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Detection of Resistant Sources of Soybean against *Sclerotium rolfsii* in Assam condition

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ABSTRACT: Among the different production constraints in Soybean, diseases are most serious. These diseases are caused by various pathogens, out of which fungi causes greatest threat in crop production and it reduces the yield of the crop to a greater extent. A study was conducted during *kharif* (June–Nov.) 2018 and 2019 under net house condition of the Department of Plant Pathology, Assam Agricultural University Jorhat, Assam, India. It aimed to compare the performance of forty genotypes received from AICRP (All India Coordinated Research Project) on Soybean. Diseased soybean seedlings were used as the source of pathogen and it was collected from ICR farm of Assam Agricultural University. Disease reaction of the isolate of *Sclerotium rolfsii* was tested by blotter paper technique. Percent disease index (PDI) was evaluated in the forty genotypes. It ranged between 70-98. Based on the score HIMSO1688, PS1347, BRAGG, JS335 are found to show moderate resistance against collar rot. 28 genotypes namely PS1637, JS21-71, MACS1566, PS24, RSC11-17, MAUS734, Dsb33, NRC138, JS2171, PS1637, AUKS176, DS3110, MACS1620, MAUS732, KS113, SL958, NRC148, RSC11-15, RVS2011-10, CAUMS1, RVSM201135, VLS97, TS59, RVS2007-4, KDS1073, NRCSL2, KDS1009, BAUS100 showed high susceptibility to the pathogen and 8 genotypes DS3109, NRC146, SL1191, GJS3, NRC139, SL1171, HIMSO1689, JS9305 were found to be susceptible with high PDI. These four moderately resistant genotypes can thus serve as materials for future breeding activities.

Keywords: Soybean, *Sclerotium rolfsii*, collar rot, resistant, Assam.

INTRODUCTION

Soybean is known for its economical importance as it plays a vital role in adjusting the protein requirement in our diet (Mondal and Wahhab 2001). The crop is well known for its nutrient composition thus overcoming problems of food and nutritional insecurity of developing countries (Sharma *et al.*, 2016). It improves soil fertility and reduces the required rate of nitrogen fertilizer (Rahman *et al.*, 2020). The production of the crop is constantly challenged by various living and lifeless factors. Therefore, solving these issues becomes necessary to ensure production and economic profitability (Bandara *et al.*, 2020). The increase in temperature during the recent decade is excessive and it creates a risk factor for the yield of Soybean crops (Novikova *et al*., 2020). Due to vigorous climate change, the pathogen is more aggressive and adaptable to the environment (Ghatak and Ansar 2017; Savary *et al.*, 2010). Beforehand prediction of pathogenicity is very important to decrease the disease spread in plants (Khalili *et al*., 2020).

Among all these diseases, collar rot is a major threat which causes a yield loss of 10–25%, but under excessive infestation, yield loss may range from 50–80% (Patil and Rane 1982). Collar rot can occur in both early and mature plants (Rahman *et al.*, 2020). *S. rolfsii*'srisk increases in areas where sudden rainfall increases moisture of the soil for long duration along with spike in temperature (Tarafdar *et al*., 2018). It is known to infect all plant tissues (Mahadevakumar *et al.*, 2015). It is a soil borne fungiwith worldwide importance (Mondal *et al.*, 2020) .Its form of survivalis mycelium in plant parts and sclerotia in the soil (Tarafdar *et al*., 2018) (Fig. 6-14). Sclerotia are whitish in immature stageand with maturity become reddish to dark brown, with size of 1-3mm in diameter (Cer and Morca, 2020). Sclerotia formation at the end of the Sclerotium sp. Disease life cycle aids in the survival of the fungus on dead plant material in the soil; therefore sclerotia serve as the primary inocula for the initial infection of host plant (Abd Allah *et al.*, 2013). It is a polyphagous, omnivorous, ubiquitous and virulent fungus in areas where rainy season is coupled with increase in temperature (Naresh *et al.*, 2017). It has frequently been reported to cause root diseases in at least 500 species of dicotyledonous and monocotyledonous plants, which represent 100 families (Ciancio and Mukherji 2007). The incidence decreases with the age of the crop (Akram *et al.*, 2016). Diversity in virulence is the result of morphological and phenotypic variance and it is important for the

