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In vitro and field screening of okra cultivars against *Fusarium* wilt disease

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ABSTRACT: Eleven varieties and thirteen hybrids of okra were screened both in laboratory and field conditions against okra wilt caused by *Fusarium oxysporum f.* sp. *vasinfectum* (FOV). None of the varieties showed immune-like response (SI) and high level of resistance (HR). However, seven varieties showed intermediate resistance (IR), and three varieties recorded intermediate susceptibility (IS) reaction. The maximum disease index was recorded in Parbhani Kranti (53.57%) followed by Varsha Uphar (53.33%) varieties in comparison to susceptible check Pusa sawani (76.70%). In field trial, artificial sick plot was created. Among the okra varieties, Arka Abhay recorded lowest wilt incidence (25.62%) followed by Aruna (27.78%). Whereas as, susceptible check Pusa Sawani recorded highest wilt incidence of 71.49 per cent. Rest of varieties showed on par disease incidence. The data also revealed that rest of varieties showed moderate disease incidence with on par with each other in comparison to the susceptible variety. However, Supriya hybrid showed moderate susceptible reaction and eight hybrids exhibited susceptible reaction and four hybrids showed the highly susceptible reaction.

Keywords: *Abelmoschus esculentus*, okra wilt, *Fusarium oxysporum f.* sp. *vasinfectum*, genetic resistance, okra, screening

INTRODUCTION

Okra , *Abelmoschus esculentus* Moench, family Malvaceae) is an important vegetable crop. and is vulnerable to wilt disease caused by the fungus *Fusarium oxysporum* f. sp. *vasinfectum (*Atk) Snyder & Hansen, The genus *Fusarium* includes saprophytic species, as well as plant pathogens, being widely distributed throughout tropical and subtropical areas of the world (Burgess, 1981). The pathogen causes vascular wilt in okra and cotton (Cia and Salgado, 1997). The first report of *Fusarium oxysporum* f. sp. *vasinfectum* was occurred in 1935, infecting the cotton variety 'Texas' in Paraíba State (Silva *et al.* 2007b). The disease has been responsible for significant yield losses in many areas where this crop is grown (Silva *et al.,* 2007a). The management of *Fusarium* wilt in Malvaceae hosts is very difficult and the main control strategy is being the prevention of introduction of the pathogen to new planting areas. Other disease management methods are crop rotation and seed treatment with fungicides (Davis *et al.,* 2006). In the literature, there is a lack of information about the worldwide use of okra cultivars with genetic resistance to *Fusarium* wilt. Furthermore, new cultivars are continually being released by okra breeding programs. Therefore, it would be of practical interest to evaluate and characterize the reaction of okra varieties and hybrids which are presently available in the market to diseases of potential economic threat, such as *Fusarium* wilt.

Therefore, present study was conducted to evaluate (both greenhouse conditions and field conditions) the okra germplasm collections and released varieties aiming to identify sources of resistance to native isolates of *F. oxysporum* f. sp. *Vasinfectum.*

MATERIALS AND METHODS

The okra cultivars were evaluated by two methods *viz.*, under controlled and field condition for the identification of resistant sources for wilt.

Screening under controlled condition: Eleven public sector varieties and thirteen private sector hybrids were screened for *Fusarium* wilt. Five mm mycelial disc of the pathogen was inoculated separately into the conical flask and incubated in to 100ml sterilized PDA broth for seven days at 25ºC. Later, the mycelial mat was removed from the flask and macerated in a warring blender along with 100 ml of sterilized distilled water for a minute. The inoculum was later collected in a beaker. Conidial concentration was estimated and then adjusted to 10^6 conidia/ml. For inoculation, the plantlets (with two pairs of fully open leaves of 21 days old) were taken and the substrate adhered to the roots was washed using sterile water. After that, the root tips (at approximately 5 cm down from the stalk) were cut with sterile scissors and then immersed into the conidial suspension for two minutes. After this time, the inoculated plantlets were transplanted into plastic bags containing 2 kg of sterile

substrate (mix of 100 L of soil; 200 g of NPK fertilizer; 100 g of lime; 40 g of ammonium sulphate and 20 L of burnt rice husks). After transplanting, 5 ml of the conidial suspension was also placed in the soil around the stalks.

