

SCREENING SPECTRUM OF BRINJAL GENOTYPES AGAINST WHITE ROT DISEASE CAUSED BY *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY

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ABSTRACT

Brinjal has a relatively long growth period, and it is exposed to a broad range of pests and diseases. Among the various diseases, sclerotinia blight is an important disease which causes loss in both quality and quantity of fruits. *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most devastating soil borne necrotroph with wide host range and has the ability to survive for long periods in the form of sclerotia. Chemicals are widely used with some limitations, to check the growth of various diseases, but their correct dosage is very important to control the pathogen. Use of resistant varieties and understanding the physiology of pathogen can serve as an effective tool, in controlling the diseases in a cost effective and environment friendly approach, which can thereby reduce the usage of chemical fungicides. Under the present investigation an experiment conducted for screening different brinjal genotypes against sclerotinia blight out of fifteen genotypes, six genotypes were found moderately resistant, three genotypes were found moderately susceptible, four genotypes were found susceptible and two genotypes were found highly susceptible to the sclerotinia rot attack. None of the genotypes were found resistant to the sclerotinia blight incidence. Percent disease index values ranged from 85.42 % to 8.33%. The highest PDI was recorded in the genotype Sel 91-2-1 (85.42 %) and lowest PDI was recorded in Sel 91-2 (8.33%). The maximum average lesion length on stem was found in the genotype BRBL – 02 (7.32 cm) and it was minimum in genotype Sel 91-2 (0.37 cm).

KEYWORDS: Brinjal, genotypes, sclerotinia blight, resistant, screening, *Sclerotinia sclerotiorum*

1. INTRODUCTION

Vegetables are considered as the essential sources of the phyto-nutrients and provide a wide range of health benefits. They can ensure a secure supply of food and nutrients needed for the healthier diets. In order to prevent chronic diseases and to provide the necessary micronutrients, such as calcium, iron, iodine, vitamin A, and zinc, the World Health Organization has advised a minimum intake of 400 g of fruits and vegetables per day.

Brinjal is considered as the king of vegetables. It is one of the most common vegetable crops grown in tropical and subtropical type of climatic conditions. Brinjal belongs to the family Solanaceae, also known as nightshades, are cousins of potatoes, tomatoes, etc. It contributes 9% of the total vegetable production of the country (Sidhu et al., 2006). Eggplant is rich in dietary fiber, Vitamin B1 and copper. Other nutrients like manganese, vitamin B6, niacin, potassium, folate and Vitamin K are also found in good quantities. It also plays an important role in combating malnutrition in under nourished regions. According to the Food and Agriculture Organization of the United Nations (FAO, 2017), a minimum raise of 50% in agricultural food production is required in a scenario of modest economic growth. So, we have to focus on increasing the yield per area and decreasing the yield losses (FAO, 2017, Mauser et al., 2015; Pradhan et al., 2015). Insect pest infestation is one of the most limiting factors for accelerated yield production in brinjal.

Among all the diseases, Sclerotinia blight is one among the important diseases of brinjal which results in loss of quality and quantity of fruits (Sahni et al., 2018). The fungal genus *Sclerotinia* is mostly associated with vegetables which are caused by *Sclerotinia sclerotiorum* and *Sclerotinia minor*. *Sclerotinia minor* has a narrow host range while *Sclerotinia sclerotiorum* has a wide host range. *Sclerotinia sclerotiorum* pathogen is reported to infect 408 plant species belongs to 278 genus and 75

families (Boland and Hall, 1994). *Sclerotinia sclerotiorum* belongs to the family Sclerotiniaceae, order Helotiales, class Leotiomycetes and division Ascomycota. Hyphae are hyaline, septate, branched, and multinucleate, appearing white to tan in color. Asexual conidia are not produced but persist in the soil for extended periods in the form of sclerotia (Purdy, 1979; Willetts and Wong, 1980).

