R. Kadapa, Andhra Pradesh. These varieties were also screened in lab and net house (pot) condition. Out of 30 varieties two varieties (Salem and Prathibha) showed resistance to rhizome rot and five (TCP-129, VK-9, IC-330113, IC-033007 and IC-212606) were moderately resistant, providing good material for developing rhizome rot resistant turmeric varieties.

Introduction

Turmeric (*Curcuma longa* L.) is an important spice crop of India and all over the world. It plays a significant role in earning valuable foreign exchange. India is the world's largest producer and exporter of turmeric which accounts for 80 per cent of the international trade followed by China, Myanmar and Bangladesh (Satishkumar, 2005). It is used in medicine as a carminative and antibiotic as an effective cure for several diseases. Besides, the use of turmeric colouring pigment (curcumin) and volatile oil (tumerol) in various food and drink items has increased its economic importance in the global market. Turmeric is vulnerable to a number of fungal diseases of both soil and air borne nature.

Rhizome rot of turmeric caused by *Pythium aphanidermatum* is a major constrain for the production of healthy rhizome (Fig. 1) (Rathiah, 1987, Nageshwar Rao, 1994, Ramarethinam and Rajagopal, 1999). Chemical control of this pathogen is not practical because of high cost of chemicals, occasional break down of resistance, environmental pollution, deleterious effect to non-target beneficial soil micro-organism and ultimately the choice of the consumer for an organic product (Pandey *et al.*, 2010). Hence, development of resistant varieties and their incorporation in IDM schedule is a viable alternative for management of this pathogen. College of Horticulture, Anantharajupeta,

Y.S.R. Kadapa, A.P. maintains a large collection of turmeric from Kerala, Himachal Pradesh and West Bengal, in the germplasm conservatory, offering great scope for identification of resistant sources. Keeping the above in view, the available 30 turmeric varieties were evaluated for their resistance/susceptibility. The main objective was to screen in more controlled and tested conditions by using proper standard inocula in artificial methods in triplicate to get authentic result. Standard scoring method was followed to identifying the disease reactions in all the conditions with a comparative analysis.

Materials and Methods

Standardization of inoculum density

The mycelia suspension of the *P. aphanidermatum* was prepared by harvesting the mycelia mats from a 7 day old culture raised on potato dextrose broth. The mats were homogenized in a blender for 1 min and strained through double layered muslin cloth and diluted with sterile distilled water so as to contain 15-20 mycelial bits per microscopic field. The inoculum was added to 45 days old healthy plants raised in sterilized pots, at 2, 4, 6, 8, 10 and 12 %. Healthy plant was maintained as control. Three replications were set for each treatment. The percentage of disease incidence and that of rhizome rot incidence were recorded (Table 1).

Screening of turmeric germplasm under field conditions

Screening of 30 turmeric varieties against the rhizome rot was carried out for one year during 2016–2017 at College of Horticulture (COH), Anantharajupeta (Kadapa, A.P., India) located at 13^{o}99'0" N and 79^{o}33'0" E. Screening was done at different time intervals in 2016-17 particularly from the month of September to December. Planting was done in the month of April–May 2016-17. Selected sample material was harvested and collected in the month of March–April in the respective year for further investigation. The experiment was laid out in a randomized block design (RBD) with three replications (Plot size = 3 m x 3 m). Germination percentage counts at 45 days after planting (DAP) and percentage of rhizome rot incidence at 150 DAP, at harvest were performed. Finally on the basis of percent disease incidence (PDI) the germplasms were screened and categorically grouped into different types of reactions as follows.

Screening of turmeric germplasm under laboratory conditions

Pathogenicity of the isolate was tested on each turmeric variety. For this purpose, apparently healthy turmeric rhizomes (Fig. 1) were collected from the field which was carefully checked. Wounded and bruised ones were discarded. Rhizomes were then fumigated with 4 % formalin for 1 h and surface sterilized with 0.1 % mercuric chloride. The isolates of fungi were raised on potato dextrose agar (PDA) slants. Six to eight day old fungal culture was used for inoculation purpose. For artificial inoculation (Granger and Horne, 1924), a bore well was made on each rhizome with the help of a sterilized cork borer. A bit of the mycelial mat with spore was aseptically introduced into the bore well, and plugged again with the tissue removed earlier and sealed with vaseline. Control bore wells were not inoculated with fungi. Each combination was placed in separate polythene bags containing absorbent cotton moistened with sterile water and incubated for 48 h. After which they were transferred to card board boxes, as such, and incubated in a BOD incubator for 7 days at 25^{0}C. At the end of incubation period, the test organism producing typical symptoms as observed in naturally infected material were

