

Original Research Article

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Isolation of Single Spore and Identification of Resistance against Soybean Anthracnose

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ABSTRACT

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The genus *Colletotrichum* comprises ~600 species attacking over 3,200 species of monocot and dicot plants. *Colletotrichum* species are fungal pathogens that devastate crop plants worldwide. One hundred thirty eight soybean entries received from AICRP (All India Coordinated Research Project) on soybean, were evaluated at Raipur under natural condition during the year 2017-18 to identify sources of genetic resistant against pod blight disease incited by the fungus *Colletotrichum truncatum*. The single spore was isolated from infected pod of soybean collected from research farm at fruiting stage of the crop and maintained on PDA for further process. Out of 138 soybean entries only 10 genotypes (IVT-24, IVT-26, IVT-39, TMS-38, RSC-10-52, IVT-04, RSC-10-70, IVT-13, NRS-125, and RSC-10-71) were observed as absolutely resistant response.

Introduction

Soybean (*Glycine max* L. Merrill) is a species of legume native to East Asia. The plant is classed as an oilseed rather than a pulse. Soybean is one of the world's most important sources of oil and protein. It has highest protein content among leguminous crops belonging to the family leguminaceae. Besides its economic value, it is highly nutritious food to human being as its 40 per cent protein content, 20 per cent carbohydrates and 23 per cent oil, holds a great promise in meeting most of the need in human diet (Chandel, 2002). Soybean contains valuable amino acids. In addition, it

contains a good amount of minerals (Ca, Mg, P and Fe), salts and vitamins (Thiamine and Riboflavin and D), hence called 'poor man's meat'.

In Chhattisgarh, research work on important aspects of the anthracnose disease of soybean. Information on crop loss assessment, epidemiological studies and integrated disease management strategies are lacking. A number of management approaches viz., use of tolerant varieties, application of fungicides, cultural practices and combination of approaches leading to integrated management of the disease have been evaluated. It is necessary to know the severity of the disease

and factors associated with disease. Host plant resistance is considered as most practical, feasible and an economical method of plant disease management. Generally in soybean, the disease is severe in more prevalent at all stages of development particularly from bloom to pod fill. Raddish brown spot appears on the pods and later they turn black. Fruiting bodies (Acervulli) on infected pods resemble small pin cushions surrounded by minute blackish brown setae (Han *et al.*, 1995). The disease causes considerable damage by reducing plant stand, seed quality, seed germination and yield (Rodriguez and sinclair 1978).

The only practicable and cost-effective control for such a devastating seed-borne pathogen is selection of cultivars. Therefore, the present study was conducted to screen the soybean entries for the identification of resistant sources.

Materials and Methods

Single spore isolation and maintenance of culture

Colletotrichum spp. will be isolated from the different naturally infected hosts collected from Raipur (C.G.). Single spore which were spread on 4% water agar, after germination will be directly picked using capillary needle and transferred on PDA slants. Purified isolates will be maintained on water agar slants at 26±1°C in BOD incubator for further use.

Screening of soybean entries under natural conditions

A field experiment was conducted to know the resistance levels in soybean entries during 2017-18. All the agronomic practices were followed except plant protection measures. The severity of pod blight was recorded at

beginning of the maturity using a disease rating scale 0 to 9 given by Mayee and Datar (1986). Thereafter per cent disease index (PDI) was calculated.

Per cent disease index =

$$\frac{\text{Sum of all disease rating}}{\text{Total no. of rating} \times \text{Maximum disease grade}} \times 100$$

On the basis of PDI calculated the germplasm was categorized as mentioned below.

Results and Discussion

Procedure for isolation of single spore progenies

Observing an acervuli, under the Nikon stereobinocular microscope spores were picked using a fine pointed capillary needle. Spores were then placed on the 4% water agar. Using a bent fine capillary glass needle the spores were then separated as far as possible from each other. The plates were then incubated at 27° C for 4-6 hr. to allow the spores to germinate. The germ tube was allowed to proliferate and penetrate the agar.

