In vitro screening and characterization of taro for *Phytophthora* leaf blight disease

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One hundred seventy two diversified taro [Colocasia esculenta (L.) Schott] accessions were screened for taro leaf blight using cell wall glucan elicitor and spore suspension cultures on leaf discs. Based on the results of hypersensitive reactions developed by the elicitor molecules and spot appeared by spore inoculation, 14 lines showed resistance of leaf blight disease. Those 14 lines along with 2 susceptible lines were selected for further evaluation and characterization for leaf blight disease resistance under in vitro epiphytic conditions. Of the 16 accessions tested in vitro, three lines viz. DP-25, Jhankri and Duradim showed resistance of Phytophthora disease with lesion diameter below 2 cm and number of sporangia in a microscopic field below 2 under 10 × after 96 h of inoculation. Five lines considered as moderately resistant with lesion diameter below 3 cm and number of sporangia below 20. The lesion diameter below 3.5 cm and number of sporangia below 25, recorded in five lines were considered as moderately susceptible. The lesion diameter more than 3.5 cm and number of sporangia more than 25 were observed in three susceptible lines viz. Kuchidori, Megh-19 and N-118. The accessions Duradim, DP-25 and Jhankri have been isolated to augment breeding program to develop resistant lines for Phytophthora leaf blight disease.

Key words: Phytophathora spore, glucan elicitor, disease incidence

INTRODUCTION

Taro [Colocasia esculenta (L.) Schott] is one of the important tuber crops grown in the tropics of the world. The versatility of this crop is reflected not only by its corms but stems and leaves, which are also consumed as seasonal vegetables. Its corms are rich source of carbohydrates and minerals (Chandra, 1984). Taro has been devastated by leaf blight disease resulting 25-60% yield loss in many countries (Johnston, 1960; Kay, 1973 and Parris, 1941). Leaf blight, caused by Phytophthora colocasiae Raciborski, is the most destructive disease of taro than any other diseases of this crop. The leaf blight disease has been reported by Raciborski in Java in 1900. Although, many resistant varieties have been developed in India, the present status and availability of these varieties are not known (Thankappan, 1985). However, reasonable degree of resistance has not been found in varieties of taro growing in other countries (Parris, 1941; Hicks, 1967; Gollifer and Brown, 1974 and Jackson and Gollifer, 1975). The present study has been conducted with an objective to isolate resistant lines for *Phytophthora* leaf blight disease.

MATERIALS AND METHODS

Fifty seven diversified taro accessions were collected from different parts of Assam, Meghalaya, Nagaland, Tripura, West Bengal and coastal Orissa and were maintained at the Regional Centre of Central Tuber Crops Research Institute, Bhubaneswar along with 115 existing lines of taro. Three detached leaves of all 172 accessions were inoculated at 3 spots with 50 µl of elicitor suspension at the perpherial region. Cell wal glucan elicitor was iso-

lated from Phytophthora colocasiae Racib. following the methodology of Sriram et al. (2003). Phytophthora spore suspension (50 µl) was inoculated at the centre of the leaves. The cut ends of the leaf petiole were covered with moist cotton. The leaves were incubated in polythene bags at room temperature. Lesions indicating hypersensitive reaction due to elicitor molecule and blight spots due to Phytophthora spores were recorded at 24 h interval. The total hypersensitive area, percentage hypersensitive area and percentage spot area (blight incidence) were calculated following the methodology as described by Sahoo et al. (2002), Sriram et al. (2003) and Velayudhan et al. (2000). Based on the results of hypersensitive reactions and disease incidence, 14 lines along with 2 susceptible lines were selected for further evaluation and characterization under in vitro epiphytic conditions.

Evaluation and characterization of taro for leaf blight disease resistance

The selected 16 (14 resistant + 2 susceptible) accessions were evaluated under in vitro conditions using spore suspension on the leaf discs. Six leaf discs of 4 cm diameter from each line collected from the same age plant, were placed between moist blotting papers in petridishes. Fifty micro litres of standard spore suspension (15000 ml⁻¹) was inoculated at the centre of each leaf disc. The leaf discs with inoculum were incubated at room temperature (30-35°C) for 4 days. Appearance of disease, time taken for infection, diameter of the lesion and the number of sporangia produced was recorded at 24 h intervals. For counting of spores, the sporulated leaf pieces were washed throughly in distilled water. A drop of washed suspension was placed on haemocytometer and observed under a microscope. The experiment was conducted twice with six replications. The taro accessions were categorized as resistant or susceptible by using the 0-4 scale as described by Dey et al. (1993).

RESULTS AND DISCUSSION

The hypersensitive reactions in resistant cultivars were detected within 48-72 h of incubation. Percentage hypersensitive area was observed to be significantly higher in Bhengabari (2.77%)

followed by Puri (1.75%), Phagupur (0.99%), Begunia (0.96%), Tripura Local-3 (0.73%), Kamakanda (0.69%), Sree Reshmi (0.68%), Megh-49 (0.67%), Padmapatria (0.65%), DP-25 (0.61%), Jhankri (0.57%), Duradim (0.56%), Kadma local (0.56%) and Kuchidori (0.54%) [Fig. 1], whereas the same was observed in the range of 0 to 0.51% in susceptible lines. In general, the hypersensitive response due to elicitor treatment in the susceptible cultivars was absent or delayed (Sriram et al., 2003). The total hypersensitive area was recorded in the range of 0.93 to 2.70 sq cm in resistant accessions like Jhankri, Phagupur, DP-25, Duradim, Megh-49, Bhengabari etc.; where as in the susceptible lines (N-118 and Megh-19), no hypersensitive reactions were recorded (Fig. 1). As per the scale described by Velayudhan et al. (2000), the blight incidence was observed to be low (less than 5%) in 9 lines, medium (5.01-10.0%) in 32 lines and high (more than 10%) in 131 lines. Based on the results of total hypersensitive area, percentage hypersensitive area due to elicitor treatment and spot area due to spore inoculation, 14 accessions along with 2 susceptible lines were selected for further evaluation and characterization.