The fungus produces white to gray mycelium and spherical to irregular and black sclerotia. The sclerotium produces one to several apothecia. Asci are cylindrical and 8-spored. Ascospores are hyaline, ellipsoid to ovoid. The disease symptoms occur on the stem, branches, leaves and fruits. Water-soaked lesions appear on the stem which gradually increase in length and reach to the base of branches which causes the entire wilting of the plant. Sometimes branch infection results in partial wilting of the plant. The infected portion will be covered by the white cottony mycelial growth under cool and humid conditions. Sclerotia develop on the mycelia, which are white in the initially, later become more or less black in colour. Now a day huge losses by sclerotinia rot disease of brinjal in Bihar.

2. MATERIALS AND METHODS

The present investigation entitled “Screening Spectrum of Brinjal Genotypes against White Rot Disease caused by *Sclerotinia Sclerotiorum* (Lib.) De Bary” was undertaken in the Department of Plant Pathology, Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur, Bihar.

2.1 Isolation and identification of the test pathogens

Sclerotial bodies were isolated from the infected branches of the brinjal plant. Sterilization of the plantsamples (sclerotia) was done by dipping in 1 % sodium hypochlorite solution for 30 seconds and then rinsed with the sterile distilled water for 3 times. Then they are placed on sterile filter paper for allowing it to dry. Surface sterilized sclerotia were cut into small pieces and are inoculated aseptically onto the sterilized petriplates containing PDA medium amended with streptomycin. The inoculated plates were incubated at $25\pm 1^{\circ}\text{C}$ for 5 days so as to provide optimum conditions for the growth of the fungus. The fungus thus obtained was sub-cultured using the hyphal tip technique. The maintenance of purified cultures was done by preparing slants which were stored at 4°C for future studies. After obtaining the pure culture of the pathogen, it was characterized for the various parameters such as radial mycelial growth (mm), colour of the mycelium, days to sclerotia formation, number of sclerotia formed after 25 days after inoculation.

Koch’s postulates were employed to prove the pathogenicity of *S. sclerotiorum* after purifying the sample into fresh culture. The actively growing mycelium was cut into small bits of 5 mm² and was inoculated on the brinjal fruit and covered with a small piece of cotton so as to provide optimum moisture for the development of pathogen. Brinjal fruit covered with cotton, without any fungal inoculation was kept as control. After development of disease symptoms on fruit, the pathogen was re-isolated from infected fruit and the culture thus obtained was compared with the original culture to confirm the identity of pathogen. Pathogen was re-isolated from infected fruit and the culture thus obtained was compared with the original culture to confirm the identity of pathogen.

2.2 Preparation of potting mixture and rising of the crop

The experiments were carried out in the laboratory in the in-vitro conditions and the arrangement of seeds, potting mixture, plastic glasses were made well in advance. Different genotypes of brinjal (*Solanum melongena* L.) were procured from the Department of Vegetable Science, Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur, Bihar INDIA. The potting mixture was prepared well in advance before sowing of the seeds in the plastic glasses. Fine soil was collected and clods were broken to make it into a fine tilth. Potting mixture was prepared by mixing the soil and vermin compost in the ratio of 1:1 and was sterilized using solar radiation and fumigated with 4% formaldehyde solution. Polythene sheet were removed after five days and soil was exposed to open environment for atleast 15 days to remove the traces of formaldehyde present in the soil. The plastic glasses of 150 ml were filled with sterilized potting mixture and then brinjal seeds were sown and watered regularly.

2.3 Screening of brinjal genotypes against *Sclerotinia sclerotiorum* causing white rot disease of brinjal

Fifteen genotypes of brinjal were collected from the Department of Vegetable Science, Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur, Bihar INDIA. The genotypes were screened for identifying source of resistance against *Sclerotinia* rot of brinjal and to assess the *Sclerotinia* rot intensity in in-vivo conditions.

Seeds of different brinjal varieties were sown in the plastic glasses with the sterilized potting mixture prepared as mentioned above with soil and vermicompost in 1:1 ratio. 50 days old plants were selected and cut stem method was employed for inoculating the pathogen as given Kull et al., 2003.

Mycelial plugs of 5 mm² were cut from the actively growing margins of the culture and they are used for inoculating the plants. 50 days old plants were given a cut horizontally using a sterile razor blade 0.5 cm above the node and single mycelial plug was placed so that the pathogen is in contact with the plant. The cut wound is wrapped with a small piece of moist cotton with the help of the cello tape so as to provide optimum conditions for the growth of pathogen.

