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COMPARISON OF INOCULATION METHODS FOR CHARACTERIZING PATHOGENIC AGGRESSIVENESS AGAINST SUDDEN DEATH SYNDROME IN SOYBEAN

Manoj Kumar Sahu*, K. P. Verma, Nand Kishore Pandey and Krishna Maravi

Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492 012 (C.G.), India.

Abstract

The research was conducted under greenhouse at Indira Gandhi Agricultural University, Raipur (C.G.), India during 2014 to determine inoculation methods for characterizing pathogenic aggressiveness against sudden-death syndrome in soybean [*Glycine max* (L.) Merr.]. *Fusarium solani* f. sp. glycine is the primary etiological agents of sudden-death syndrome (SDS) of soybean. Twenty isolates of F. solani were tested for pathogenic aggressiveness in soybean, using a test tube and soil inoculation method. All the isolates were showed pathogenic variability in both the methods. In test tube method, most of the isolates were caused 100% seedling mortality as comparison to soil inoculation method. The highest mortality 100% was found in only SF3 and SF7 isolates and least 20% was found in SF17 isolates in soil inoculation method.

Key words : *Glycine max, Fusarium solani,* SDS, pathogenicity.

Introduction

Soybean [*Glycine max* (L.) Merr.] is a legume of the family Fabaceae, subfamily Faboideae. The plant is classed as an oilseed rather than a pulse by the Food and Agricultural Organization (FAO). Soybean seed contains 40% protein, 20% oil, 30% carbohydrates and excellent amounts of dietary fibre, vitamins & minerals. It contains high level of amino acids such as lysine, leucine, lecithin and large amount of phosphorus. Now it is being used for manufacturing of nutritional product *i.e*., milk, curd, cheese, backed soybean, roasted soybean etc. and can be afforded by poor class people also. Soybean builds up the soil fertility by fixing large amounts of atmospheric nitrogen through root nodules. Soybean being the richest, cheapest and easiest source of best quality of protein, it has multiplicity of use as food and industrial product, which made it as a "wonder crop".

In India, it's occupies an area (*kharif*) of 108.83 lakh ha. with a production of 104.36 lakh mt and productivity of 959 kg/ha (Anonymous, 2014). The major soybean growing districts in Chhattisgarh are Kabirdham, Bemetra, Rajnandgaon, Durg, Mungeli and Raipur. In Chhattisgarh soybean is grown during kharif season and it's suffer from a number of diseases such as many fungal, bacterial and viral diseases which are responsible for low producing. Among the fungal pathogens, Fusarium wilt is very common and important disease of soybean. These pathogens cause significant loss in yield and primarily responsible for wide gap in the yield levels in farmers field (Zape *et al*., 2014). SDS foliar symptoms are thought to be induced by a low molecular weight toxin (Jin *et al*., 1996) and include mottling of leaves on the upper part of the plant, interveinal chlorosis, necrosis and defoliation (Hartman *et al*., 1999). Additional symptoms include root rot, crown rot, vascular discoloration of the stem, pod abortion and gray to red coloration on the basal stems, although the pith remains white. Because soybean germplasm exhibits various levels of resistance to F. solani, increasing resistance to *F. solani* is an important objective of soybean cultivar development. Screening for SDS resistance has been conducted under field conditions, both in natural (Rupe, 1991, 1995; Wrather *et al*., 1995) and artificially infested soil (Melgar *et al*., 1994; Scherm & Yang, 1996). Even when cultivars are screened in artificially infested soil, disease incidence is unpredictable due to the sensitivity of symptomology to environmental factors (Schuerger & Mitchell, 1993; Rupe *et al*., 1996; Farias *et al.*, 2008). Methods for assessing aggressiveness in greenhouse studies include using soil infestation by growing the pathogen on sorghum grain (Hartman *et al*.,

Author for correspondence : Email: manoj99.sahu@gmail.com

1997, 1999; Huang & Hartman, 1998; Farias Neto *et al*., 2008), culture filtrates (Jin *et al*., 1996; Li *et al*., 1999). Development of an accurate disease for screening resistance to these pathogens, in a rapid and uniform way in the greenhouse, is crucial for developing soybean cultivars with broad based resistance to the SDS pathogens. Although, various inoculation methods for testing pathogenic aggressiveness of *F. solani* isolates and evaluating soybean response to the pathogen within a greenhouse have been reported, little information is available regarding direct comparisons of the aggressiveness of *F. virguliforme* isolates, formerly named *F. solani* in soybean. Thus, the present study was initiated to assess the pathogenic aggressiveness of *F. solani* isolates on susceptible soybean cultivar, using two greenhouse inoculation methods.

Materials and Methods

Isolation, purification and identification of *Fusarium solani*

Fresh diseased roots of soybean plant samples showing wilt symptoms were collected from different location of Chhattisgarh plain area and washed properly with tap water. Infected roots and stems were cut in small pieces with the help of sterilized blade in such a way that each of them contained healthy as well as diseased tissues. These pieces were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for one minute followed by three subsequent washing in sterilized water to remove traces of mercuric chloride ($HgCl_2$). The pieces were transferred aseptically to Petridishes containing PDA. Inoculated Petridishes were kept in incubator at 25 ± 2°C and were examined at frequent intervals to see the growth of fungus developing from different pieces. Isolation was made and the isolated culture was purified by single spore isolation (Kotasthane and Agrawal, 2014). Morphological characteristics of purified isolates were compared with standard description (Seifert, 1996 and Aoki *et al*., 2005). Finally, twenty purified isolates of *F. solani* were observed pathogenic aggressiveness for further experiment.

