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Assessment of relative performance of different genotypes of brinjal against fruit rot infection by artificial inoculation of *Phomopsis vexans*

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Abstract
During crop production, brinjal is prone to various diseases. Among them, leaf blight and fruit rot disease caused by *Phomopsis vexans* is one of the most detrimental diseases causing a huge loss in terms of production, productivity including quality of the crop which is still a major challenge to pathologists and breeders. The most suitable approach is the use of resistant genotypes as a source for the development of high-yielding cultivars to avoid seed-borne disease incidence. Many researchers have identified resistant sources but limited success has been achieved in developing resistant cultivars. Regular identification of resistant sources is necessary for successfully protecting the crop from disease. In the present studies, an extensive screening of thirty-four genotypes which includes a few elite lines, a few local genotypes, a few hybrids and many released varieties has been done against fruit rot pathogen through artificial inoculation of conidial suspension. Based on the screening, the wide spectrum of genotypes was classified into five different categories namely highly resistant (Pant Samrat, G-204, G-175, G-131, G-9 and DB-7) resistant (G-203, G-31, Pusa Upkar, Arka Nidhi and Bhangar Local) moderately resistant (G-43, Kashi Sandesh, Pusa Uttam, G-23, DB-9, Pusa Safed, Pant Rituraj and Arka Kusumarkar) susceptible (G-160, G-145, G-65, G-10, G-5, G-22, Pusa Shymla and Pusa Kaushal) and highly susceptible (G-17, G-109, G-60, G-181, G-164, Pusa Kranti and Pusa Bindu) on the basis of the percentage of fruit infection. Screening against pathogens and then classifying them into different categories based on the level of response to pathogens gives complete information to breeders to utilize in the development of resistant genotypes.

Keywords: Brinjal, *Phomopsis vexans*, fruit rot, disease severity, genotypes

Introduction
Brinjal (*Solanum melongena* L.) which is also known as the “king of vegetables” is an important solanaceous crop of sub-tropics and tropics [1]. In India, brinjal accounted for 12.8 million tonnes of production in an area of 0.749 million hectares [2]. West Bengal is the leading producer of brinjal, followed by Maharashtra and Bihar. The cultivated brinjal is of Indian origin [3] and because of its popularity, its cultivation spread worldwide rapidly. It is rich in Vitamins A and B and consumed by all sections of people [4]. Brinjal is known to be invaded by many phytopathogens which are major constraints for the limited production and productivity of this crop. Among them, fruit rot caused by *Phomopsis vexans* is considered to be the most destructive disease of brinjal. The pathogen has been reported in warmer regions in most countries [5]. Being a seed-borne pathogen, *Phomopsis vexans* causes damping-off of seedlings, and seedling blight at the nursery stage and as the disease progress elongated blackish to brown lesion appears on the stem and branches. On leaves, it forms small circular, buff olive to cinnamon-buff spots with irregular blackish margins and on fruits, the disease appears as minute sunken greyish spots with a brownish halo, which later enlarge and produce concentric rings with yellow and brown zones [6]. The disease is reported to cause over 50% loss in production and productivity in various parts of the world [7]. As far as resistant genotypes are concerned, the variety which is resistant today will become susceptible after a few years due to the evolution of new virulent races of the pathogen. Therefore, there is a need to search for new resistant genotypes so that we don’t compromise with yield. Therefore, screening of genotypes should be done continuously and inclusion of resistant and susceptible lines should be done for better comparison of the results.

Materials and Methods
Study area
The present study was undertaken in the Division of Seed Science and Technology ICAR-Indian Agricultural Research Institute, New Delhi, India between 2017–2018 and revalidation was done between 2019-20 by using healthy fruits from thirty-four brinjal genotypes collected from a field of the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi.

Table 1: List of brinjal genotypes used in the screening against brinjal fruit rot.