Table 1: Experiment layout

Design	Completely Randomized Design (CRD)
No. of Replication	3 (Each replication consisting of 4 plants)
No. of Genotypes	15

Location	BAU, Sabour, Department of Plant Pathology
Age of plant at the time of inoculation	50 days

The different genotypes screened against *Sclerotinia sclerotiorum* of brinjal are given below (Table:2)

Table 2: List of Brinjal Genotypes under investigation for screening against *Sclerotinia sclerotiorum*

S.No	Name of Genotypes
1.	Swarna Mani
2.	Haritha
3.	Mukta Keshi
4.	Pant Samrat
5.	IIHR - 563
6.	BRBL - 01
7.	BRBL – 02
8.	BRBL – 04
9.	BRBL – 07
10.	BRBL – 10
11.	Sel 91-2-1
12.	Sel 91-2
13.	Rajendra Brinjal -02 (RB- 02)
14.	71-19
15.	PB-67

The plants were observed for a period of 14 days to identify the symptoms produced by the pathogen. The diseased and resistance genotypes were differentiated after the appearance of the symptoms. Lesion length (cm) on stem was considered as a parameter to assess the *Sclerotinia* rot intensity, by using the rating (0-4) scale given by Lesovoi et al., 1987; Sansford, 1995; Azad et al., (Table: 3)

Per cent disease index (PDI) was calculated by using the formula as given by McKinney, 1923.

$$PDI = \left(\frac{\text{Sum of all disease ratings}}{\text{Total no. of plants observed} \times \text{Maximum disease rating}} \right) \times 100$$

Table 3: Disease rating scale for *Sclerotinia* blight of brinjal (Lesovoi et al., 1987; Sansford, 1995)

Lesion Length in cm.	Rating to be given	Disease reaction
No visible lesion	0	Resistant
0.1-2 cm	1	Moderately resistant
2.1-4 cm	2	Moderately Susceptible
4.1-6 cm	3	Susceptible
> 6 cm	4	Highly susceptible

3. RESULTS

3.1 Growth and colony morphology

After obtaining the pure culture by the hyphal tip method the mycelial bits of 5 mm² were cut and placed on the PDA, white mycelial growth of the pathogen was noticed after 48 hours of inoculation. The entire plate was filled with the white mycelial growth within 4-5 days after inoculation 25 °C ±1°C. Small shiny protusions with water droplets appeared 6 days after incubation, from which sclerotia began to form. Within a period of 5-8 days hard, black sclerotia were formed at the periphery of the Petri plates which were semi-spherical to round or irregular in shape measuring 5-7 mm X 4-6 mm in size. These results were in par with that obtained by Goswami et al., (2012) where the pathogen produced creamy white to light brown colony on PDA and mycelial growth was uniform and sparse in the initial stage which later became fluffy and compact with irregular margin confirming the identity of the pathogen. Brown to black semi spherical sclerotia measuring 4.35 × 3.25 mm were formed at the periphery of the petriplates on PDA that is typical characteristics of the pathogen.

3.2 Pathogenicity test

The collected isolate was tested for its pathogenicity, by inoculating the brinjal fruit with the mycelium. Upon inoculation initially water-soaked lesions appeared on the fruit which later enlarged and become elliptical in shape. Sclerotia were also formed on the lesions 12 days after inoculation. These symptoms were similar to the symptoms which appeared in the field and the symptoms produced on the fruit were in accordance to the symptoms described by Jagger (1920), Gupta et al., (2015) and Prasad et al., (2017).

3.3 Screening of brinjal genotypes against *Sclerotinia sclerotiorum* causing white rot disease of brinjal

Fifteen genotypes of brinjal collected from Department of Vegetable Science, Bihar Agricultural College, Bihar Agricultural University, Sabour were screened for their reaction to *sclerotinia* blight under in-vivo conditions for grouping the genotypes into various categories of resistant and susceptible on the basis of the lesion length on the stem using cut stem method

(Plate:1) The genotypes are screened using the 0-4 scale given by (Lesovoi et al., 1987; Sansford, 1995) and the results taken after 14 days of artificial inoculation are summarized in the data table below (Table: 4, Plate: 2.1 and 2.2).