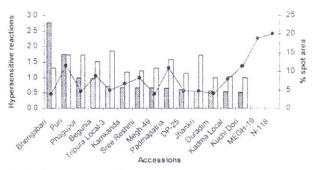


Fig. 1: Response of taro accessions towards hypersensitive reaction and disease incidence

In vitro evaluation of taro for leaf blight disease resistance

The results of the experiments under *in vitro* epiphytic conditions revealed that there was no significant difference in time taken for infection in the leaf discs (Table 1). Similar result was also reported by Paharia and Mathur (1964) and Dey *et al.* (1993). Appearance of spot was delayed (Fig. 2) in resistance lines DP-25 (50 h) followed by Jhankri

Table 1: Reactions of Phytophthora leaf blight under in vitro conditions

Accessions	Time taken for infection Size of the lesion at 96 h						Number of sporangia		
	(hours)			(cm)			per microscopic field at 96		d at 96 h
	Exp. I	Exp. II	Agerage	Exp. I	Exp. II	Agerage	Exp. I	Exp. II	Agerage
Begunia	40	42	41.0	3.48	2.92	3.20	13.33	13.17	13.25
Bhengabari	36	40	38.0	3.42	1.54	2.48	11.67	4.17	7.92
DP-25	54	46	50.0	2.13	1.48	1.81	1.83	1.00	1.42
Duradim	39	43	41.1	2.32	1.53	1.92	1.50	1.17	1.33
Jhankri	51	38	44.5	2.20	1.46	1.83	1.67	2.17	1.92
Kadma Local	36	36	36.0	3.37	3.27	3.32	21.67	20.33	21.00
Kamakanda	36	46	41.0	2.90	3.05	2.98	15.00	12.33	13.67
Kuchidori	36	46	41.0	2.90	3.05	2.98	15.00	12.33	13.67
Megh-19	36	36	36.0	4.30	2.98	3.64	26.67	23.67	25.17
Megh-49	41	44	42.4	2.43	2.02	2.23	8.33	2.78	5.58
N-118	36	36	36.0	4.30	2.98	3.64	26.67	15.33	21.00
Padmapatria	38	36	36.0	3.98	3.55	3.77	32.17	26.00	29.08
Phagupur	38	40	39.0	3.13	1.94	2.54	19.17	4.17	11.67
Puri	36	40	38.0	3.65	3.18	3.42	21.67	23.67	22.67
Sree Reshmi	44	44	44.0	2.00	2.20	2.10	2.17	1.83	2.00
Tripura Local-3	36	40	38.0	3.82	2.50	3.16	18.33	8.17	13.25
CD at 1% level	NS	NS		1.66	0.58		17.04	5.84	7.1

(44.5 h), Sree Reshmi (44 h), Megh-49 (42.4 h) and Duradim (41.1 h). However, in the susceptible lines like N-118, Megh-19 etc., the infection among the accessions were observed at 48, 72 and 96 h of incubation. The lesion diameter was recorded significantly low in DP-25 (1.81 cm). followed by Jhankri (1.83 cm), Duradim (1.92 cm), Sree Reshmi 2.10 cm), Megh-49 (2.23 cm) and Bhengabari (2.48 cm) after 96 h; where as the diameter was significantly high as 3.77 cm, 3.64 cm and 3.57 cm in the accessions N-118, Megh-19 and Kuchidori respectively (Table 1). The number of sporangia per microscopic field was observed to be significantly low in Duradim (1.33), DP-25 (1.42) and Jhanki (1.92) whereas more number of sporangia were produced in the leaf discs of the accessions N-118 (29.08) followed by Kuchidori (25.17), Puri (22.67) and Megh-19 (20.00) [Table 1]. Of the 16 lines tested, none of the line was found to be immune. Three lines viz. DP-25, Jhankri and Duradim showed resistance to Phytophthora leaf blight disease with lesion diameter below 2 cm and number of sporangia in a microscopic field at 10 × below 2 after 96 h of inoculation. Five line were considered as moderately resistant with lesion diameter below 3 cm and number of sporangia below 20. The lesion diameter below 3.5 cm and number of sporangia below 25 recorded in five lines were considered as moderately susceptible. The lesion diameter more than 3.5 cm and number of sporangia more than 25 were observed in three

susceptible lines viz. Kuchidori, Megh-19 and N-118.

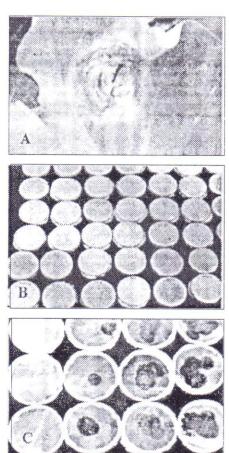


Fig. 2: A-C *In vitro* evaluation for leaf blight disease in taro
A. Source of inoculum. B. Incubation,
C. Reactions in resistant and susceptible lines

Of the 172 lines tested under *in vitro* epiphytic conditions, three lines – DP-25, Duradim and Jhankri showed resistance of leaf blight disease with low infections. The results of the present study are encouraging to augment breeding programme in taro to develop resistant lines to *Phytophthora* blight.

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