Bhangar Local	G-175	Kashi Sandesh	Pusa Shymla	G-109
G-204	G-160	Pant Samart	Pusa Upkar	G-60
G-203	G-145	Pant Rituraj	Pusa Uttam	G-181
G-5	G-131	Pusa Kaushal	Pusa Safed	G-164
DB-7	G-65	Pusa Kranthi	G-23	G-17
Arka Nidhi	G-31	Pusa Bindu	G-43	G-22
Arka Kusumarkar	G-10	G-9	DB-9	------------

Preparation of fungal inoculum
The *Phomopsis vexans* pathogen used for inoculation was collected from diseased fruit samples from the field of Indian Agricultural Research Institute, New Delhi and cultured on potato dextrose agar (PDA) media. The sporulation pathogen culture plate was flooded with 10 ml sterile double distilled and gently scraped the colony surface using a spatula. The mycelial fragments were removed through filtration of suspension using cotton gauze and spores' concentrations were determined hemocytometereter. Depending on the amount of spore present, the concentration of the resulting suspension was adjusted to 1×10^4 conidia per ml.

Pathogenicity Test
In order to ensure and confirms the identity of the disease and its causal agent, a pathogenicity test was conducted on the brinjal cultivar Pusa Kaushal using the pinpricking method to prove Koch's postulates. Fruits were pinched using a sterilized needle and then spot inoculated @ 20µl with freshly prepared spore suspension. Fruits were observed for symptom development ten days after inoculation. Re-isolation was made from infected fruits.

Severity assessment
Thirty-four brinjal genotypes were collected from the brinjal field Pusa, New Delhi and surface sterilized using 70% alcohol(Table:1). The fruits were pinched using a sterilized needle and then spot inoculated @ 20µl with freshly prepared spore suspension. Three replication were kept for all the genotypes and each replication contain a total of five fruits. Inoculated fruits were incubated at 27±1°C in an incubator. Fruits were observed daily for the percent of fruit rot and the area of lesions of a particular genotype was recorded. Percent of fruit infection was estimated by measuring the diseased area of the fruits. Re-isolation was made from infected fruits on PDA. For the assessment of *Phomopsis* disease incidence in brinjal, the disease scale has been constructed by McKinney [8] and Hossain *et al.* [9] was used.

PDI= (Sum of numerical values/total number of fruits observed × maximum grading) × 100.

Numerical values were obtained by multiplying the number of infected fruits by their respective grades. Based on PDI, each fruit was identified with the following grades. I - 0% PDI: free from the disease; II - 0.1–5.0% PDI: poorly affected; III - 5.1–20.0% PDI: moderately affected; IV – 20.1–50.0% PDI: severely affected; and V - >50.1% PDI: very seriously affected by the disease.

Results and Discussion

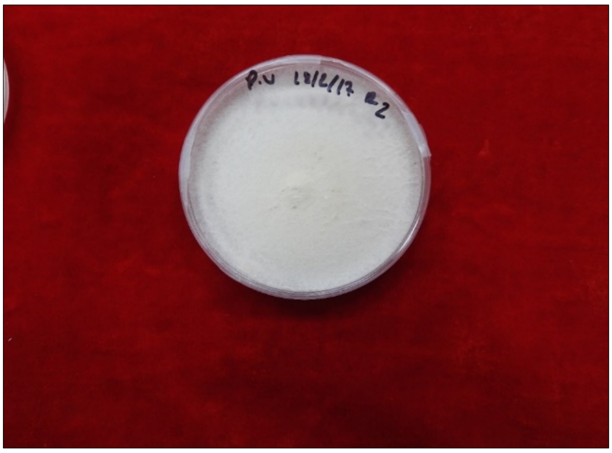
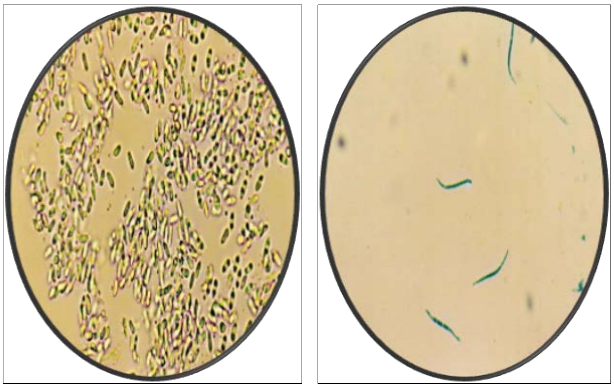


Fig 1: Pure culture of *Phomopsis vexans*



Alpha conidia Beta conidia

Fig 2: Conidia structure of *Phomopsis vexans*

Cultural and Morphological character
The pathogen exhibits a circular white-color colony on the PDA media (Fig 1). Alpha and Beta conidia of *Phomopsis vexans* were observed on a compound microscope. Alpha conidia were globose and fusoid and Beta conidia were hyaline and filiform shaped (Fig: 2). This report was in conformity with [10] who described that the *Phomopsis vexans* (Perfect stage: *Diaporthe vexans*), is reported to produce alpha and beta conidia. Various studies revealed that the formation of conidia in the pycnidia of *P. vexans* is temperature-dependent. At 10–16 °C the pathogen produces beta conidia and at 25-28 °C, the alpha conidia. These two forms of conidia get interconverted when subjected to a specific temperature.