resistance breeding programs (Paparu *et al.,* 2020). The disease control measure include mainly cultural methods (Damicone and Jackson, 1994). Control efforts are less successful due to large host range, excessive growth and ability to survive in soil for several years (Punja, 1985). Genetic resistance is the most economic control measure for this pathogen (Babariya and Nath 2021). Searching for resistant sources require easy disease evaluation techniques (Bowen and Schapaugh 1989). With this objective in background, the present research was aimed to screen soybean genotypes against collar rot resistance.

MATERIALS AND METHODS

The Study was conducted during *kharif* (June–Nov) 2018 and 2019 at the Department of Plant Pathology, Assam Agricultural University Jorhat, Assam, India under proper net house condition

A. Collection of fungal isolate

Diseased plants exhibiting characteristic symptoms of collar rot were selected and isolation was done at Dept of Plant Pathology, Assam Agricultural University, Jorhat (26°45′0.00″N latitude and 94°13′12.00″E longitude) on 18th April 2018. Storage of the samples was done in refrigerated condition (4°C) for 1–2 days. For isolation parts like infected stalk, seed, root or collar region was used.

B. Fungus isolation

In each petriplate, Potato dextrose agar medium (15-20ml), supplemented with streptomycin sulphate was poured. Treatment with 0.1% NaOCl for 30 seconds followed by washing in sterilized distilled water (SDW) for three times was done to remove NaOCl solution from the infected samples (~5 mm size). These infected samples was placed in Petri plate containing PDA (Fig. 4 and 5). Incubation was done at 27±1°C for *S. rolfsii*. Sub-culturing of the pathogen was done on PDA slants after 2-3 days and it was kept to incubate for nearly a week at above stated temperatures (Fig. 3). Storage of these purified slants were done under refrigerated condition at 4°C and used whenever required.

C. Collection of germplasm

Seeds of forty different germplasm of Soybean was collected from AICRP on soybean and seedlings were raised in sterilized soil inside the net house of Department of Plant Pathology, Assam Agricultural University Jorhat in 2018 and 2019 (Fig. 7).

D. Infection of Sclerotium rolfsii

Soil infection method (Sahni *et al.*,2008) and blotter paper technique (Nene *et al*.,1981) was used to test the reaction of the sample isolates of *Sclerotium rolfsii.* The sterilized sandy–loam soil mixed with 30 days old suspension culture of *S. rolfsii* @15 sporeshole^{-1} of the seed trays (Fig. 2). Surface sterilization of the Seeds of soybean was done with 1% NaOCl for 1 min followed by rinsing with sterile water. Seeds were sown in the sterilized seed trays. Regular moistening of the trays was done. Proper observation was done of the mortality rates. A control was also set for the experiment.

In the blotter paper technique, seedlings were inoculated with suspension of mycelial mat of *Sclerotium rolfsii*. Inoculation was done by dipping in the suspension for 5 mins and then using the wet blotter paper for careful wrapping (Fig. 8-11). The control was treated with sterile water and were incubated at 35°C with 12-hour photoperiod. The assessment was made after 8 days of inoculation.

E. Experimental design, disease assessment and data analysis

Calculation of the mean values of pathogenicity and frequencies of reactions of resistance/ susceptibility was done. Complete randomized design (CRD) was used and analysis of variance was done and were separated by the critical difference at P=0.05, where the effect of variation among the isolates in disease development were identified. Disease rating scales of Le *et al*. (2012) (1–5) was used for identifying the disease severity.

1 = no disease symptom;
2 = disease symptoms without visible fungal outgrowth;
3 = disease symptoms with visible fungal outgrowth;
4 = partial wilting of plant;
5 = complete wilting and death.

