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Identification of Host Plant Resistance to Anthracnose in Greater Yam (*Dioscorea alata L.*)

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A B S T R A C T

Anthracnose, caused by *Colletotrichum gloeosporioides* Penz., is one of the most serious leaf and vine epiphytotic disease of greater yam (*D. alata*) in India. Forty greater yam accessions were screened in the field for resistance to anthracnose disease by whole plant area scoring method. The accessions were also evaluated for resistance in the laboratory using an excised leaf technique with pure isolates of *Colletotrichum gloeosporioides* Penz. Significant differences in the lesion/disease scores among greater yam genotypes, ranging from 0 (immune) to 5 (susceptible), were observed under both laboratory and field conditions. Resistance evaluations under both conditions consistently classified the genotypes as resistant and susceptible lines which is useful for future breeding programmes. Highly resistant lines *viz.* Da 110, Jas 2 and TCR 142 were identified for further evaluation and use in resistance breeding programmes.

Introduction

Yams (*Dioscorea* spp) are multi-species, polyploid and vegetatively propagated tuber crops cultivated widely in the tropics and subtropics. *Dioscorea alata* L., commonly known as water yam is one amongst the top ten most important yam species, listed based on staple food and agricultural perspectives (Lebot, 2009). The wide adaptation and cultivation of water yam makes it one of the most important *Dioscorea* species used for food in tropical and subtropical regions (Tay, 2013). Greater yam tubers possess a high nutritional content with an average crude protein content of 7.4%, starch content of 75±84%, and vitamin C content ranging from 13.0 to 24.7 mg/100g (Muzac-Tucker *et al.*, 1993).

The major limitation to the sustainable production of *D. alata* is it's susceptibility to anthracnose disease and it can cause yield reduction of upto 80% (Nwankiti and Ene,

1984). Water yam (*D. alata*) was thought to be more susceptible to anthracnose than other yam species (Amusa, 1997). *Gloeosporium pestis* Massee was first described as the pathogen causing yam anthracnose from yam in Fiji (Winch *et al*., 1984). It was later reported from *D. alata* in India (Prasad and Singh, 1960; Singh *et al.*, 1966) and subsequently classified as *Colletotrichum gloeosporioides*.

Typical symptoms of the anthracnose disease include necrotic spots on shoots, young leaves, petioles, fruits and stems, leaf and stem distortion, and shot holes. It spreads in the rainy season when temperature and moisture are favourable for the development of the disease. A detailed description of the different symptoms produced by *C. gloeosporioides* and how these develop on *D. alata* was provided by Winch *et al*., (1984). Genetic improvement programmes were undertaken by International Institute of Tropical Agriculture (IITA, Nigeria) and ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI, India) for developing high yielding varieties of *D. alata* and *D. rotundata* with pest and disease resistance to meet farmers' requirements. More than 1000 germplasm accessions of yams have been collected, conserved and elite accessions are being used for yam improvement in these institutions and other yam improvement programmes in different countries. In all these breeding programmes, the main thrust was given for the identification of host plant resistance to anthracnose disease in greater yam among prebreeding lines and landraces.

The present investigation was carried out to determine host plant resistance for anthracnose in greater yam based on different screening techniques and the results are reported herein. This knowledge will be beneficial for developing varieties with anthracnose resistance in future.

Materials and Methods

The plant materials used for the study comprised of forty accessions of *D. alata* conserved in the National repository of tuber crops germplasm at ICAR-CTCRI, Sreekariyam, Thiruvananthapuram. The screening was carried out during 2017-2018. The list of greater yam accessions used for the study is given in Table 1.

Field screening

The 40 accessions were screened in the field for anthracnose resistance. Whole plant area scoring method was done in which the lesions were rated visually on a 0-5 scale based on the percentage of lesion appeared on leaves and vines, where 0= no infection, 1= 1-10%, 2= 10-25%, 3= 25-50%, 4= 50-75% and 5= >75% infection (Table 2).

Laboratory screening

Excised leaf assay was carried out using 40 yam genotypes, collected from germplasm collections maintained in the field gene bank at ICAR-CTCRI, Thiruvananthapuram, with varying resistance levels to anthracnose. Resistance screening was performed with pure isolates of *Colletotrichum gleosporioides*, which were isolated from infected leaves. Leaves of similar age and size (leaves at nodes 3 to 4, counted from the top) were collected from the field. The leaves were washed with running tap water. Monoconidial cultures of *Colletotrichum gloeosporioides* grown on potato dextrose agar (PDA) were scraped with a sterilized inoculation loop and transferred to a vial containing 9ml sterile distilled water. It is then agitated using a rotary shaker to obtain a uniform, homogenized spore suspension. A drop of the suspension was observed under 10X objective lens of light microscope to ensure the presence of adequate count of live spores. The

suspension was diluted with sterile water so as to get 10^4 cfu ml^{-1}. Exactly 25µl of spore suspension was inoculated onto the test leaves arranged in petriplates. Sterile water was kept as control. The petioles of the leaves were kept in contact with the folded wet tissue paper arranged inside the petriplates to prevent drying. Wet tissue paper was also placed on the lid of petriplates for maintaining humidity. The plates were incubated at room temperature. Lesions formed were rated visually after 3 days of inoculation on a 0-5 scale based on the percentage of lesion per inoculated droplet, where 0= no infection, 1= 1-10%, 2= 10-25%, 3= 25-50%, 4= 50-75% and 5= >75%. The reactions of the accessions were assigned as given in table 3.