The evaluation was carried out nine days after inoculation, when external (yellowing, wilt and leaf fall) and internal symptoms (darkening of the veins) appeared on the accessions. The internal symptoms were observed after cutting the plant stalks longitudinally, with the help of a razor blade. An ordinal disease severity scale, adapted from Reis *et al.* (2004), was used to evaluate the plant responses. The grades of this scale ranged from 0 to 4, where : 0 = plants with no symptoms; 1 = plants with no symptoms of wilt or yellowing, but with darkened vascular bundles; 2 = plants with intensely darkened vascular bundles : 3= yellowing and premature leaf drop; 4 = dead plants.

From the grades, a disease index (DI) was calculated.

$$DI\ (\%) = \frac{100.\Sigma\ [(f\ \mathbf{X}\ v)}{(n\ \mathbf{X}\ x)} \times 100$$

Whereas DI = disease index; f = number of plants with the same grade; v = observed grade,
n = total number of plants evaluated and x = maximum grade on the scale.

The DI data were grouped into classes according to the reaction to the pathogen observed in each accession: 0% = similar to an immune-like response (SI); 0.01-25% = high level of resistance (HR); 25-50% = intermediate resistance (IR); 50-75% = intermediate susceptibility (IS); and 75-100% = high level of susceptibility (HS) (Reis *et al*., 2004).

Table 1. Screening of okra genotypes to *F .oxysporum* f. sp. *vasinfectum* under controlled conditions

Variety	Disease index (%)	Disease reaction	Hybrid	Disease index (%)	Disease reaction
Co-1	39.06	IR	Adhunik	32.35	IR
MDU-1	51.92	IS	Panchali	41.67	IR
Parbhani Kranti	53.57	IS	Varsha	51.60	IR
Aruna	32.14	IR	Vijay	33.33	IR
Arka Anamika	37.50	IR	No.1	32.81	IR
Arka Abhay	29.69	IR	No. 7	37.50	IR
Punjab Padmini	42.86	IR	No. 8	51.80	IR
Lam	42.19	IR	No. 10	42.19	IR
Varsha Uphar	53.33	IS	No.11	30.00	IR
Pusa A-4	36.67	IR	Nath Shobha	39.06	IR
Pusa Sawani(Check)	76.70	HS	Sun Gro-35	54.69	IS
			Supriya	28.13	
			US-7109	53.13	
			Pusa Sawani (Check)	78.60	

Screening under field conditions: A total of eleven public sector varieties and thirteen private sector hybrids were screened in wilt sick plot at CHES, Hirehalli, during *Kharif* 2013-14 and 2014-15. Each cultivars was sown in three meter row length. At the time of sowing, giant culture of *F. oxysporum* f. sp. *vasinfectum* of Tumkur isolate (TMK-6) was also incorporated to the soil to improve sickness of the soil. Observations on per cent wilt incidence were recorded.

The cultivars were categorized into different disease reaction as per Anon, 2013.

RESULTS AND DISCUSSION

To identify the sources of resistance, 11 public sector varieties and 13 private sector hybrids of okra were screened against okra wilt caused by *F. oxysporum* f. sp. *vasinfectum* under controlled as well as field conditions at CHES, Hirehalli during *kharif* 2013 and 2014 as explained in material and methods.

a. Screening under controlled conditions

Among the varieties (Table 1), none of the varieties showed immune-like response (SI) and high level of resistance (HR). However, seven varieties showed intermediate resistance (IR), three varieties *viz.,* MDU-1, Parbhani Kranti and Varsha Uphar recorded intermediate susceptibility (IS) and susceptible check Pusa Sawani showed high level of susceptibility (HS). The maximum disease index was recorded in Parbhani Kranti (53.57%) followed by Varsha Uphar (53.33%) varieties compared to susceptible check Pusa sawani (76.70%).