Table4: Percent disease index and Average lesion length in brinjal genotypes against reaction to Sclerotinia rot under in-vitro assessment

S.No.	Genotype	Avg lesion length on stem (cm)*	Percent disease index
1.	Swarna Mani	2.97	44.44 (41.81)
2.	Haritha	1.17	19.44 (26.16)
3.	Mukta Keshi	6.53	83.33 (65.09)
4.	Pant Samrat	1.34	24.31 (29.53)
5.	IIHR – 563	3.14	52.08 (46.19)
6.	BRBL – 01	1.2	25.69 (30.45)
7.	BRBL – 02	7.32	74.31 (59.54)
8.	BRBL – 04	5.9	78.47 (62.35)
9.	BRBL – 07	1.04	19.44 (26.16)
10.	BRBL – 10	4.72	72.92 (58.64)
11.	Sel 91-2-1	5.93	85.42 (67.55)
12.	Sel 91-2	0.37	8.33 (16.77)
13.	Rajendra Brinjal-02(RB-02)	1.78	30.56 (33.55)
14.	71-19	4.86	74.31 (59.54)
15.	PB-67	2.17	37.50 (37.76)
	SEm±	0.82	10.21
	CD (0.01)	2.379	29.62

* Average of three replications (Figures given in parenthesis are angular transformed values)

Disease index (PDI) was calculated using the formula as given by McKinney, 1923. PDI values range from 85.42 % to 8.33%. The highest PDI was recorded in Sel 91-2-1 (85.42 %) followed by Mukta Keshi (83.33%) and BRBL – 04 (78.44%). Lowest PDI was recorded in Sel 91-2 (8.33%) followed by Haritha (19.44%) which was in par with BRBL - 07(19.44%) and Pant Samrat (24.31%) (Fig: 1). The maximum average lesion length was in genotype BRBL – 02 (7.32 cm), followed by Mukta Keshi (6.53 cm) and Sel 91-2-1 (5.93cm). The minimum average lesion length was found in genotype Sel 91-2 (0.37 cm) (Fig:2)

Out of fifteen genotypes, six genotypes viz., Haritha, Pant Samrat, BRBL – 01, BRBL – 07, Sel 91-2, Rajendra Brinjal -02 (RB-02) were found Moderately Resistant, three genotypes viz., Swarna Mani, IIHR-563, PB-67 were found Moderately susceptible, four genotypes viz., BRBL – 04, BRBL – 10, Sel 91-2-1, 71-19 were found susceptible and two genotypes viz., Mukta Keshi, BRBL-02 were found highly susceptible to the sclerotinia rot attack. None of the genotypes were found resistant to the sclerotinia rot (Table: 5).

Table: 5 Disease reaction and grade of brinjal genotypes against Sclerotinia rot

S.No.	Lesion Length (cm.)*	Rating to be given	Disease reaction	No. of the genotypes	Name of the genotypes
1.	No visible lesion	0	Resistant	0	Nil

2.	0.1-2 cm	1	Moderately resistant	6	Haritha, Pant Samrat, BRBL – 01, BRBL – 07, Sel 91-2, Rajendra Brinjal- 02 (RB-02)
3.	2.1-4 cm	2	Moderately Susceptible	3	Swarna Mani, IIHR-563, PB-67
4.	4.1-6 cm	3	Susceptible	4	BRBL – 04, BRBL – 10, Sel 91-2-1, 71-19
5.	> 6 cm	4	Highly Susceptible	2	Mukta Keshi, BRBL-02