Pathogenicity Test by fruit inoculation
Susceptible fruits of the Pusa Kaushal genotype inoculated with *Phomopsis vexans* using the pin-prick method developed small, roughly circular, soft, light yellow color lesions surrounded by a brown ring after 8 days of inoculation

whereas complete fruit rotting was recorded between 12th days after inoculation. Typical fruit rot symptoms developed on the fruits, which were similar to those observed by [11, 12] who proved the pathogenicity of *P. vexans* causing fruit rot with the above methods.

Disease severity measurement

1. Pant samrat 2. G-203

Fig 3: Highly Resistant and Resistant genotype of Brinjal

Pusa shymla Pusa bindu

Fig 4: Susceptible and Highly Susceptible Genotype of Brinjal

Out of 34 genotypes studied six genotypes namely Pant Samrat, G-204, G-175, G-131, G-9 and DB-7 and showed highly resistant against *Phomopsis vexans* in which the mean value infection level was 0% and five genotypes G-203, G-31, Pusa Upkar, Arka Nidhi and Bhangar Local showed mean value of 1.92%, 2.65%, 3%, 4.45% and 4.56% respectively. Which are classified under resistant categories. Eight genotypes such as G-43, Kashi Sandesh, Pusa Uttam, G-23, DB-9, Pusa Safed, Pant Rituraj and Arka Kusumarkar showed a mean value of infection levels of 5.36%,6.78%,8.12%, 9.16%, 9.68%, 10.98%, 13.71% and 13.75% respectively which were classified under moderately resistant categories (Fig 3). Eight genotypes namely G-160, G-145, G-65, G-10, G-5, G-22, Pusa Shymla and Pusa Kaushal were susceptible and G-17, G-109, G-60, G-181, G-164, Pusa Kranti and Pusa Bindu were found to be highly susceptible against *Phomopsis vexans* (Fig 4). Genotypes like G-160 G-145, G-65, G-10 G-17, G-109, G-60, G-164 and Pusa Bindu started to show infection from the 8th day after inoculation and the entire fruit got an infection on the 13th day after inoculation, whereas G-5, G-22 and G-181 started to show infection from 9th day after inoculation and the entire fruit got an infection on the 16th day after inoculation. In Pusa Shymla, Pusa Kaushal and Pusa Kranti the infection started to appear from the 9th day after inoculation and the entire fruit got infection between the 19th to 20th day after inoculation. Kalda *et al*. [13] reported that out of 300 entries tested against *Phomopsis vexans* which includes *solanum spp*, brinjal cultivars and F1 hybrids two *Solanum melongena* lines were resistant. However, the rest other *solanum spp* were highly resistant and F1 hybrids showed variable reaction resistance to Phomopsis blight governed by recessive genes. The observation from our work revealed that different genotypes have different responses against pathogen infection during crop duration. Even among the susceptible genotypes the relative expression of the diseases to the pathogen infection varies. Resistant genotypes are the primary source and most important method of controlling fruit rot disease by producing preformed toxins infecting the immune cells of pathogens and protecting the plant from disease. A wide-ranging diversity of brinjal genotypes and recent advances in genome sequencing of brinjal help in accelerating the breeding of high-yielding variety (s) which are resistant to Phomopsis blight. This work provides insight into the breeding approach for the identification of resistance sources including identification of resistance source, inheritance of resistance and application of advanced breeding tools for utilization breeding program. Host resistance is the most important and practical method which is also an environment-safe control method against this pathogen. Therefore, there is an urgent need for screening the wild and elite varieties of Brinjal resistant to fruit rot and leaf blight disease.

Conclusion

Therefore, in conclusion, we can say that screening wide spectrum genotypes in the field under natural conditions and then classifying them into different categories namely highly resistant, resistant, moderately resistant, susceptible and highly susceptible gives complete information to breeders to utilize them in the development of resistant genotypes.

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