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Screening of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) genotypes for resistance to *Colletotrichum gloeosporoides*

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Abstract: Anthracnose is one of the most destructive fungal diseases caused by *Colletotrichum gloeosporoides* in yard long bean, leading to complete crop loss at all stages and its parts like hypocotyls, stem, peduncle, flowers, leaves and pods were seriously affected. Few bush type cowpea cultivars have been earlier identified as reliable sources of resistance while trailing types are susceptible, but high yielding. Breeding resistant varieties is suggested as the only practical strategy, especially under hot and humid condition. Fifty-yard-long bean genotypes belonging to bush, semi erect and pole types were screened against anthracnose disease through artificial inoculation under pot culture. The present study identified the resistant varieties of vegetable cowpea through artificial inoculation followed by detached leaf assay. Among the 50 varieties of yard long bean observed, Kanakamony, dual purpose yard long bean was found highly resistant with disease severity of 3.67% followed by Arimbra local.

Keywords: Anthracnose, *Colletotrichum gloeosporoides*, yard long bean.

INTRODUCTION

Yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt), a trailing type of vegetable cowpea ($2n= 24$) is one of the most popular and remunerative vegetable crops traditionally grown in Kerala. Due to the favourable agro climatic conditions, the crop has gained much importance and has come to occupy a prime position among the vegetable crops raised in the state. But the production of vegetable cowpea is hindered by an array of diseases that cause growth suppression or death of plants, leading to reduction in yield and productivity. Sreeja (2014) conducted periodical survey in the potential cowpea growing areas of Thiruvananthapuram district and reported that among the six major fungal diseases, anthracnose was found to be the most predominant one (0-55%). Disease index ranged between 0 - 33.30 and considerable yield losses were attributable to the disease.

Anthracnose is a destructive fungal disease caused by *Colletotrichum* spp. In India, the incidence of anthracnose disease was first reported from Maharashtra (Rao, 1966). According to Emechebe and Lagoke (2002),

all stages of the crop and its parts like hypocotyls, stem, peduncle, flowers, leaves and pods were seriously affected. Previous attempts to identify reliable sources of resistance have led to the identification of few bush type resistant cowpea (Kumar, 1999), as most of the trailing cultivars are susceptible to anthracnose, but high yielding compared to the bushy or semi-trailing types.

The disease is pan-tropical in distribution and is being widely recorded in regions where conditions are wet and humid. Once the infection is incited under favourable condition, its management using fungicides is difficult. Breeding resistant varieties is suggested as the only practical strategy, especially under hot and humid condition. The present study identified the resistant varieties of vegetable cowpea through artificial inoculation followed by detached leaf assay.

MATERIALS AND METHODS

Fifty yard-long bean genotypes belonging to bush, semi erect and pole types were screened against anthracnose disease through artificial inoculation under pot culture. Seeds of vegetable cowpea genotypes were collected from different parts of India, including the released varieties of SAUs and ICAR institutes and stored at refrigerated conditions for the study. The most virulent isolate of *C. gloeosporoides* was used for artificial inoculation (Fig. 1 1). To isolate the fungal spores for artificial inoculation, infected plant parts were collected from field, washed in tap water, and surface sterilised using 0.1% mercuric chloride followed by washing thrice with autoclaved distilled water.

The fungus was cultured in potato dextrose agar medium and mycelial growth was observed through microscope. All Petri dishes were incubated at room temperature ($25\pm 20^\circ\text{C}$). Petri dishes were examined for the growth of the pathogen and the morphological characteristics were observed. Inoculum was prepared from secondary culture of 7 dayold culture, by scrapping off the mycelium and spores and suspended in 100 ml sterile distilled water.

Seven seeds each of fifty genotypes were sown in two pots. After the germination, five healthy seedlings were retained and excess thinned out. Artificial inoculation was done on 20 days old seedlings kept in pots inside the greenhouse. Each plant was sprayed with 20 ml of spore suspension of virulent strain of *C. gloeosporoides* having a concentration of 10^6 spores ml⁻¹ by following serial dilution method. The plants were covered with moistened polythene covers to maintain high humidity (Fig. 2). Inoculated plants were observed daily for disease incidence. Observations on disease incidence were taken on three, five, seven, nine, fifteen, twenty, twenty-five and thirty days. Final observation was taken when the disease was well expressed. Disease severity was assessed using the scale 0-5 reported by Latunde and Dada (1990).