* Average of three replications

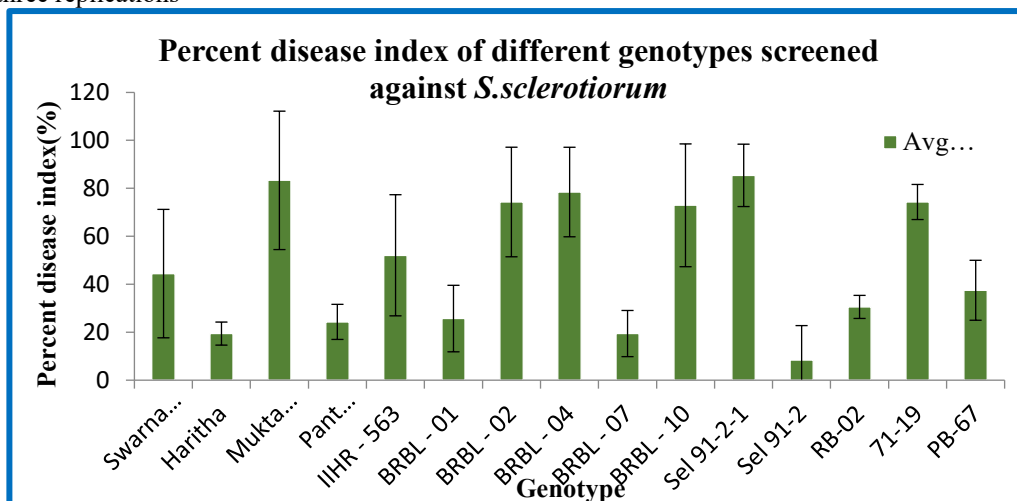


Fig 1: Percent disease index of different genotypes screened against *S.sclerotiorum* after 14 days of inoculation

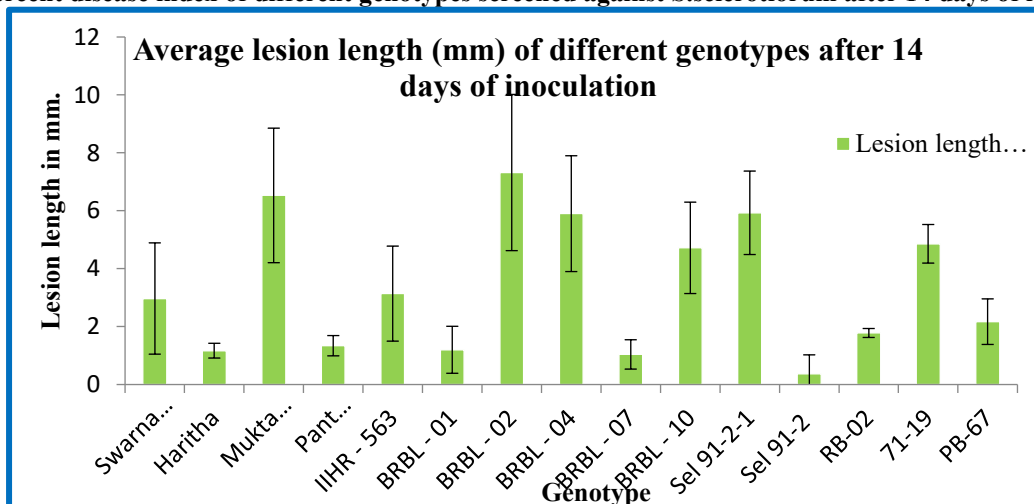


Fig 2: Average lesion length on stem (mm) of different genotypes 14 days after inoculation with *S. sclerotiorum*