Two methods were used for evaluate the pathogenic aggressiveness against different isolate of F. solani in susceptible soybean cultivar.

Test tube inoculation method

The above mentioned fungus was multiplied on Potato Dextrose Agar in Petriplates. Two discs of 5 mm were cut from 10 days old culture and placed in test tube, already filled with 10 ml sterilized water and well prepared of spore suspension. Three test tubes were taken as three replications. One plant of fifteen day old was placed in suspension of the fungal culture. The test varieties used in this study *i.e.* PS 1543. Tubes containing sterilized water without culture were served as check. Observations on wilt incidence were recorded after 2 days and second observation was taken after 5 days.

Soil inoculation method

The above mentioned fungus F. solani was multiplied on Potato Dextrose Agar in Petriplates. Mass inoculum of F. solani was prepared on sorghum grains. The 50 g sorghum grains were soaked in distilled water for overnight in a 250 ml Erlenmeyer flask. The floating sorghum seed and debris were removed. Then after, the grains were washed with tap water for three to five times. Excess water from grains was drained and autoclaved for 60 min at 121°C on consecutive days and allowed to cool. After cooling, 5 disc of 5 mm diameter, containing mycelial of *F. solani* on PDA were added to the flask. The flask was incubated at 25° C in BOD for fifteen day.

The plastic pots (12 × 10 cm) were taken and filled with 80% sterilized soil, which was previously treated by 4% formalin. Soil was infested by placing the test fungus colonized sorghum grain (Scandiani *et al*., 2011). The soil of pots was infested by placing of 3 gm infested sorghum grains and were distributed in a layer in each pot then covered with 2 cm layer of sterilized soil. Then after 5 soybean seeds were planted in each pot and covered with 2 cm of soil, each pot representing 1 replicate and three replications were used. Observations on wilt incidence were recorded on the basis of seedling mortality per cent after 21 days and characterized of mortality into three category *viz*. highly pathogenic (91-100%), moderately pathogenic (50.1- 90%) and least pathogenic (1-50%).

Results and Discussion

Pathogenic aggressiveness of different isolates of F. solani by test tube method

The pathogenic aggressiveness of different isolates of *F. solani* were studied by test tube method under greenhouse condition. The results are obtained and presented in table 1. After 2 days of inoculation, 100% chlorotic plants were observed in isolates SF7 and next were SF3 and SF10 (33.33%). Other isolates did not showed chlorotic symptoms.

After 5 days of inoculation, SF2, SF3, SF5, SF7, SF8, SF9, SF10, SF11, SF12, SF13, SF14, SF16, SF18, and SF20 gave 100% seedling mortality, while 66.66% and 33.33% mortality was recorded in SF1, SF4, SF6, SF17

Table 1 : Pathogenic variation of different isolates of *Fusarium solani* by test tube method.

Isolates	No. of chlorotic plant (%) *	Seedling mortality (%) *
	2 DAI	5 DAI
SF1	0.00	66.66
SF2	0.00	100.00
SF3	33.33	100.00
SF4	0.00	66.66
SF5	0.00	100.00
SF6	0.00	66.66
SF7	100.00	100.00
SF8	0.00	100.00
SF9	0.00	100.00
SF10	33.33	100.00
SF11	0.00	100.00
SF12	0.00	100.00
SF13	0.00	100.00
SF14	0.00	100.00
SF15	0.00	33.33
SF16	0.00	100.00
SF17	0.00	66.66
SF18	0.00	100.00
SF19	0.00	33.33
SF20	0.00	100.00
Control	0`00	0.00

* Mean of three replications.

and SF15, SF19 respectively. No seedling mortality was noticed in control. Out of twenty isolates, 14 isolates were highly pathogenic, 4 moderately pathogenic and 2 least pathogenic. Characterized of mortality into three category *viz*. highly pathogenic (91-100%), moderately pathogenic (50.1- 90%) and least pathogenic (1-50%). Similar type of pathogenic ability of Fusarium species had been reported by Robert and Kraft (1971).

Pathogenic aggressiveness of different isolates of *Fusarium solani* by soil infestation method

All isolates of *F. solani* were found as pathogenic and seedling mortality as compared to the control and results are presented in table 2. Each isolates was also higher produced typical SDS foliar symptoms, which included chlorosis, necrosis and seedling mortality. The symptoms developed chlorosis after 15th days of inoculation. The highest seedling mortality was observed after 21 days of inoculation (DAI). Isolates SF3 and SF7 showed 100 per cent seedling mortality followed by 86.66 % in SF12 and SF18.

The lowest seedling mortality 20% was found in

Table 2 : Pathogenic variation of different isolates of *Fusarium solani* by soil infestation method.

Isolates	Seedling mortality (%) *
SF1	27.77
SF2	80
SF3	100
SF4	25
SF5	80
SF6	21.66
SF7	100
SF8	25
SF9	53.33
SF10	80
SF11	53.33
SF12	86.66
SF13	60
SF14	26.66
SF15	46.66
SF16	46.66
SF17	20
SF18	86.66
SF19	33.33
SF20	25
Control	0

* Mean of three replications.

isolates of SF17 followed by 21.66% mortality was SF6 and 25% in SF4, SF8 and SF20. Out of the 20 isolates, where 2 isolates were highly pathogenic (91-100%), 8 were moderately pathogenic (50.1-90%) and 10 were least pathogenic (1-50%). Similar type of reports on characterizing aggressive of *F. virguliforme* and *F. tucumaniae* causing sudden death syndrome had been observed using the soil infestation technique by Scandiani *et al*. (2011).

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