0. No infection

0.5. Hypersensitive spots on main stem only

- 1: Trace of infection – Small anthracnose lesions on main stem, petioles of lower leaf only
2. Slight infection – Lesions on stem, petioles and branches
3. Moderate infection – Advanced anthracnose lesions on stem, petioles, branches, veins on the abaxial surface of leaves
4. Severe – Advanced anthracnose lesions on stem, petioles, branches, leaves, veins and peduncles
5. Very Severe – Advanced anthracnose lesions on stem, petioles, branches, leaf veins, spreading lesions on peduncle and pods

Based on the percentage of plant area infected, disease severity/intensity was calculated using the following formula (Wheeler, 1969).

Per cent disease severity =

$$\frac{\text{sum of all numerical ratings}}{\text{total no. of plants taken for observation}} \times \frac{100}{\text{maximum disease category}}$$

Per cent disease incidence was calculated by using the following formula.

Per cent disease incidence =

$$\frac{\text{no. of plants infected}}{\text{total no. of plants observed}} \times 100$$

Based on the per cent disease severity, the genotypes were grouped into 5 categories as adopted by Rajkumar et al. (1995).

Disease severity	Category
0	Immune
1 – 10	Highly resistant
10.1 – 25	Moderately resistant
25.1 – 50	Moderately susceptible
Above 50	Highly susceptible

Five plants of the resistant genotype identified through artificial inoculation were grown in the field and detached leaf assay was done just before flowering to confirm resistance.

RESULTS

Observations on disease incidence were recorded at 10 days after infection. The infection was first observed in leaves, reddish brown streaks were very prominent on veins and veinlets. Prominent mildew symptoms were also observed on the leaf lamina (Fig. 3). Finally, leaves became chlorotic and detached from the infected plant. Reddish brown lesions were also developed on the stem. These individual lesions coalesced to form large sunken lesions and covered the whole stem causing drying up of the veins which is known as vine blackening. The pods were rotted, grey in colour, covered with black fruiting bodies of fungus. According to

the visible symptoms, the plants were awarded disease scores. Among the 50 genotypes tested, Kanakamony, a dual purpose yard long bean variety was found to be highly resistant with disease severity of 3.67%, followed by Arimbra local with

Table 1:
Percent disease severity and disease reaction of yard long bean genotypes for anthracnose incidence

Sl.No.	Treatments	Source	Severity	Percent Disease	Disease Reaction
1	Anaswara	KAU		22.15	Moderately resistant
2	Bagyalakshmi	KAU		13.90	Moderately resistant
3	Kanakamony	KAU		3.67	Highly resistant
4	Arimbra local	KAU		9.58	Highly resistant
5	Vyjayanthi	KAU		56.84	Highly susceptible
6	Mithra	KAU		46.90	Moderately susceptible
7	Githika	KAU		56.70	Highly susceptible
8	Lola	KAU		54.90	Highly susceptible
9	Manjari	KAU		38.80	Moderately susceptible
10	Sharika	KAU		63.80	Highly susceptible
11	Vellayani Jyothika	KAU		55.90	Highly susceptible
12	KAU Deepika	KAU		50.90	Highly susceptible
13	CO6	TNAU		24.70	Moderately resistant
14	Pusa Beej	IARI		66.45	Highly susceptible
15	Kashi Kanchan	IIVR		47.64	Moderately resistant
16	Arka Garima	IIHR		23.90	Moderately resistant
17	Arka Mangala	IIHR		24.30	Moderately resistant
18	Arka Samradhi	IIHR		21.60	Moderately resistant
19	FH 31	Farm House, Trivandrum		56.50	Highly susceptible
20	FH 55	Farm House, Trivandrum		53.50	Highly susceptible
21	FH 7	Farm House, Trivandrum		70.78	Highly susceptible
22	VS 38	KAU		64.99	Highly susceptible
23	VS 53	KAU		50.12	Highly susceptible
24	VS 58	KAU		44.64	Moderately susceptible
25	VS 16	KAU		70.00	Highly susceptible
26	TCR 17	NBPGR		69.80	Highly susceptible
27	TCR 18	NBPGR		41.50	Moderately susceptible
28	TCR 19	NBPGR		56.00	Highly susceptible
29	TCR 50	NBPGR		47.25	Moderately susceptible
30	TCR 53	NBPGR		26.80	Moderately susceptible
31	TCR 54	NBPGR		36.00	Moderately susceptible
32	TCR 55	NBPGR		78.91	Highly susceptible
33	TCR 56	NBPGR		36.00	Moderately susceptible
34	TCR 60	NBPGR		59.49	Highly susceptible
35	TCR 61	NBPGR		54.00	Highly susceptible
36	TCR 63	NBPGR		59.45	Highly susceptible