considered as pathogenic. Such fungi were re-isolated on PDA slants from the artificially infected rotten rhizomes and studied for confirmation of the identity of the organism inoculated in each case. The isolates of test fungi namely, *Pythium aphinidermatum* were raised on PDA in petri-plates for securing fungal discs for experimental purposes. In another set, petri dishes were sterilized after making a cross mark on the lower lid for indicating its centre. Antibiotic rich PDA was prepared. The required dose of chemical (s) for each treatment was weighed separately, suspensions of these chemicals in sterile water were incorporated into the medium.

Screening of turmeric germplasm under green house condition

Earthen pots (30 cm in diameter) containing sterile soil mixed up thoroughly with fungal mass culture (one flask for each pot) and incubated for 7 days being covered with black polythene sheet. After incubation rhizome of turmeric were planted in the pot.

After emergence of sprouting, the inoculum was grown in sand maize meal and was inoculated in potted plant. Watering was done to maintain proper moisture level. After emergence of sprout from the turmeric, close observation was kept for 45 days.

Statistical analysis

The data thus obtained was subjected to statistical analysis using PAST (Hammer *et al.,* 2001). The mean and standard deviation of disease incidence percent in each variety was taken for calculating pathogen susceptibility ratings.

Results and Discussion

Out of all the turmeric cultivars screened under field conditions Prathibha, Salem, PTS-8, CL-10, NB-60, IC-033007, IC-211641, Kasturi, ACC-79 were resistant to rhizome rot, showed 0.0 % diseases incidence, whereas BSR-2 (08.37%), TCP-64 (10.00%), TCP-70 (08.12%), TCP-129 (08.00%), NDH-8 (05.26%), IC-330113 (04.36%), IC-212606 (08.37%), IC-211647 (07.91%) were moderately resistant to the pathogen (Table 4). Rajendra Sonia (37.00%), KTS-6 (22.58%), KTS-7 (33.00%) and Gl- Puram (28.76%) were susceptible to rhizome rot whereas RH-9/90 (62.50%) was highly susceptible. CL-12 (12.97%), IC-416941 (12.76%), Morthapuzta (11.76%), Roma (13.79%), CL-1 (16.66%), VK-9 (11.42%), Sports (15.62%) and CLI-335 (18.75%) were moderately susceptible to rhizome rot.

Under laboratory conditions out of all the turmeric cultivars screened, Prathibha, Salem were resistant to rhizome rot with 0 % rotting. VK-9, IC-330113, IC-033007, IC-212606 were moderately resistant to the pathogen with 5 %, 9%, 6%, 20% rotting. Rajendra Sonia (60.00%), Morthapuzta (60.00%), KTS-6 (60.00%), TCP-70 (60.00%), BSR-2 (70.00%), TCP-64 (70.00%), CL-1 (70.00%), CL-10 (70.00%), Kasturi (60.00%), ACC-79 (60.00%), IC-416941 (7.000%), IC-211647 (70.00%), IC-211641 (70.00%), RH-9/90 (60.00%), NB-60 (60.00%) were susceptible to rhizome rot whereas CL-12 (80.00%), CLI-335 (80.00%), NDH-8 (80.00%), Roma (90.00%) were highly susceptible to the pathogen (Table 2). KTS-7 (30.00%), TCP-129 (30.00%), PTS-8 (40.00%), Sports (30.00%) and Gl-Puram (30.00%) were moderately susceptible to the pathogen.

Under nethouse conditions out of all the turmeric cultivars screened, Prathibha, Salem were resistant to rhizome rot with 0 % infection. IC-212606 (08.12%), IC-330113 (10.00%), Sports (6.25%), VK-9 (20.00%), KTS-7 (20.00%), TCP-129 (20.00%) were moderately resistant to the pathogen.

