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Research Article

CHARACTERIZATION OF RESISTANT AND SUSCEPTIBLE GENOTYPES OF TURMERIC LEAF BLIGHT CAUSED BY *ALTERNARIA ALTERNATA* (FR.) KEISSLER

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ABSTRACT

The fungal pathogen *A. alternata* Keissler is highly responsible for severe economic-loses in major turmeric growers of South-Karnataka, India, due to leaf-blight disease eight genotypes of turmeric (*Curcuma longa* L.) mainly cultivated in South-Karnataka region have been screened for disease severity of the pathogen. The rhizomes of turmeric were grown in nursery bags and the spore-suspension of the pathogen were inoculated to the turmeric plant. Among the tested Varieties Hassan-8, was resistant (9.3%) to the pathogen whereas, Maddur-2 was highly susceptible showing 73.4% severity while Hangla-1, Kollegal-5 and B. Halli-7 were susceptible to the disease with 45.5059%, 44.2708%, 42.1875% respectively and P. Pura-3, B. Pura-4 and M. Pura-6 were moderately resistant showed 24.7%, 23.9%, 24.6% respectively.

INTRODUCTION

Turmeric (*Curcuma longa* L.) is an herbaceous perennial plant belongs to the family Zingiberaceae, order Scitaminae. It is also called nature's precious gift and commonly known as 'National Heritage' (Maurya *et al*., 2011). It has high medicinal value as well as important spices all over the world. It is considered as good spice in human life (Gorawar and Hegde, 2006). The underground rhizome is used in many ways *viz*., condiments in food preparation, coloring agents in textiles, in cosmetics (facial preparations and creams) and in Ayurvedic drug preparations. Around 80 species are reported genus *Curcuma* from Indo Malayan region and about 40 of them are native to India (Velayudhan *et al*., 1999). Turmeric has various biological activity such as anti-bacterial, anti-inflammatory, hypolipidemic, hepatoprotective, lipoxygenase, cycloxygenase, protease inhibitory effects, besides being effective as active oxygen species scavengers and lipid peroxidase inhibitors. Its coloring properties are usually more important than its flavor attributes (Sekar 2004). India, ''the land of spices'' is the largest producer, consumer and exporter in the world and accounts for 80 per cent of the world's production. Turmeric is susceptible to various diseases such as leaf blight, anthracnose and rhizome rot (Kavitha *et al*., 2011). Among these the leaf blight caused by *Alternaria alternata* (Fr.) Keissler is important foliar disease commonly found in Karnataka damaging the crop to a greater extent by reducing size and weight of the rhizome. The disease in India was first reported by Chowdhury (1969). *alternaria* is a of fungi belonging to ascomycete, there are 299 species in the genus *Alternaria* (Kirk *et al.,* 2008; Nowicki-Marcin *et al*., 2012). The disease is recently gaining importance due to its severity (Gaddanakeri and Kulkarni, 1998) causing considerable damage to the plant in almost all turmeric growing areas of Northern Karnataka. Mallikarjun (1996) Aim of the study is to find out the best resistant and susceptible genotype.

MATERIALS AND METHODS

Collection of samples

Turmeric samples were collected from Puttunpura, Hangla Malliyaapura and Basavanpura from Gundulpet taluk, Hampapur from K. R. Nagar, Bogarahally from Hassan district, Paalya from Kollegal taluk and Goravanahally from Maddur taluk of Karnataka, India.

Screening of turmeric Varieties for its resistant and susceptibility against Alternaria alternata leaf blight by Standard Blotter Method (SBM)

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Sterilized Petri plates were provided with three layers of sterile blotter discs to hold the moisture, From the infected leaf materials, typical spots were cut into 1cm^2 pieces, surface sterilized with 2% Sodium hypochlorite solution for two minute and was washed with distilled water thrice to remove the traces of sodium hypochlorite and then the leaf bits were transferred aseptically to the Petri plates. Inoculated plates were incubated for 6-7 days at room temperature with 12/12 alternative light and dark periods. After 6-7 days of incubation the plates were screened for the sprouting/development of the pathogen and microscopic examination was carried out and characteristic features were recorded to identify the pathogen.

