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Screening of soybean germplasm against frogeye leaf spot of soybean caused by *Cercospora sojina* Hara in Himachal Pradesh

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Abstract

Frogeye leaf spot caused by *Cercospora sojina* Hara, an important disease of soybean (*Glycine max* L. Merill) is generally managed by different strategies but use of resistant varieties is the best approach. This study was conducted to find resistant source(s) in soybean against frogeye leaf spot caused by *Cercospora sojina*. Out of two hundred soybean accessions evaluated under natural epiphytotic conditions, 5 lines showed absolute resistance (AR) whereas 20 accessions were highly resistant (HR). Seventy four germplasm lines were found moderately resistant (MR) to the disease and remaining accessions were either susceptible (S) or highly susceptible (HS). Per cent Disease Index (PDI) ranged between 0.00 to 77.81 per cent. The accessions found resistant (disease reaction of 0, 1 and 2) under field conditions were further evaluated under greenhouse conditions with the diverse pathotypes. Four soybean accessions viz., Harder, JSM 285, CAT 195 and GP 465 exhibited absolute resistance against Frogeye leaf spot (FLS). Hence the identified resistance source can be further used in disease resistance breeding programme.

Key words: *Cercospora sojina*, frogeye leaf spot, epiphytotic conditions, resistance, pathotypes

Soybean being an oil seed crop also provides vegetable protein for millions of people worldwide. It is cultivated as *kharif* crop in India. This crop is vulnerable to the attack of various biotic and abiotic stresses which hinder the yield potential of soybean. Among these, frogeye leaf spot caused by *Cercospora sojina* causes yield losses to the tune of 66 per cent during epidemic years (Dogra 2015, Mittal 2001 and Mian *et al*. 1998). The severe leaf infection leads to defoliation and reduces the seed size (Akem and Dashiell 1994). In some varieties, this disease causes pod-shattering and the extent of yield losses depends upon resistance level of a variety (Tiwari and Bhatnagar 1988). Many fungicides as well as alternative management practices are available for managing the disease, however, host resistance provides long term and eco-friendly solution (Das *et al*. 2017). In this study soybean germplasm was evaluated under field and greenhouse conditions to identify the sources of resistance against different *Cercospora sojina* pathotypes.

Materials and Methods

Field screening

Two hundred genotypes of soybean were obtained from the Department of Genetics and Plant Breeding. Experiment was laid out in field conditions during *Kharif* 2020-21 and 2021-22. Two hundred soybean accessions obtained from the Department of Genetics and Plant Breeding were sown at research farm of Department of Plant Pathology, CSKHPKV, Palampur Himachal Pradesh as per standard cultural practices. Soybean lines were critically observed entire seasons for the occurrence of disease. Data on disease severity was recorded during *kharif* 2021 and 2022 on 0-9 scale (Yorinori 1981) given as follow:

Disease reaction	Disease Score/Rating	Severity Scale /Disease Severity
Absolute Resistance (AR)	0	No disease
Highly Resistance (HR)	1	1 % leaf area is covered with the small brown spots/lesions
Moderate Resistance (MR)	3	1.1-10% leaf area is covered with brown spots/lesions; no spots on stem
Moderate Susceptible (MS)	5	10.1-25% leaf area is covered with brown spots, no defoliation; little damage
Susceptible (S)	7	25.1-50% leaf area covered with circular brown spots; damage conspicuous
Highly susceptible	9	More than 50 % leaf area covered with circular brown spots

The per cent disease index (PDI) was also calculated with the following formula:

$$PDI = \frac{\text{Sum of all the ratings}}{\text{Total number of plants examined} \times \text{maximum disease score}} \times 100$$

The screened lines during 2021 and 2022 *kharif* were categorized into different resistant and susceptible categories by adopting following PDI and grade description:

PDI	Categories	Disease grade
0.0	Absolute Resistance / Immune (AR)	0
0.0-11.11	Highly Resistance (HR)	1
11.12-33.33	Moderately Resistance (MR)	3
33.34-55.55	Moderately Susceptible (MS)	5
55.56-77.77	Susceptible (S)	7
77.78-100.00	Highly Susceptible(HS)	9