Observing an isolated germinating spore under the Nikon stereobinocular microscope, agar surrounding the germinating was cut using a sterilized fine pointed inoculation needle (pony No. 12). The agar block containing a single germinating spore was then aseptically transferred to the Potato Agar slants and incubated at 27° C. Efficiency of isolation of single spore was almost 100% by this method. By this procedure in a single attempt as many as 60-80 spore could be obtained. In 3-4 days at 27 ° C growth appeared on Potato dextrose agar slants. The sequences of steps involved in single spore isolation are depicted in fig.1. Genetic analysis, tagging, mapping and isolating the

gene are specialized approaches and essentially require isolating microorganisms in pure form or genetically pure form. It is possible to isolate different microorganisms by enrichment technique but require an inordinate amount of time, labour, and money. Medium selection, diluents, incubation conditions, and sample handling determine the number and types of microorganisms from plant, soil, and water (Hansen, 1926; Hildebrand, 1938; Collins *et al.*, 1977; Choi *et al.*, 1999; Gupta *et al.*, 2011). Therefore, what is isolated from the defined ecosystem or for a defined specialized approach is a reflection of the isolation procedure and conditions set by researcher. The conventional method of isolating well separated spores streaked on the agar medium

under a stereoscopic microscope was used by Tuite (1969). Kumar *et al.*, (2007) reported a technique for monoconidial culture of the most aggressive isolate in a given population of *Bipolaris sorokiniana*, to facilitate the evaluation of spot blotch resistance in wheat and barley.

The efficiency of single spore isolation representing a true mitotic event by the proposed method is almost cent percent and justifies the generation of genetically pure population (>90 progenies) for specialized approaches in a single attempt. The increased size of the micro-spore due to well-proliferated germ-tube is the basis of this technique (Table 1 and 2).

Table.1 Genotype categorization based on rating

S.No	Grade	Per cent disease incidence(PDI)	Category Reactions
1	0	0-5	Highly resistant
2	1	6-10	Resistant
3	3	11-25	Moderately resistant
4	5	26-50	Moderately susceptible
5	7	51-75	Susceptible
6	9	76-100	Highly susceptible

Table.2 Reaction of soybean genotypes to *C. truncatum* under natural conditions during 2017-18

S.No	Score	Disease Reaction	Genotypes
1	0	Highly Resistant	NIL
2	1	Resistant	IVT-04,RSC-10-52, TMAS-38, IVT-39, IVT-26, IVT-24, RSC-10-71, NRS-125, IVT-13, RSC-10-70
3	3	Moderately resistant	RSC-10-46, NRC-7, CG-SOYA-1, JS(SH)2003-2, IVT-2, JS-20-19, IVT-37, IVT-7, IVT-5, MAVS-612, AMS-323, IVT-40, NRC-82, JS-20-39, KDS-354, IVT-19, KS-3, IVT-34, IVT-10
4	5	Moderately susceptible	KDS-344, IVT-27, IVT-33, IVT-15, IVT-38, IVT-8, IVT-30, IVT-17, IVT-20, IVT-21, IVT-43, IVT-32, PS-1477, IVT-42, IVT-24, JS-20-34, IVT-16, IVT-35
5	7	Susceptible	IVT-25, IVT-41, IVT-28, IVT-39, IVT-1
6	9	Highly Susceptible	IVT-29, IVT-36, IVT-6, IVT-2, IVT-3, IVT-4, IVT-18, IVT-11, IVT-12, IVT-9, IVT-31, IVT-13