Table.1 Standardization of inoculum density for artificial inoculation

Sl. no.	Percent inoculum Level	Percent disease incidence Percent	rhizome rot
1	0	0.00	0.00
2	2	15.00	20.20
3	4	22.33	25.44
4	6	32.44	42.22
5	8	72.66	80.16
6	10	100.00	100.00
7	12	100.00	100.00
S.Em (±)		0.304	
CD at 1 %		0.815	

Table.2 Rhizome rot reaction in laboratory conditions of turmeric germplasms in Andhra Pradesh

Sl. no.	Name of the Germplasm	Rotting %in the laboratory (M ± SE)	Disease Reaction
1.	Rajendra Sonia	60 ± 0.45	S
2.	Prathibha	0 ±0	R
3.	Morthapuzta	60 ± 0.51	S
4.	Salem	0 ±0	R
5.	KTS-6	60 ± 0.15	S
6.	KTS-7	30 ± 2.08	MS
7.	BSR-2	70 ± 0.26	S
8.	TCP-64	70 ± 0. 27	S
9.	TCP-70	60 ± 0.43	S
10.	TCP-129	30 ± 0.37	MS
11.	PTS-8	40 ± 0.32	MS
12.	Roma	90 ± 0.2	HS
13.	CL-1	70 ± 1.5	S
14.	CL-10	70 ± 0.15	S
15.	VK-9	5 ± 0.12	MR
16.	RH-9/90	60 ± 0.43	S
17.	NB-60	60± 0.37	S
18.	NDH-8	80 ± 0.26	HS
19.	Sports	30 ± 0.37	MS
20.	CLI-335	80 ± 0.2	HS
21.	Gl-Puram	30 ± 0.32	MS
22.	Kasturi	60 ± 0.43	S
23.	ACC-79	60 ± 0.26	S
24.	IC-416941	70 ± 0.26	S
25.	IC-330113	9 ± 0.21	MR
26.	IC-033007	6 ± 0.35	MR
27.	IC-212606	20 ± 11.11	MR
28.	IC-211647	70 ± 0.15	S
29.	IC-211641	70 ± 0.26	S
30.	CL-12	80 ± 0.05	HS

Table.3 Rhizome rot reaction in nethouse conditions of turmeric germplasms in Andhra Pradesh

Sl. no.	Name of the germplasm	% of infection	Disease reaction
1.	Rajendra Sonia	80 ± 19.24	HS
2.	Prathibha	0 ±0	R
3.	Morthapuzta	80 ± 11.11	HS
4.	Salem	0 ±0	R
5.	KTS-6	100 ±0	HS
6.	KTS-7	20 ± 11.11	MR
7.	BSR-2	80 ± 19.24	HS
8.	TCP-64	80 ± 22.22	HS
9.	TCP-70	50 ± 11.11	MS
10.	TCP-129	20 ± 11.11	MR
11.	PTS-8	50 ±0	MS
12.	Roma	100 ±0	HS
13.	CL-1	100 ±0	HS
14.	CL-10	100 ±0	HS
15.	VK-9	20 ± 11.11	MR
16.	RH-9/90	64.42 ± 0.28	HS
17.	NB-60	80 ± 11.11	HS
18.	NDH-8	100 ±0	HS
19.	Sports	06.25 ± 0.02	MR
20.	CLI-335	64.42 ± 0.28	HS
21.	Gl-Puram	55.00 ± 0.57	HS
22.	Kasturi	37.00 ± 0.57	S
23.	ACC-79	33.00 ± 0.57	S
24.	IC-416941	62.50 ± 0.12	HS
25.	IC-330113	10.00 ± 0.57	MR
26.	IC-033007	50 ±0	MS
27.	IC-212606	08.12 ± 0.11	MR
28.	IC-211647	55.00 ± 0.57	HS
29.	IC-211641	28.76 ± 0.1	S
30.	CL-12	64.42 ± 0.28	HS

Table.4 Rhizome rot reaction and yield parameter in natural field condition of turmeric germplasms in Andhra Pradesh