37	TCR 68	NBPGR	31.70	Moderately susceptible
38	TCR 69	NBPGR	69.22	Highly susceptible
39	TCR 70	NBPGR	36.23	Moderately susceptible
40	TCR 74	NBPGR	26.89	Moderately susceptible
41	TCR 75	NBPGR	44.47	Moderately susceptible
42	TCR 77	NBPGR	35.50	Moderately susceptible
43	TCR 80	NBPGR	37.95	Moderately susceptible
44	TCR 84	NBPGR	54.25	Highly susceptible
45	TCR 86	NBPGR	33.63	Moderately susceptible
46	TCR 91	NBPGR	55.20	Highly susceptible
47	TCR 92	NBPGR	33.30	Moderately susceptible
48	TCR 93	NBPGR	65.33	Highly susceptible
49	TCR 96	NBPGR	39.82	Moderately susceptible
50	TCR 125	NBPGR	37.49	Moderately susceptible
	SE (m)±	-	1.13	-
	C.D. (0.05)	-	3.22	-



Fig. 1



Fig. 2

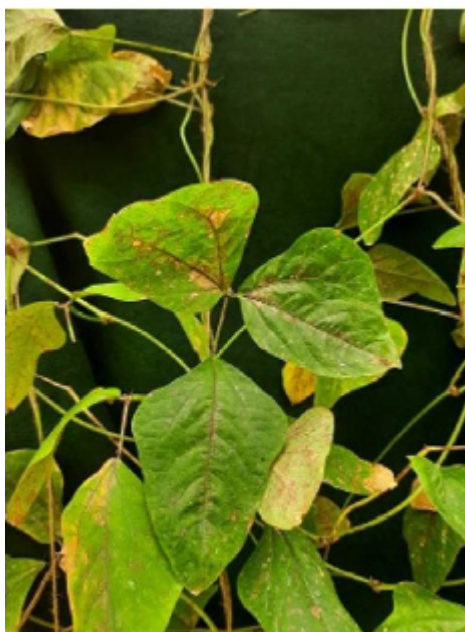


Fig. 3



Fig. 4

9.58% disease severity. TCR 55(78.91 %) was found to be highly susceptible followed by FH-7 (70.78 %) (Table 1). Among the genotypes evaluated, eight showed moderately resistant reaction from 13.90 to 23.40%. In the eighteen moderately susceptible genotypes, much difference could not be noticed in disease severity values and most of them were pole types. In detached leaf assay, the leaves of Kanakamony was symptomless, and the resistance was confirmed in vitro (Fig. 4).

DISCUSSION

Anthrachnose is one of the most destructive fungal diseases caused by *Colletotrichum gloeosporioides* in yard long bean, leading to complete crop loss in all stages and its parts like hypocotyls, stem, peduncle, flowers, leaves and pods were seriously affected. *C. gloeosporioides* has a wide host range including *Brassica campestris*, legumes, pigeon pea, soybean, brinjal, pumpkin, cucumber, tomato, spinach, mung bean, broad bean, cowpea, etc. (Sharma and Kulshrestha, 2015). Symptomatology studies on anthracnose of cowpea was conducted by Sreeja (2014). The initial symptoms appeared as minute, circular to irregular spots on the leaves which later increased in size and turned light to dark brown in colour and coalesced together to form large, necrotic spots on the leaves with shot holes. On the stem and vines, symptoms appeared as spindle shaped lesions with light grey centre and reddish-brown margin which enlarge upto 10-12 mm in length. In the later stages, small, irregular deep-seated reddish-brown spots appear on the pods also. Isolation, characterization and identification of the pathogen revealed that *Colletotrichum gloeosporioides* was associated with anthracnose.

Adebitan and Olufajo (1998) reported that grain types exhibited better resistance to anthracnose. Artificial inoculation of fifty genotypes confirmed the resistance in Kanakamony, followed by Arimbra local, hence these varieties are valuable sources for anthracnose resistance breeding programme. Kanakamony is a dual-purpose cowpea (grain cum vegetable) belonging to the sub-species *cylindrica*. Shiny et al. (2015) reported that susceptible bush-type cultivar Pusa Komal and pole type cultivar Lola showed 100 and 68.80 % disease severity while the immune

bush type cultivar Kanakamony was free from the symptoms and pole type Arimbra Local showed 8.80 % disease severity using SDS-PAGE. These results further support that Kanakamony is highly resistant to *C. gloeosporoides* causing anthracnose. Since fungicides and chemicals are not fully effective, the transfer of resistance from the cultivars to trailing types can only be a durable solution. Hence the results revealed that the highly resistant genotypes Kanakamony and Arimbra local could be recommended for crop improvement programmes in trailing type vegetable cowpea for enhancing the resistance to anthracnose.

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