Isolation of Alternaria alternata on Potato Dextrose Agar (PDA) medium

Potato dextrose agar (PDA) media was used to isolate the fungal pathogen if any from the infected leaf material. PDA media was prepared according to the standard procedure. 200 g of peeled potato was boiled in 500 ml of water and potato infusion was filtered and the volume was made up to 1000ml by adding distilled water. To the potato infusion, 20g of Dextrose, 15g of agar-agar was used as solidifying agent and 0.5mg of antibiotic (Chloromphenicol) was added to discourage the growth of bacteria and to isolate pure fungal colony. All the components of medium were mixed and homogenized in a micro oven and sterilized in a pressure cooker at 121˚C, 15lb pressure for 20 min. and Petri plates were also sterilized in similar way. Infected leaf materials were cut into 1cm^2 and were surface sterilized with 2% sodium hypochlorite ($NaOCl_2$) solution for two min. followed by washing with sterile distilled water thrice. The media was poured into sterilized Petri plates and was allowed to solidify. After solidification, the plates were inoculated with leaf materials and were incubated at room temperature at an alternative period of 12/12 light and dark period for 7 days. After 7th day of incubation the plates were screened for the growth of the fungus. The fungal colonies observed on plates were subcultured on fresh PDA plates to establish pure cultures. Pure cultures were used to identify the organism associated with leaf blight of turmeric.

Koch's postulates to confirm the association of a pathogen

The isolated pathogen(s) were tested for their ability to cause disease on one month old turmeric seedlings under green house conditions. Sporangial suspension was used for the pathogenicity. The conidia were harvested from the seven-day old culture of fungal pathogen and the conidial suspension was prepared (1x10^5 conidia/ml) and sprayed on turmeric leaves and covered with plastic covers for 24 hrs. After five days of post inoculation, the inoculated plants were observed for the appearance of disease symptoms. Plants sprayed only with sterile distilled water served as control.

Screening of turmeric Varieties

The turmeric samples collected from different places of Karnataka, India namely, Puttunpura, Hangla Malliyaapura and Basavanpura from Gundulpet taluk, Hampapur from K. R. Nagar, Bogarahally from Hassan district, Paalya from Kollegal taluk, Goravanahally from Maddur taluk. The fungal pathogen, *A. alternata* was isolated following Standard Blotter Method (SBM) using Potato Dextrose Agar media. Further samples of different genotypes of turmeric viz., resistant and susceptible were raised in two Kg plastic bags and filled with sand, soil and compost in the ratio 3:1:1 the plants were frequently watered and kept at optimum growth condition. Five plants were raised for each genotype.

The spore suspension of *A.alternata* was made 1×10^5 conidia/ml in distilled water and sprayed to the plants at two leaf stage (twenty days). Four replicates were maintained against each genotypes. The control plants were sprayed with distilled water alone. The data on development of the disease was observed weekly. The disease severity was calculated as PDI by using following formula (Wheeler, 1969).

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves}} \times 100$$

Note: Scale Categories Percent leaf area infected, are categorized as - 0 immune no disease, 1 resistant up to 10%, 2 moderately resistant 11-25%, 3 susceptible 26-50%, 4 highly susceptible more than 50%.

RESULTS

Collection of turmeric samples from agricultural fields

Turmeric grown fields were surveyed around South-Karnataka region and collected 10 different samples and labelled. Packed in sterile bags and brought to the laboratory for further studies. Infected leaves of turmeric plants showing the symptoms of leaf blight were also collected and labelled for the isolation of pathogen.