Results and Discussion

Field screening was done by whole plant area scoring method in which the lesions appeared on leaves and vines were rated visually on a 0-5 scale. TCR 142 and Da 340 were found to be immune to anthracnose since they didn't show any trace of infection (Table 3). Many other accessions *viz.* Da 110, Da 293, Jas 2, TCR 113, Sree Keerthi *etc*. could be in the resistant category as the percentage of infected area in these plants were only 1-10% (Score 1). Maximum infection was observed in Sree Neelima, DaH 24-6-3 and also Orissa Elite, classifying them as highly susceptible accessions (Score 5). Five percent of the total samples were immune to anthracnose whereas more than 32% were highly resistant and 25% were resistant to anthracnose in the field. Only 5% were found to be susceptible or highly susceptible in the field.

Laboratory screening was done by excised leaf assay using 40 greater yam genotypes and the lesions formed were rated visually at 3 days after inoculation on a 0-5 scale. JAS- 2 and Da 110 were found to be immune under laboratory conditions (Score 1), whereas Da 820, TCR 282, TCR 342, TCR 43, Da 509 and DaH 24-6-3 showed extensive infection, categorizing them as highly susceptible. As in field screening, only 5% of the samples were observed as immune in laboratory screening assay. But more than 17% were given a score of 5, classifying them as highly susceptible and 15% of the accessions were moderately susceptible.

The laboratory screening evaluation methodology was supported by Onyeka *et al.,* (2006) and ICAR-CTCRI (2016) where tissue culture derived whole plant assay was used for assessing anthracnose resistance in greater yam. Similar laboratory and field evaluation study was carried out by Poolsawat *et al*., (2012) to identify host plant resistance in grape wine to anthracnose.

Resistant varieties Sree Keerthi, TCR 113, DaH 58 FG and DaH 9-196 showed similar grade of infection, all with a score of 1 in both field screening and laboratory screening, ensuring the effectiveness of laboratory screening assay in predicting the field responses of greater yam to *Colletotrichum gleosporioides.* The scores obtained were tabulated in Table 3.

It is concluded that, by using both laboratory and field disease assessments, 19 accessions were classified as resistant genotypes while the rest 21 were classified as susceptible genotypes.

The released varieties *viz*. Sree Karthika, Sree Keerthi and Sree Swathy showed resistant scores in the study. Also the susceptible varieties including Sree Neelima and Orissa Elite expressed susceptible scores both under field and lab screening, indicating the effectiveness of the tests done. Identification of resistant yam genotypes *viz.* Da 110, Jas 2 and TCR 142 will be beneficial for yam breeding to achieve anthracnose resistance and also to identify the resistant genes in future (Fig. 1 and 2).

Table.1 List of greater yam accessions used for screening against anthracnose

Sl. No.	Genotype	Place of Collection
1.	Da 110	Dandakaranya
2.	Da 198	Thiruvananthapuram
3.	Da 200	NBPGR, Thrissur
4.	Da 209	NBPGR, Thrissur
5.	Da 210	Coimbatore
6.	Da 293	Pathanamthitta
7.	Da 374	Pathanamthitta
8.	Da H 9-196	Hybrid, ICAR-CTCRI
9.	Da H 22-2-3	Hybrid, ICAR-CTCRI
10.	Da H 58 FG	Hybrid, ICAR-CTCRI
11.	TCR 308	NBPGR, Thrissur
12.	TCR 319	NBPGR, Thrissur
13.	Sree Karthika	Released variety, ICAR-CTCRI
14.	Sree Keerthi	Released variety, ICAR-CTCRI
15.	Sree Swathy	Released variety, ICAR-CTCRI
16.	Da 264	Thiruvananthapuram
17.	Da 340	Thiruvananthapuram
18.	Da 817 V	Pathanamthitta
19.	JAS 2	Pre-breeding line, ICAR-CTCRI
20.	TCR 226	NBPGR, Thrissur
21.	TCR 64	NBPGR, Thrissur
22.	Da 12	Kottayam
23.	Da 377	Kollam
24.	Da 489	Kottayam
25.	TCR 113	NBPGR, Thrissur
26.	Da 820	Idukki
27.	Da H 17-5	Hybrid, ICAR-CTCRI
28.	Da 810	Thiruvananthapuram
29.	Orissa Elite	Released variety, ICAR-CTCRI
30.	Sree Neelima	Released variety, ICAR-CTCRI
31.	TCR 208	NBPGR, Thrissur
32.	TCR 282	NBPGR, Thrissur
33.	Da 508	Assam
34.	TCR 102	NBPGR, Thrissur
35.	TCR 342	NBPGR, Thrissur
36.	Da 503	Pre-breeding line, ICAR-CTCRI
37.	Da H 24-6-3	Hybrid, ICAR-CTCRI
38.	TCR 142	NBPGR, Thrissur
39.	Da 509	Thiruvananthapuram
40.	TCR 43	NBPGR, Thrissur