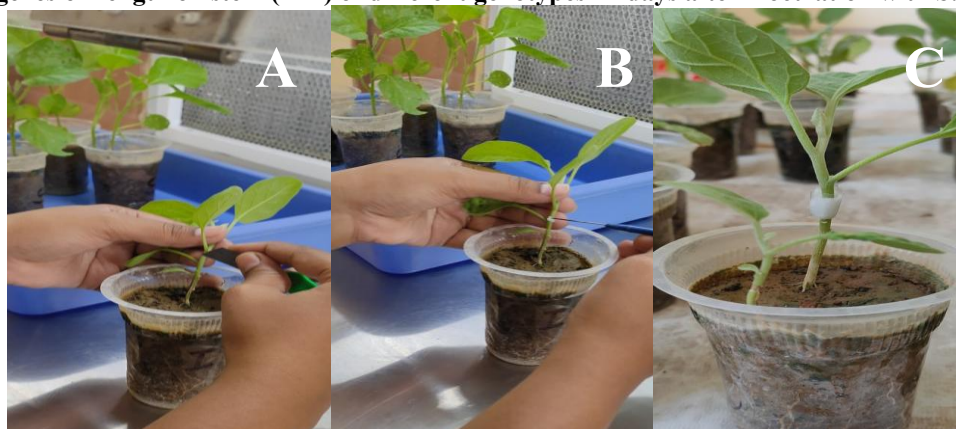


Plate 1: Cut stem method for inoculation of *S. sclerotiorum* on brinjal plants

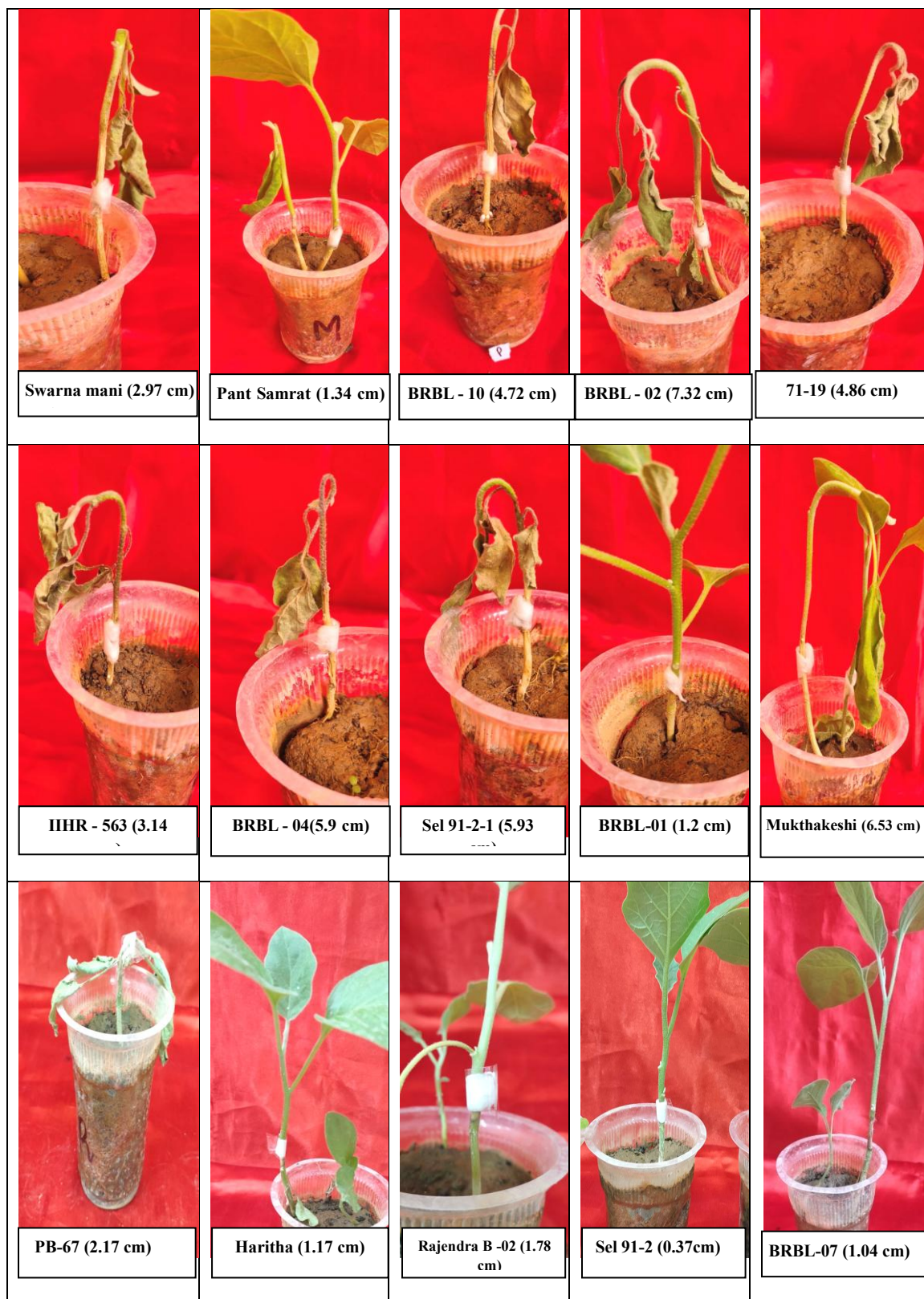


Plate 2.1: Average lesion length among different genotypes 14 days after inoculation with *S. sclerotiorum* brinjal plants