Among the hybrids, (Table 1) none showed immune-like response (SI) and high level of resistance (HR). However, eleven hybrids showed intermediate resistance

Table 2. Screening of okra varieties and hybrids to *F. oxysporum* f. sp. *vasinfectum* under field conditions

Variety	Disease index (%)	Disease reaction	Hybrid	Disease index (%)	Disease reaction
Co-1	34.12 (35.73)	S	Adhunik	32.35 (34.66)	S
MDU-1	45.05 (42.14)	S	Panchali	41.67 (40.21)	S
Parbhani Kranti	45.83 (42.60)	S	Varsha	51.60 (45.92)	HS
Aruna	27.78 (31.72)	MS	Vijay	31.33 (34.04)	S
Arka Anamika	33.46 (35.30)	S	No.1	32.81 (34.95)	S
Arka Abhay	25.62 (30.40)	MS	No. 7	37.50 (37.76)	S
Punjab Padmini	35.33 (36.36)	S	No. 8	50.80 (45.46)	HS
Lam	37.60 (37.79)	S	No. 10	42.19 (40.51)	S
Varsha Uphar	40.95 (39.79)	S	No.11	30.00 (33.21)	S
Pusa A-4	30.44 (33.47)	S	Nath Shobha	39.06 (38.68)	S
Pusa Sawani (Check)	71.49 (58.19)	HS	Sun Gro-35	54.69 (47.69)	HS
			Supriya	28.13 (32.03)	MS
			US-7109	53.33 (46.91)	HS
			Pusa Sawani (Check)	78.60 (62.44)	HS

* Figures in parentheses are angular transformed values

(IR), two hybrids *viz*., Sun Gro-35 and US-7109 recorded intermediate susceptibility (IS) and susceptible check Pusa Sawani showed high level of susceptibility (HS).The maximum disease index was recorded in Sun Gro-35 (54.69%) followed by US-7109 (53.13%) hybrids compared to susceptible check Pusa sawani (78.60%). Reaction of different varieties and hybrids against *Fusarium* wilt revealed that none of the varieties and hybrids showed immune to high resistance. Similar studies were conducted by Frederick Mendes Aguiar *et al*. (2013). They reported that about 72 per cent of the accessions were classified as having high and intermediate resistance to *F. oxysporum* f. sp. *vasinfectum* isolate'Fus-194' when screened under glasshouse condition. Screening studies of present investigation mainly depends on the scale of which internal symptoms are the main criteria. A distinctive characteristic of *Fusarium* wilt mainly depends on discoloration of the root and stem xylem. However, there is no consensus regarding the diagnostic importance of this vascular discoloration for evaluation of the host germplasm reaction to *Fusarium* wilt. In support of the above report, Salgado *et al.* (1994) used vascular discolorations as a criterion for judging susceptibility of tepary bean seedlings to *Fusarium* wilt.

Present studies also indicate that most of the varieties and hybrids showed intermediate resistance and intermediate susceptibility. These results were confirmed with the findings of Drame (2004) who reported okra cultivars exhibited intermediate susceptibility with five African isolates of *F. oxysporum* sp. *vasinfectum.* In the present study, the seedlings were considered slightly susceptible if they showed internal discolorations even though they were free of external symptoms. Thus, the seedlings were considered resistant only if they were completely free of any internal and external symptoms. Present studies are also in agreement with Waheed Akram *et al*. (2014). They studied basal susceptibility of tomato varieties against *Fusarium* wilt by using ten different isolates of *F. oxysporum* f. sp. *lycopersici* and reported that none of the varieties was completely resistant or immune against *F. oxysporum* f.sp. *lycopersici*. Similarly, Chopada *et al*. (2014) screened ten different tomato varieties against *F. oxysporum* f. sp. *lycopersici* isolate SGFOL-7 and reported that three varieties *viz.,* NS-2535, Heamsona and GT-2 were found moderately resistant and none of the variety showed complete resistant reaction.

b. Screening under field conditions:

The disease incidence and yield of 11 public sector varieties and 13 private sector hybrids were recorded and presented in Table 2. Arka Abhay recorded lowest wilt incidence and highest yield with 25.62 per cent followed by Aruna showed wilt incidence of 27.78 per cent. Whereas as, susceptible check recorded highest wilt incidence of 71.49 per cent. Rest of varieties showed on par disease incidence. The soil type, moisture, inoculum level and other optimum environmental factors favour the disease development. Under these conditions, it is unlikely that any cultivar would have escaped from the infection. The present studies are confirmed with the findings of Drame (2004), Mahesh (2008) and Iqbal *et al*., (2010).

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