The per cent disease index was calculated according to formula

$$\text{PDI (\%)} = \frac{\text{Sum of disease ratings} \times 100}{\text{Total no. of ratings} \times \text{Max disease grade}} \times 100$$

RESULTS AND DISCUSSION:

A. Pathogenicity of Sclerotium rolfsii on different genotypes of Soybean

To identify resistant lines against plant disease germplasm screening is an important aspect. Elite lines with high degree of resistance can be further used in breeding programs. With this view, disease causing capacity of the isolate of the pathogen was tested in lab condition by artificial inoculation on forty genotypes of Soybean. Disease rating for each genotype was done using the 1–5 disease rating scale of Le *et al.* (2012) for *S. rolfsii*. Standard procedure of Sahni *et al.* (2008) was followed for in planta screening. Observation showed that the artificial infection produced considerable infection on roots (Fig. 12-13). Disease index of *S. rolfsii* ranged from71.4–100.0% on the susceptible genotype of tomato as reported by Curtis *et al*. (2010). Similar study was also done on common bean where 1-6 disease rating scale was used by Paparu *et al.* (2020).

In our study, the disease reaction of the pathogen varied based on the genotype. Kator *et al*. (2015) observed variation in the degree of virulence of a single isolate of *Sclerotium rolfsii* on three different tomato genotypes. The PDI values ranged from high to low. Among all the genotypes, 28 genotypes showed high susceptibility to the pathogen, 8 genotypes susceptibility and 4 genotypes were found to be moderately resistant. The 28 genotypes showing high PDI and maximum grade of 5 (Table 1) were PS1637, RSC11-17, MAUS734, Dsb33, NRC138, MACS1566, JS2171, PS1637,

AUKS176, PS24, DS3110, MACS1620, MAUS732, KS113, SL958, NRC148, RSC11-15, RVS2011-10, CAUMS1, RVSM201135, VLS97, TS59, JS21-71, RVS2007-4, KDS1073, NRCSL2, KDS1009, BAUS100. The 8 genotypes showing high PDI and low maximum grade of 4 were DS3109, NRC146, SL1191, GJS3, NRC139, SL1171, HIMSO1689, JS9305.The 4 genotypes showing comparatively lower values of PDI and lowest value of maximum grade of 3 were HIMSO1688, PS1347, BRAGG, JS335. The genotypes were mostly susceptible to the selected isolate of the pathogen and only a few genotypes showed moderate resistance. Similar results were reported by Farooq *et al*. (2011); Eslami *et al*. (2015); Paparu *et al*. (2020).

Table 1: Scoring of each plant and max grade and PDI of each genotype.