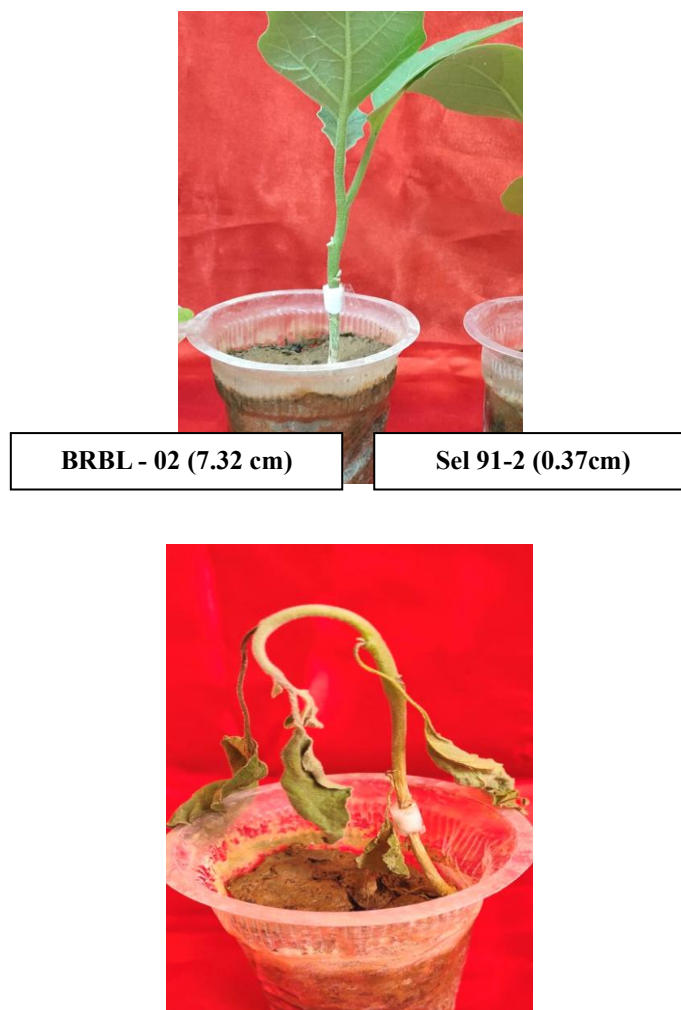


Plate 2.2: Difference between genotypes showing maximum average lesion on BRBL - 02 (7.32 cm) and minimum average lesion on Sel 91-2 (0.37cm) 14 days after inoculation

Similar screening method was followed by different workers for the inoculation of the pathogen (Kull et al., 2003; Singh et al., 2022). Kull et al., (2003) showed that cut stem-inoculated plants showed symptoms after 3 DAI and concluded that cut stem inoculation method was statistically better than the detached leaf and cotyledon methods for inoculating the pathogen. Singh et al., (2022) obtained similar results in brinjal varieties against sclerotinia blight under natural field condition following 0-4 rating scale. They reported that the disease range from 0 % in BRLVAR-3, BRRVAR-1, BRRVAR-15, to 33.33 % in BRLHYB-5 and BRLHYB-7. Thirty eight germplasms were found moderately resistance, 4 germplasms found moderately susceptible and none of germplasms found susceptible to highly susceptible.

4. DISCUSSIONS

4.1 Screening of brinjal genotypes against *Sclerotinia sclerotiorum* causing white rot disease of brinjal.

Fifteen genotypes of brinjal were screened for their reaction to sclerotinia rot under in-vivo conditions, taking into account the length of the lesion on the stem and by using the 0-4 rating scale given by Lesovoi et al., 1987 and Sansford, 1995.