Figure 1 Field of turmeric surveyed for the collection of Samples/ Varieties

Isolation of pathogen and pathogenicity test

Leaf blight pathogen was isolated and identified by its morphological and conidial characters by comparing with Marthur's manual. Further this was tested for its pathogenicity by detached leaf assay using Sterile Distilled Water treatment as control. The leaf inoculated with pathogen showed leaf blight symptoms after two days of inoculation with pathogen inoculum and the pathogen was re-isolated from the infected leaf.

Screening of turmeric Varieties

The Varieties of turmeric used for screening showed difference in tolerance or resistance to *A. alternata.* When the susceptible plants were observed, the disease appeared initially as small brownish spots on the upper surface of leaf lamina and later, dark brown colored lesions were produced on upper surface of

the leaves. These lesions gradually increased and formed larger patches. Leaf blight and complete drying of the affected plants were notable symptoms in advanced stage the disease.

Figure 2 Detached leaf assay of turmeric leaves inoculated with *A. alternate* spore suspension to study its pathogenicity as well as to screen resistant variety

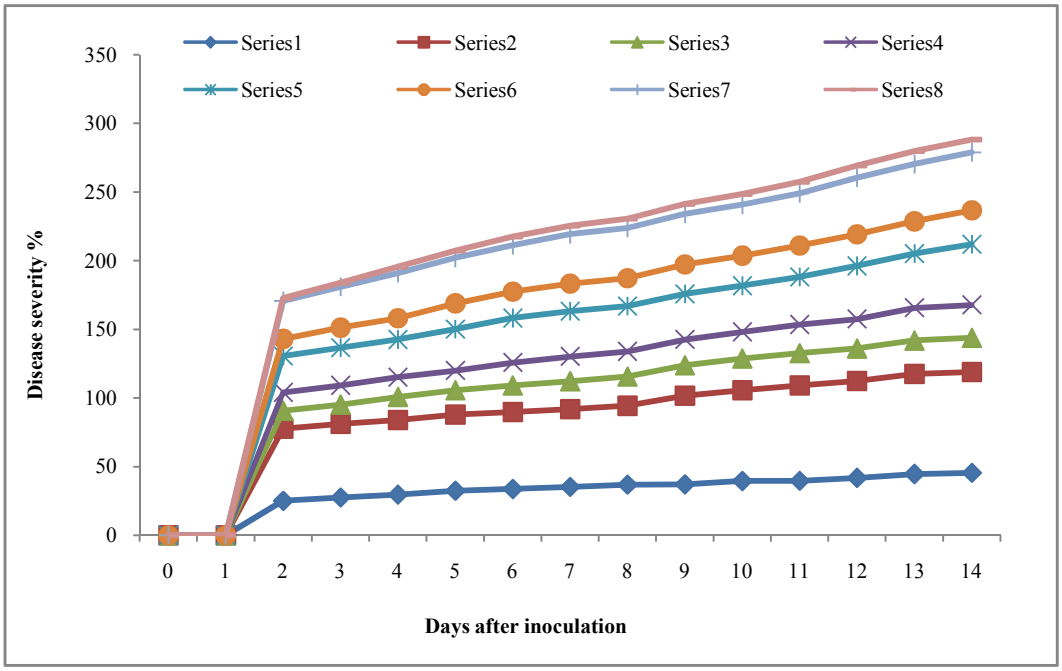

Figure 3 Disease severity (%) incited by *A. alternata* (Fr.) Keissler in eight different genotypes of turmeric. Values are the mean of 5 replicates each at 0 to 1-15 weeks after inoculation with pathogen.

Table 1 Turmeric varieties collected from South Karnataka region

Variety. No.	Varieties of Turmeric collected	Place of collection	Taluk/ District
1	P. Pura-3	Puttunpura	Gundlupet Taluk, Chamaraja Nagar District
2	Hangla-1	Hangla	
3	M. Pura-6	Malliyaapura	
4	B. Pura-4	Basavanpura	
5	B. Halli-7	Bogarahally	K. R. Nagar Taluk, Mysore District
6	Hassan-8	Hampapur	Hassan District
7	Kollegal-5	Paalya	Kollegal Taluk, Chamaraja Nagar District
8	Maddur-2	Goravanahally	Maddur Taluk, Mandya District

Hassan-8 showed no symptoms remains healthy even after 15th week after inoculation with spores of *A. alternata.* The plant remained to grow healthy without any future signs for disease development.