Table.2 Categorisation of resistance against Anthracnose disease in greater Yam

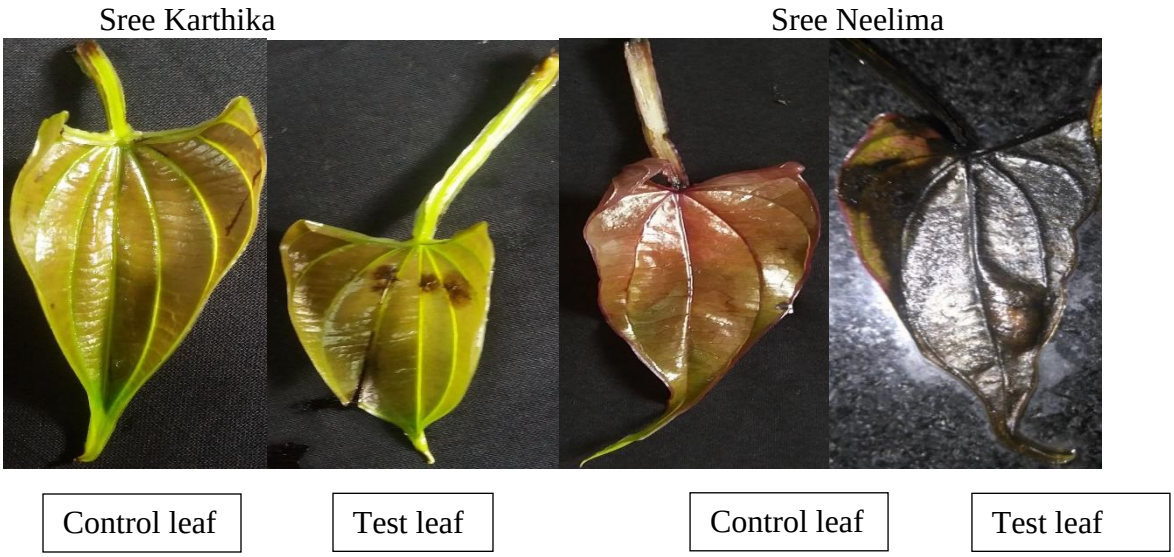
Score index	Category
0	Immune
1	Highly resistant
2	Resistant
3	Moderately susceptible
4	Susceptible
5	Highly susceptible

Table.3 Scores obtained by field and lab screening

Sl. No.	GENOTYPE	FIELD SCORE	LAB SCORE	R/S
1	Da 110	1	0	R
2	Da 198	2	3	S
3	Da 200	2	1	R
4	Da 209	3	1	R
5	Da 210	3	1	R
6	Da 293	1	2	R
7	Da 374	2	2	R
8	DaH 9-196	1	1	R
9	DaH 22-2-3	2	2	R
10	DaH 58FG	1	1	R
11	TCR 308	2	2	R
12	TCR 319	2	2	R
13	Sree Karthika	3	1	R
14	Sree Keerthi	1	1	R
15	Sree Swathy	1	2	R
16	Da 264	2	2	R
17	Da 340	0	3	R
18	Da 817 V	1	4	S
19	JAS 2	1	0	R
20	TCR 226	1	4	S
21	TCR 64	1	3	S
22	Da 12	1	4	S
23	Da 377	3	2	S
24	Da 489	2	2	R
25	TCR 113	1	1	R
26	Da 820	4	5	S
27	Da H 17-5	3	3	S
28	Da 810	3	3	S
29	Orissa Elite	4	3	S
30	Sree Neelima	5	4	S
31	TCR 208	3	1	R
32	TCR 282	3	5	S
33	Da Assam	2	4	S
34	TCR 102	1	4	S
35	TCR 342	3	5	S
36	Da 503-O	2	5	S
37	DaH 24-6-3	5	5	S
38	TCR 142	0	1	R
39	Da 509	3	5	S
40	TCR 43	3	5	S

Fig.1 Scoring chart for Yam Anthracnose

Fig.2 Lab screening of highly resistant Sree Karthika and highly susceptible Sree Neelima



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