Fig.1 Single spore isolation technique followed

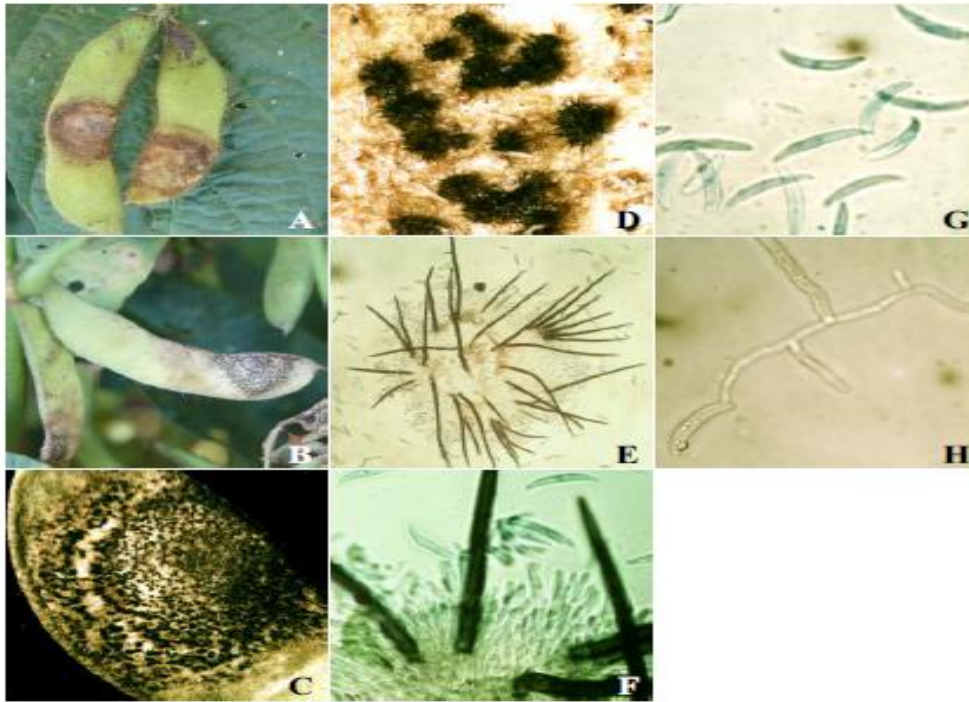


Fig.2 Resistant and susceptible phenotypes observed during screening of soybean genotype against pod blight



One can be very certain that the spore being isolated is clearly discernable and directly lifted from the source and the minimized use of water justifies the procedure to be direct, simple and rapid.

Screening of genotypes for their resistance to pod blight under natural conditions during 2017-18

Anthraco-nose of soybean was first reported from Korea in 1917, but now occurs in all soybean-growing areas. The disease can cause 16 to 100 per cent losses in yield depending upon the cultivar and environmental conditions. The infected pods either have very small, shriveled, brown, moldy seeds or produce no seed. Anthracnose of soybean can be managed by the use of resistant varieties, clean cultivation, and pathogen-free seeds. In the present investigation 138 soybean entries were evaluated for anthracnose resistance during the wet season of 2017-18. Evaluation for anthracnose resistance indicated no immune genotype, 10 genotypes (IVT-24, IVT-26, IVT-39, TMAS-38, RSC-10-52, IVT-04, RSC-10-70, IVT-13, NRS-125, RSC-10-71) were classified as resistant (Score1) and 12 as highly susceptible. Rest of the 116 entries were evaluated MR to Susceptible group. There are few studies that have evaluated anthracnose resistance in soybean. Several studies reported on the evaluation of 414 soybean germplasm accessions to *C. truncatum* (Manandhar *et al.*, 1986 a & b; Manandhar *et al.*, 1988; Hossain *et al.*, 2001). These studies found no immune genotypes; however, 10 genotypes were classified as resistant. Breeding efforts to produce anthracnose-resistant soybean cultivars are lacking despite the potential for yield losses due to this disease. In addition, there is a lack of knowledge about soybean resistance in different tissues (from roots to seed) and whether any described resistance would be effective against the several species of

Colletotrichum that cause anthracnose. The soybean genotype Mandarin was among the soybean genotypes reported to have resistance to anthracnose (Manandhar *et al.*, 1986 a & b). Ten genotypes which were classified as resistant in our present investigation will be reconfirmed following recently reported artificial inoculation technique to screen soybean for resistance to anthracnose by Yang and Hartman (1986). Several lines of evidence indicate screening of soybean genotypes for resistance to anthracnose of soybean. Gawade *et al.*, (2009) reported that out of 184 soybean genotypes, 109 genotypes were expressed moderately resistant reaction, whereas Madhusudhan (2002) reported 27 moderately resistant genotypes out of 60 screened against *C. truncatum*. Similarly seven out of 16 soybean varieties expressed resistant reaction under natural conditions (Khalko 2011; Kaushal and Paul 1991).

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