The Varieties Hangla-1, Kollegal-5, B. Halli-7 were susceptible to the disease till 15th week whereas Maddur-2 was highly susceptible till 15th week and P. Pura-3, B. Pura-4, M. Pura-6 were Moderately resistant to the pathogen *A. alternata*. Among all these varieties, Hassan-8 showed 9.375% of infection even after 15th week of inoculation. This suggests that Hassan-8 is a resistant variety when compared to the rest of the variety of *Curcuma longa* L. Varieties used for screening.

DISCUSSION

In this study the difference in disease severity of eight different varieties/cultivars of *C. longa* L. to the disease leaf blight caused by *A. alternata*. Hassan-8 was resistant to the disease, three of the *Curcuma longa* L. Varieties P. Pura-3, B. Pura-4, M. Pura-6 were found to be moderately resistant to the disease, Hangla-1, Kollegal-5, B. Halli-7 were Moderately Resistant

and Maddur 2 was Highly susceptible to disease. The most important yield contributing character in turmeric is the number of rhizomes and their size (Chadha, 2001).

Table 2 Reaction of turmeric genotypes to leaf blight caused by *Alternaria alternate*

Genotypes	Disease severity week after pathogen inoculation (%)															Disease Response
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Hangla-1	0	0	25.10	27.49	29.58	32.49	33.75	35.31	36.87	37.08	39.58	39.73	41.87	44.68	45.50	S
Maddur-2	0	0	52.49	53.72	54.47	55.35	56.04	56.56	57.5	64.58	66.14	69.37	70.62	72.81	73.43	HS
P. Pura-3	0	0	13.02	13.85	16.66	17.70	19.27	20.20	21.14	22.08	22.91	23.43	23.43	24.27	24.77	MR
B. Pura-4	0	0	13.33	14.06	14.37	14.37	16.66	18.15	18.33	18.74	19.37	20.83	21.56	23.85	23.95	MR
Kollegal-5	0	0	26.56	27.5	27.5	30.20	32.5	32.99	33.12	33.33	33.75	34.79	38.75	39.58	44.27	S
M. Pura-6	0	0	12.5	14.58	15.41	18.75	19.27	20.10	20.31	21.35	21.87	22.91	22.91	23.43	24.68	MR
B. Halli-7	0	0	27.60	29.68	32.91	33.12	33.75	35.93	36.48	36.87	37.08	37.91	41.24	41.66	42.18	S
Hassan-8	0	0	2.08	3.12	4.68	5.20	6.24	6.25	6.77	7.29	7.81	8.33	8.85	9.37	9.37	R

Note: Scale Categories Per-cent leaf area infected- 0 immune no disease (I), 1 resistant up to 10% (R), 2 moderately resistant 11-25%(MR), 3 susceptible 26-50%(S), 4 highly susceptible more than 50%(HS).

Chadha (1994) reported 4 to 7 rhizomes in variety Suguna and found the varieties, Suvarna and Sudarshan with rhizome length 6 cm to 8.8 cm respectively. Ramakrishna *et al.* (1995) reported 191.50 q/ha fresh rhizome yield in variety Suguna and Hegde *et al.* (1997) obtained the fresh rhizome yield of 215.50 and 196.30 q per ha from Sudarshan and Suguma respectively. Since the pathogen *A. alternata* is mainly air-borne in this study we have adopted the foliar spray method with the conidial suspension of the pathogen and the disease severity have been noted, which showed differences in susceptibility of the pathogen when compared to the above studies

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