Sl. no.	Name of the Germplasm	Germination (%) (M ± SE)	Percent disease incidence (%) (M ± SE)	Yield (kg/plot) (M ± SE)	Reaction category
1.	Rajendra Sonia	80.00 ± 0.57	37.00 ± 0.57	18.74 ± 1.29	S
2.	Prathibha	95.00 ± 0.57	00.00 ± 0	12.30 ± 0.1	R
3.	Morthapuzta	85.00 ± 1	11.76 ± 0.68	7.44 ± 0.67	MS
4.	Salem	65.00 ± 0.57	00.00 ± 0	9.50 ± 0.56	R
5.	KTS-6	77.50 ± 0.52	22.58 ± 0.04	14.00 ± 2.78	S
6.	KTS-7	90.00 ± 0.57	33.00 ± 0.57	6.50 ± 2.37	S
7.	BSR-2	87.50 ± 0.57	08.37 ± 0.07	42.30 ± 0.36	MR
8.	TCP-64	70.00 ± 0.57	10.00 ± 0.57	12.00 ± 0.8	MR
9.	TCP-70	85.00 ± 0.57	08.12 ± 0.11	15.80 ± 0.79	MR
10.	TCP-129	67.50 ± 0.33	08.00 ± 0.57	14.50 ± 0.48	MR
11.	PTS-8	92.50 ± 0.65	00.00 ± 0	15.48 ± 0.17	R
12.	Roma	72.50 ± 0.57	13.79 ± 0.09	6.00 ± 0.37	MS
13.	CL-1	80.00 ± 0.57	16.66 ± 0.13	22.00 ± 0.33	MS
14.	CL-10	82.50 ± 1. 67	00.00 ± 0	17.84 ± 0.26	R
15.	VK-9	87.50 ± 0.09	11.42 ± 0.19	9.40 ± 1.6	MS
16.	RH-9/90	80.00 ± 0.57	62.50 ± 0.12	16.00 ± 1.64	HS
17.	NB-60	83.33 ± 0.54	00.00 ± 0	5.76 ± 0.27	R
18.	NDH-8	95.00 ± 0.57	05.26 ± 0.08	7.22 ± 0.22	MR
19.	Sports	80.00 ± 0.57	15.62 ± 0.49	12.90 ± 0.69	MS
20.	CLI-335	80.00 ± 0.83	18.75 ± 0.04	8.50 ± 1.31	MS
21.	Gl-Puram	84.00 ± 0.57	28.76 ± 0.1	14.40 ± 2.23	S
22.	Kasturi	88.00 ± 0.57	00.00 ± 0	22.50 ± 0.08	R
23.	ACC-79	78.50 ± 1.49	00.00 ± 0	18.47 ± 0.14	R
24.	IC-416941	88.00 ± 1.76	12.76 ± 0.38	9.42 ± 0.56	MS
25.	IC-330113	89.00 ± 0.46	04.36 ± 0.09	9.82 ± 0.33	MR
26.	IC-033007	63.38 ± 0.74	00.00 ± 0	6.76 ± 0.97	R
27.	IC-212606	87.50 ± 0.57	08.37 ± 0.07	42.30 ± 0.36	MR
28.	IC-211647	67.50 ± 0.42	07.91 ± 0.08	22.60 ± 0.80	MR
29.	IC-211641	78.00 ± 0.32	00.00 ± 0	20.53 ± 0.17	R
30.	CL-12	82.50 ± 0.67	12.97 ± 0.08	8.00 ± 0.53	MS

Table.5 Comparative study on disease reaction data from field in natural condition, data from storage infection and data from inoculation of pathogen in pot culture

