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Identification of new resistant varieties to Phomopsis fruit rot of eggplant (*Solanum melongena*)

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ABSTRACT

Eggplant (*Solanum melongena* L.) is one of the supreme members of the solanaceous group which is widely grown in India. It is considered as the single and economic host of the fungus *Diaporthe vexans* causing fruit rot, stem canker and blight disease. The present study was carried at the vegetable research farm of ICAR-Indian Agricultural Research Institute, New Delhi during 2019–20 and 2020–21 to screen the eggplant lines (18 cv.) for resistance against this pathogen. The pathogen was isolated from the infected fruits and confirmed by morphological and sequencing information. Artificial inoculation using pin prick method revealed the presence of variability in the lines for disease. The studied lines were categorized based on Per cent disease index (PDI) and the highest PDI value of 80–100% recorded in cultivars Pusa Shyamla, Pusa Kranti, DBL-186, DBL-187, Muktokeshi and DBSR-52 and thus categorized as highly susceptible. The cultivars NDB-25, Kashi Sandesh, Kalo Solia, DBL-175 and Debjhuri Hazari were reported to be susceptible with PDI value of 60%. In the present study, first time we have report a number of highly resistant lines, viz. BR 112, Swarna Mani, BR 40-7 and IC-112992 with 0% PDI as novel source of resistance against *P. vexans.* These resistant lines have significant importance in large scale cultivation as resistant varieties by farmers. They can also be further utilized as a parent in population development for the identification of gene (s)/ QTL (s) associated with the resistance.

Keywords: Diaporthe, Eggplant, Pathogen, Resistance, Screening, Variability

Eggplant (*Solanum melongena* L.) is one of the most common vegetable crops in India and occupies the position of "King of Vegetables" (Daunay and Janick 2007). Asian countries produce 87% of the world's output while occupying 90% of the land area. Eggplant has a quite fascinating ethno-botanical history with its uses as food crop, medicinal properties and also as an ornamental crop (Bhanushree *et al.* 2021). It is a rich source of antioxidants due to the presence of phenolics i.e. cryogenic acid, flavonoid and anthocyanin (Nandi *et al.* 2021). The crops suffers immensely due to the attack of various disease causing pathogens and insect pests. Among the diseases, Phomopsis fruit rot caused by the pathogen *Diaporthe vexans* (Sacc. & Syd.) Harter affects the crop at any stage of its growth and development, subsequently results in complete loss, and the crop is the only economic host of the pathogen (Karmakar and Singh 2017). The earliest evidence of incidence of this disease in India dates back to 1914 from Gujurat (Harter 1914). The pathogen is externally and internally seed-borne causing damping-off of seedling, infection on leaves appear as small, irregular olive coloured spots upon which the pycnidia of the fungus arises (Harter 1914). The characteristics disease symptoms on the fruit are noticed as circular to oval sunken areas which appear as pale yellow and brown zones covering the fruit surface upon which pycnidia of pinhead size are formed (Islam *et al.* 2010). There is heavy toll in yield and quality of eggplant upto 10–50% (Kumar *et al.* 2020). It is very difficult to control the disease by traditional way as the inoculum survives in the crop debris and in dried fruit for about 14 months (Jayaramaiah *et al.* 2013). The disease control by chemical measure is found to be inconsistent and insufficient. Thus, the most sustainable and long lasting measures is the identification of resistant source and their use in development of resistant varieties and/or hybrids. In view of the above fact, large number of eggplant lines were screened through artificail inoculation for resistance to Phomopsis fruit rot.

MATERIALS AND METHODS

Plant materials: A total of 18 lines including released varieties, indigenous and exotic germplasm, landraces, wild accessions were grown at the vegetable research farm of ICAR-Indian Agricultural Research Institute, New Delhi

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during 2019–20 and 2020–21 and screened for resistance against Phomopsis fruit rot. Seedlings of all lines were raised in seedbed and transplanted during rainy (*kharif)* season. The cultivation practices were followed as per Singh and Kalda (2003).