Genotypes	1	2	3	4	5	6	7	8	9	10	sum	max grade	PDI
DS3109	2	4	2	1	4	5	1	1	4	4	28	4	70
NRC146	4	5	4	1	4	4	1	1	1	4	29	4	72.5
PS1637	5	3	5	4	4	1	4	5	3	5	39	5	78
JS21-71	5	5	1	5	3	5	1	4	5	5	39	5	78
MACS1566	3	5	5	3	5	4	5	5	4	4	43	5	86
SL1191	4	2	4	2	4	4	1	4	1	4	30	4	75
HIMSO1688	1	3	1	3	3	1	2	2	2	3	21	3	70
PS24	5	5	3	5	4	5	1	5	5	5	43	5	86
RSC11-17	5	5	5	5	5	3	5	1	5	5	44	5	88
MAUS734	5	1	5	3	5	5	5	5	5	5	44	5	88
Dsb33	5	4	5	5	3	5	1	5	1	5	39	5	76
NRC138	5	5	5	4	3	5	2	2	3	4	38	5	76
JS21-72	5	5	5	5	3	5	4	5	4	5	46	5	86
PS1637	5	5	5	5	5	5	4	5	5	5	49	5	98
AUKS176	5	5	5	4	4	3	2	4	1	5	38	5	76
PS1347	1	1	2	3	1	3	1	3	3	3	21	3	70
GJS3	4	4	5	4	1	1	1	4	1	4	29	4	72.5
NRC139	4	4	1	4	3	1	4	2	4	1	28	4	70
DS3110	3	3	3	2	5	5	4	5	4	5	39	5	78
SL1171	4	4	1	4	5	1	4	2	4	1	30	4	75
MACS1620	5	3	5	4	3	5	2	5	3	4	39	5	78
MAUS732	5	5	1	5	3	5	4	5	5	5	43	5	86
KS113	4	5	5	5	5	5	5	5	5	5	49	5	98
SL958	5	5	1	5	3	5	4	5	5	5	43	5	86
NRC148	5	3	3	5	5	4	4	5	3	1	38	5	76
RSC11-15	5	3	5	5	5	3	3	4	4	4	41	5	82
RVS2011-10	5	3	5	4	5	3	5	2	4	4	40	5	80
HIMSO1689	4	5	4	1	4	4	1	1	1	4	29	4	72.5
CAUMS1	5	5	5	4	5	5	5	5	5	5	49	5	98
RVSM2011-35	5	5	5	3	5	2	2	5	3	5	40	5	80
VLS97	5	5	5	5	1	5	4	5	3	5	43	5	86
TS59	5	5	5	1	1	3	4	5	1	5	35	5	70
RVS2007-4	3	3	5	4	5	5	4	5	5	5	44	5	88
KDS1073	3	1	5	4	5	4	5	5	4	5	41	5	82
NRCSL2	5	5	5	5	5	5	5	5	5	4	49	5	98
KDS1009	3	5	5	5	2	4	5	5	3	3	40	5	80
BAUS100	5	5	5	4	1	4	4	5	3	5	41	5	82
BRAGG	1	3	2	3	3	1	2	2	1	3	21	3	70
JS9305	4	4	5	4	4	1	4	1	1	1	29	4	72.5
JS335	1	3	3	3	3	1	3	2	1	1	21	3	70

Fig. 1. Preparation of suspension culture of S*clerotium rolfsii.*.

Fig. 2. Pure culture of *Sclerotium rolfsii* on PDA slant.

Fig. 3. Front view of PDA culture plate.

Fig. 4. Reverse view of PDA culture plate.

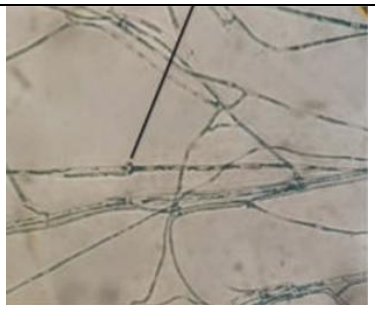

Fig. 5. Microscopic observation of *Sclerotium rolfsii*.

Fig. 6. Initiation of seedlings.

Fig. 7. Eight days old seedlings growing on sterilized soil.

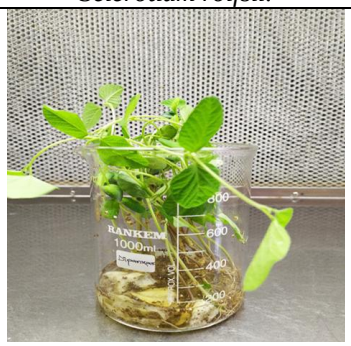

Fig. 8. Dipping of roots in suspension culture in Laminar airflow.

Fig. 9. Seedlings of cultivar were placed on blotter paper.

Fig. 10. Blotter papers were folded.

Fig. 11. Roots of soybean seedlings in which *Sclerotium rolfsii* culture was applied in laminar airflow.

Fig. 12. Sclerotia formation in seed trays in soil infection method.

Fig. 13. Collar rot infected plant.

CONCLUSIONS

HIMSO1688, PS1347, BRAGG, JS335 showed comparatively lower PDI for the pathogen *Sclerotium rolfsii* and thus have some potentiality to develop resistance against it. These genotypes has the potentiality for the control of the collar rot disease in Soybean field and can promote Soybean production in areas like Assam. Breeding works can be carried out to improve these varieties in terms of their yield potential and disease resistance by introducing useful genes which will thereby improve their potentiality to use them in disease management systems of Soybean

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