Sl.No	Name of the germplasm	Disease reaction in field	Disease reaction in storage incubation	Disease reaction in pot inoculation	Overall disease reaction
1.	Rajendra Sonia	S	S	HS	S
2.	Prathibha	R	R	R	R
3.	Morthapuzta	MS	S	HS	S
4.	Salem	R	R	R	R
5.	KTS-6	S	S	HS	S
6.	KTS-7	S	MS	MR	MS
7.	BSR-2	MR	S	HS	S
8.	TCP-64	MR	S	HS	S
9.	TCP-70	MR	S	MS	MS
10.	TCP-129	MR	MS	MR	MR
11.	PTS-8	R	MS	MS	MS
12.	Roma	MS	HS	HS	HS
13.	CL-1	MS	S	HS	S
14.	CL-10	R	S	HS	S
15.	VK-9	MS	MR	MR	MR
16.	RH-9/90	HS	S	HS	HS
17.	NB-60	R	S	HS	S
18.	NDH-8	MR	HS	HS	HS
19.	Sports	MS	MS	MR	MS
20.	CLI-335	MS	HS	HS	HS
21.	Gl-Puram	S	MS	HS	S
22.	Kasturi	R	S	S	S
23.	ACC-79	R	S	S	S
24.	IC-416941	MS	S	HS	S
25.	IC-330113	MR	MR	MR	MR
26.	IC-033007	R	MR	MS	MR
27.	IC-212606	MR	MR	MR	MR
28.	IC-211647	MR	S	HS	S
29.	IC-211641	R	S	S	S
30.	CL-12	MS	HS	HS	HS

Screening of Turmeric Germplasm under Field Conditions

Scoring scale	Percent disease incidence	Reaction
1	0	Resistant (R)
2	1-10	Tolerant/Moderately resistant (MR)
3	11-25	Moderately susceptible (MS)
4	26-50	Susceptible (S)
5	> 50	Highly susceptible (HS)

Fig.1 A. Healthy rhizome before inoculation of pathogen B. Spoiled Rhizome after inoculation of pathogen

Morthapuzta (80.00%), Rajendra Sonia (80.00%), KTS-6 (100.00%), BSR-2 (80.00%), TCP-64 (80.00%), Roma (100.00%), CL-1 (100.00%), CL-10 (100.00%), RH-9/90 (64.42%), NB-60 (80.00%), NDH-8 (100.00%), CLI-335 (64.42%), Gl-Puram (55.00%), IC-416941 (62.50%), IC-211647 (55.00%), CL-12 (64.42%) were highly susceptible (Table 3) whereas TCP-70 (50.00%), PTS-8 (50.00%), IC-033007 (50.00%) were moderately susceptible. Kasturi (37.00%), ACC-79 (33.00%) and IC-211641 (28.76%) susceptible to the pathogen.

A comparative study was conducted for overall disease reaction. Overall disease reaction showed that Prathibha, Salem were the varieties, resistant to rhizome rot whereas TCP-129, VK-9, IC-330113, IC-033007, IC-212606 were moderately resistant. Rajendra Sonia, KTS-6, Morthapuzta, TCP-64, BSR-2, CL-1, CL-10, NB-60, Gl-Puram, Kasturi, ACC-79, IC-416941, IC-211647, IC-211641 were susceptible (Table 5) whereas CL-12, Roma, NDH-8, RH-9/90, CLI-335 were highly susceptible. KTS-7, PTS-8, TCP-70 and Sports were moderately susceptible.

The study indicated that two turmeric varieties screened were resistant to rhizome rot; five varieties were moderately resistant to the disease. Since the variation in the percent of disease incidence between various varieties was wide, it was not possible to fix the upper and lower limits of each group as constant values. Hence, the mean and standard deviation of the percent of disease incidence was used for fixing various categories of resistance/susceptibility. The categorization based on the extent of variation from the mean (positive or negative) reduced the probabilities of resistant/susceptible varieties (Bhumanavar *et al.,* 1989).

Screening of 675 collections of turmeric (Curcuma longa L.) against the shoot borer at Vellanikara indicated that none of the collections were free of the pest infestation but 22 accessions were relatively tolerant to the pest (Velayudhan and Liji, 2003). Patho-Biochemical investigations of blight and wilt diseases of tomato (*Lycopersicon esculentum*) have been reported (Kumar *et al.,* 2013). Screening of 25 ginger types to the shoot borer also indicated that none was significantly different in their reaction to the pest: however, maximum shoot damage was observed in valluvanad (43.4 %) and minimum in Rio-de-Janeiro (21.3 %) (Nybe and Nair, 1979).

In this study screening was done by different methods. Screening by different methods has confirmed the performance of lines, which are now being used extensively in crossing

programs at ICRISAT and elsewhere (Nene and Haware, 1980). The present study provides the breeders with a wide choice of varieties resistant to rhizome rot.

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