Isolation of Diaporthe vexans and pathogenicity: The fruits which showed symptoms were collected from field. The fruits were cut into small pieces (5 mm) and sterilized using 2% Sodium Hypochlorite solution for 20–30 sec. Samples were repeatedly rinsed in distil water thrice for 2–3 min to remove excess Sodium Hypochlorite solution and were blot dried. Dried samples were placed on PDA media and incubated at 28 ± 2°C with 12 h of light (Prasad 2018). The pathogen takes 12–14 days for the formation of sporulating pycnidia on media. The pathogenicity of fungus was confirmed using susceptible check varieties. Culture obtained from the isolation of diseased samples was maintained by preparing slants for long term use. Sub-culturing from slants was practiced to maintain the viability of the pathogen and for further inoculation. Further, the hyphal tip of the fungus from the purified culture was taken and transferred to slants containing PDA. After a sufficient period of incubation, the pathogen is confirmed based on cultural, morphological and microscopic structure observed under microscope at magnification of 10X and 40X. Later, it was confirmed by sequencing information.

Inoculum preparation and inoculation: For the infection assay, fresh conidial suspensions from 10–14 days old pure culture were prepared by scrapping and macerating the pycnidia in double distilled water (Pandey *et al.* 2002, Jayaramaih *et al.* 2013, Bhanushree *et al.* 2021). Prepared suspension for inoculation was filtered through cheese cloth and the concentration or conidial count of alpha conidia was adjusted to 1×10^5 conidia/ml using Haemocytometre. The pin prick method of inoculation was performed for the screening purpose (Prasad 2018, Thesiya *et al.* 2020, Bhanushree *et al.* 2021). Healthy fruits were collected from field and washed with tap water and surface sterilized using 70% alcohol. Fruits were placed in sterilized trays prepared by placing a layer of non-absorbent cotton on which water is sprayed or sprinkled to maintain humidity during incubation. Fruits were pin pricked using sterilized needles and inoculated with 20 µl droplets of inoculum (1×10^5 conidia/ml). Trays were covered using polythene covers and incubated @28±1°C for about 15 days.

Table 1 Scoring of fruits as per Prasad *et al.* (2018)

Rating scale	Fruit surface affected (%)	Response/Reaction
0	0	Highly Resistant (HR)
1	1–20	Resistant (R)
2	21–40	Moderately Resistant (MR)
3	41–50	Intermediate (I)
4	51–70	Susceptible (S)
5	71–100	Highly susceptible (HS)

Scoring of fruits: Inoculated fruits were monitored daily and observations for per cent of fruit area infected were noted on the basis of fruit size by considering the area of single fruit as 100%. Disease symptoms started from 8

Fig 1 Scoring of the inoculated fruits.

days after inoculation and data were recorded regularly up to 15 days of inoculation. The fruits were scored on 0–5 rating scale, with 5 being the highly susceptible and 0 being the highly resistant as described previously by Prasad *et al.* (2018). Detailed descriptions of the disease rating scale, the severity of symptoms and disease reactions are presented in Table 1 and Fig 1. The per cent fruit infection and per cent FAD (Fruit area diseased) was estimated by the diseased area of the fruits after deducting the healthy area. The disease severity in terms of per cent disease index (PDI) was calculated as (Wheeler 1969):

PDI= (Sum of all disease rating/total number of observations × maximum disease grade) × 100.

Cross culturing of the infected fruits was done to re-isolate the pathogen on PDA media.