The Percent disease index values range from 85.42 % to 8.33%. The highest PDI was recorded in the genotype Sel 91-2-1 (85.42 %) and lowest PDI was recorded in Sel 91-2 (8.33%). The maximum lesion length was found in the genotype BRBL – 02 (7.32 cm) and minimum lesion length was found in genotype Sel 91-2 (0.37 cm). Out of 15 genotypes, six genotypes viz., Haritha, Pant Samrat, BRBL – 01, BRBL – 07, Sel 91-2, Rajendra brinjal-02 (RB-02) were found Moderately Resistant, 3 genotypes viz., Swarna Mani, IIHR-563, PB-67 were found Moderately susceptible, 4 genotypes viz., BRBL – 04, BRBL – 10, Sel 91-2-1, 71-19 were found susceptible and 2 genotypes viz., Mukta Keshi, BRBL-02 were found highly susceptible to the sclerotinia rot attack. None of the genotypes were found resistant to the sclerotinia rot incidence.

Similar screening method was followed by different workers for the inoculation of the pathogen (Kull et al., 2003; Singh et al., 2022; Choudhary et al., 2021; Kumar et al., 2015). Kull et al., (2003) showed that cut stem-inoculated plants showed symptoms after 3 DAI and concluded that cut stem inoculation method was statistically better than the detached leaf and cotyledon methods for inoculating the pathogen. Singh et al., (2022) obtained similar results in brinjal varieties against sclerotinia blight under natural field condition following 0-4 rating scale. They reported that the disease range from 0 % in BRLVAR-3, BRRVAR-1, BRRVAR-15, to 33.33 % in BRLHYB-5 and BRLHYB-7. Thirty eight germplasms were found moderately resistance, 4 germplasms found moderately susceptible and none of germplasms found susceptible to highly susceptible. Choudhary et al., (2021) evaluated ten varieties of rapeseed – mustard against

Sclerotinia rot disease using the cut stem inoculation method and reported that DRMR-72 was found resistant, 4 varieties DRMR-2035, DRMR-32, DRMR-73, and DRMR-40 were found moderately resistant, 4 varieties RMWR-09-5, DRMR-7, NRCYS-5-2 and NPC 16 showed susceptible reaction and only one variety DRMR-261 was found highly susceptible to the disease. Kumar et al., (2015) evaluated fifty nine genotypes of rapeseed-mustard against *S. sclerotiorum* by inoculating plants on third internode at the flowering stage. The degree of resistance or susceptibility towards the pathogen in different lines was studied by using 0-4 scale. 10 genotypes were found moderately susceptible, 46 genotypes showed susceptible reaction and three genotypes viz., Neelam, BSH-1 and Rohini were found highly susceptible

4. CONCLUSION

Fifteen genotypes of brinjal were screened for their reaction to sclerotinia blight under *in-vivo* conditions, taking into account the length of the lesion produced on the stem and by using the 0-4 rating scale given by Lesovoi et al., 1987; Sansford, 1995. The Percent disease index (%) values range from 85.42 % to 8.33%. The highest PDI (%) was recorded in the genotype Sel 91-2-1(85.42%) and lowest PDI (%) was recorded in Sel 91-2 (8.33%). The maximum average lesion length was found in the genotype BRBL – 02 (7.32 cm) and minimum average lesion length was found in genotype Sel 91-2 (0.37 cm). Out of fifteen genotypes, six genotypes viz., Haritha, Pant Samrat, BRBL – 01, BRBL – 07, Sel 91-2, Rajendra Brinjal - 02 (RB-02) showed moderately resistant reaction, three genotypes viz., Swarna Mani, IIHR-563, PB-67 were found moderately susceptible, four genotypes viz., BRBL – 04, BRBL – 10, Sel 91-2-1, 71-19 were susceptible and two genotypes viz., Mukta Keshi, BRBL-02 were highly susceptible to the sclerotinia rot attack. None of the genotypes were found resistant to the sclerotinia rot incidence. So, the genotypes having resistance against the pathogen can be further used in the breeding programmes for controlling the pathogen.

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