RESULTS AND DISCUSSION

Characteristic features of Diaporthe vexans and its confirmation: Variation in pathogen population can be discerned through their cultural, morphological and pathogenic characters. In the present study, the isolate of pathogen was studied for their cultural characteristics, viz. colony type, colony colour and zonation, along with pycnidial and conidial characters. The pathogen produced snow white colony having fluffy mycelial growth with irregular margins, distinct zonation when it grown on PDA media. Sequencing of the isolated fungus was also carried out and submitted to gene bank with accession number(s) SUB9325596 SeqIL1 MW784847 (Bhanushree *et al.* 2021). Bhat *et al.* (2019) also found the morpho-cultural and pathological variability of 30 isolates of *D. vexans*, isolates produced fluffy to embedded colonies with regular to irregular margins. Development of zonation by the pathogen when raised on medium was noticed, this characteristic feature is also reported by the previous workers (Divinagracia 1969, Akhtar and Chaube 2006). Our reports are in acceptance with the report of Akhtar and Chaube (2006) who mentioned that the six isolates out of 32 exhibited clear and distinct zonation when raised on medium. An insight into the data about the colour of the pycnidia produced, number of days taken to produce sporulating pycnidia, colour of the ooze discharged and distribution of pycnidia in culture plates was recorded. The isolate of our study produced sporulating black colored pycnidia in about 12–14 days after culturing on media and were seen to be randomly distributed in the colony. The *in vitro* studies purported that *D. vexans* isolates showed variation in colour of the discharged ooze ranging from yellow, brown and light grey. The data also revealed that in majority of the isolates (90%), pycnidia were located on the surface of the colony (Akhtar and Chaube 2003, Akhtar and Chaube 2006, Mahadevakumar and Janardhana 2016, Mahadevakumar *et al.* 2017, Bhat *et al.* 2019). A brief study about the morphological characteristics of conidia was conducted which revealed that the α conidia were sub-cylindrical in shape with the presence of 2 guttulae. The earlier results pertaining to morphology of α and β conidia revealed that majority of the isolates produces sub-cylindrical shaped α conidia, but there are also reports of elliptical shape in some of the isolates. Variation in number of guttulae within α conidia was very distinct nature of the isolates, which varies from 0–3 in number. Majority of the isolates were producing either one (Akhtar and Chaube 2003) or two (Bhat*et al.* 2019) guttule which varies from the reports. It was also noticed that some of the isolates does not produced any guttule, whereas some of them were able to produce 2–3 in number. The shape of the β conidia varies from curved, filiform and sickle shapes. In our study filliform shape β conidia were observed and found very less in number in comparison with the α conidia.

Screening of eggplant lines: In order to identify and decipher the resistance sources to Phomopsis fruit rot, screening of 18 elite eggplant lines was carried out through artificial inoculation. The inoculated fruits were observed on daily basis for the incidence of disease. In most of the susceptible lines, symptoms appeared 8 days after inoculation as water soaked circular to oval sunken areas with yellow and brown zones on fruit surface. As the disease progressed, pycnidia on the infected fruit surface are formed and at later stages fruits were completely rotten. The percentage disease infection (PDI) was calculated based on the damage on the fruits using disease scale developed by Prasad (2018).

The studied lines were categorized based on the PDI (Table 2). The fruit infection of the tested genotypes ranged from 0.00–100%. The highest percentage of fruit infection was recorded in cultivars Pusa Kranti, DBL-186, DBL-187 and DBSR-52 with 100% of PDI value, followed by Pusa Shyamla and Muktokeshi with 80% of PDI value. Further, these six cultivars were categorized as highly susceptible based on PDI. The cultivars NDB-25, Kashi Sandesh, Kalo Solia, DBL-175 and Debjhuri Hazari were categorized as susceptible having PDI value 60%. Some of the moderately resistant lines, viz. Pusa Purple Round and G-184 were also identified with PDI value 40%. The lines BR-112. Swarna Mani, BR-40-7 and IC-112992 did not exhibit any infection/symptoms even after 15 days of inoculation on fruit, so they were categorized as highly resistant with PDI value of 0%. To know the disease progress in each line, the data were recorded at intervals of 11, 13 and 15 days after inoculation (Table 2).

The relative expression of disease in the studied genotype varied from lines to line in different time point. The number of days taken to initiate the disease incidence and for 50% fruit to rot was recorded for each line. The cultivar Pusa Kranti started to show infection from 9th DAI (days after inoculation), 50% fruit got infection by 12 DAI, whereas in DBL-186 and DBL-187 infection started from 10th DAI, but the 50% fruit got infection in 12th and 13th DAI respectively. Pusa Shyamla and NDB-25 started to show disease symptoms from 11th DAI and 50% fruit got infection in 14th and 15th DAI, respectively. The resistant line BR-112 and Swarna Mani started to show the disease

Table 2 PDI values and disease reactions of the eggplant genotypes

Eggplant lines	Source	Fruit surface affected (%)			Rating	PDI (%)	Reaction based on PDI
		11 DAI	13 DAI	15 DAI			
Pusa Shymla	ICAR-IARI, New Delhi	2	10	60	4	80	HS
Pusa Kranti	ICAR-IARI, New Delhi	35	100	100	5	100	HS
DBL-186	ICAR-IARI, New Delhi	20	50	100	5	100	HS
DBL-187	ICAR-IARI, New Delhi	20	50	80	5	100	HS
NDB-25	NDUAT, Ayodhya, Uttar Pradesh	10	30	50	3	60	S
Pusa Purple Round	ICAR-IARI, New Delhi	0	5	30	2	40	MR
BR-112	Haryana	0	0	0	0	0	HR
Swarna Mani	Jharkhand	0	0	0	0	0	HR
Kashi Sandesh	ICAR-IIVR, Varanasi	8	20	45	3	60	S
Debjhuri Hazari	West Bengal	10	25	50	3	60	S
Kalo Solia	West Bengal	15	25	45	3	60	S
Muktokeshi	West Bengal	30	50	70	4	80	HS
BR 40-7	Haryana	0	0	0	0	0	HR
DBL-175	ICAR-IARI, New Delhi	15	30	48	3	60	S
G-184	ICAR-IARI, New Delhi	0	5	40	2	40	MR
DBSR-52	ICAR-IARI, New Delhi	30	50	80	5	100	HS
IC-112992	ICAR-NBPGR, New Delhi	0	0	0	0	0	HR

infection 19 DAI, and took 21 and 22 days respectively for 50% of fruit infection. In the cultivar BR-40-7 disease initiation was noticed after 20th DAI and took 23 days to show 50% of fruit infection. This shows that even among the both susceptible and resistant genotypes the relative expression of the diseases varies.

The present study is perhaps the first report of novel source of resistance against *D. vexans* in brinjal. Similar method of screening for disease resistance was carried out by many researchers in other cultivars and the wild relatives of eggplant which revealed the presence of differential response of eggplant cultivars to infection by *D. vexans* (Kalda *et al.* 1976, Swarup 1995, Islam *et al.* 2020, Pandey *et al.* 2002). Screening for resistance to *D. vexans* in wild *Solanum* spp. was carried out by Kalda *et al.* (1976), out of 300 entries the wild species *S. xanthocarpum, S. indicum, S. gilo, S. khasianum, S. sisymbriifolium* were highly resistant, and *Solanum melongena* lines 11a-12-2-1, 264-1-1 and 238-4-10-3 were found resistant. Thereafter, many scientists carried out screening procedures against *Phomopsis* fruit rot, of which the cultivars Black Beauty, Florida Market, Pusa Bhirav, RHRB-8, CHBR-1 and D-2-8-9 were found resistant and Ramnagar Gaint and KS-233 showed moderately resistant reaction (Swarup 1995, Pandey *et al.* 2002, Khalil *et al.* 2013). Further, screening of interspecific hybrids and their advanced progenies was carried out by Kabir (2007) and Khalil *et al.* (2013), they found that all the progenies of F_3 and F_4 generation were resistant to the disease. The recent studies on screening of eggplant germplasms was carried out by Islam *et al.* (2020) for three consecutive years (2016–2019) and they categorized Kata begun WS and a wild accession as resistant out of all the 53 eggplant germplasms studied. This finding were in acceptance with the previous reports of Meah (2003), and Islam (2005) who also reported Katabegun WS as a resistant cultivars against *Phomopsis*. A series of released varieties from the public sector were screened for the incidence of disease and found that Pusa Dwarf, Pusa Dwarf Green, Pusa Purple Round, Pusa Purple Long, Arka Nidhi, Pant Samrat, Annamalai, Pusa Upkar, Arka Kusumarkar, Kashi Sandesh, Black beauty and Thorny Monjory Gota as resistant (Khodke 1990, Prasad 2018). The findings of our study is also in consonance with Prasad (2018) who also reported the cultivars Pusa Kranti, Pusa Shymla, Pant Rituraj, Arka Neekanth, Pusa Kaushal, and Pusa Safed Baingan 1 as highly susceptible to disease.

Primarily, the crop diseases are managed by spraying fungicides, but many times there is a chance the emergence of fungicide-insensitive isolates which make management difficult. Hence, the management of the disease through host resistance is always considered the most viable approach and currently, artificial inoculation techniques are commonly followed for screening. Hence, it may be concluded from the above findings that there exists a wide range of variation to disease reaction in the present study and the identified resistant lines of eggplant have great significant importance in large scale cultivation as resistant varieties by farmers. They can also be further utilized as a parent in population development for the identification of gene (s)/QTL (s) associated with the resistance.

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