Artificial screening of germplasm

Thirty isolates of frogeye leaf spot pathogen of soybean were isolated from different major soybean growing areas of Kangra, Mandi, Hamirpur, Solan and Bilaspur districts of Himachal Pradesh during cropping seasons 2019-20, 2020-21 and 2021-22. Pathogenicity was proved with each and on the basis of morphology, pathogen was identified as *C. sojina*. Pathogen identification was also confirmed on molecular basis. These isolates were studied on a set of 10 lines viz. Himso 1685, Hardee, Hara Soya, NRC 163, KDS 1099, DSb 32, Shivalik, NRC 154, Himso 1690 and JS 20-86 and on the basis of pathogenic variability, ten diverse cultures were selected as pathotypes. Thirty (30) soybean lines showing various levels of resistance under field conditions (AR; absolute resistance, HR; high resistance and MR; moderate resistance) were screened under greenhouse conditions to confirm the resistance. The variety Shivalik was used as susceptible check. Ten sets of 30 resistant lines along with susceptible check were sown in sterilized pots. Five seeds of each variety was sown in each pot (15 cm). Three replications were kept in each set. The fungus culture of 10 pathotypes was maintained on potato dextrose agar (PDA) medium. Fourteen days old culture was used for the preparation of inoculum. Bits of fungal colony were macerated in sterilized distilled water. The mixture was then filtered with triple-layered muslin cloth and suspension was fix to the concentration of 3×10^6 conidia /mL by using haemocytometer. Spore suspension was sprayed at 3-5 leaf stage of plant, covered with plastic bags containing holes for aeration and kept under glasshouse conditions at 25±3ºC temperature. High humidity was maintained by spraying water thrice for three consecutive days. The selected ten pathotypes were inoculated on 30 soybean germplasm lines found resistant in the field evaluation during 2021 and 2022 under green house conditions and disease reaction was recorded after 7^{th}, 14^{th} and 21^{st} days of inoculation as per the following scale (Mengistu *et al*. 2020):

Disease reaction	Disease Score/Rating	Severity Scale /Disease Severity
Absolute Resistance (AR)	0	No disease
Highly Resistance (HR)	1	1 % leaf area is covered with the small brown spots/lesions
Moderate Resistance (MR)	2	1.1-10% leaf area is covered with brown spots/lesions; no spots on stem
Moderate Susceptible (MS)	3	10.1-25% leaf area is covered with brown spots, no defoliation; little damage
Susceptible (S)	4	25.1-50% leaf area covered with circular brown spots; damage conspicuous
Highly susceptible	5	More than 50 % leaf area covered with circular brown spots

Results and Discussion

Symptoms of disease start appearing in the first week of August and became severe in the month of September. PDI calculated varied from 0.00 to 95.56 percent. In the entire soybean germplasm, 10 per cent soybean genotypes were highly resistant, 35 percent were in moderately susceptible category while 36 per cent were with moderately resistant category. Two percent of the accession showed susceptible and only 3 per cent were highly susceptible (Figure 1). Out of 200 genotypes, five were having absolute resistance (AR), 20 were highly resistant (HR), 74 were moderately resistant (MR), 70 were moderately susceptible, 28 were in category of susceptible (S) and three were highly susceptible (HS) (Table 1).

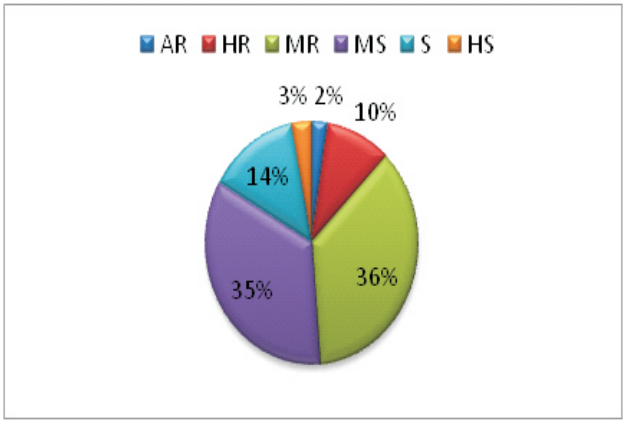

Figure 1. Disease reactions of soybean genotypes

Artificial Screening

Disease symptoms appear after 7th day of inoculation under greenhouse conditions using 10 pathotypes. Data on disease severity on 30 soybean accessions which were found resistant under field conditions with diverse pathotypes (PCS-01 to PCS-10) are presented in Table 2. Four accessions viz; Harder, CAT 195, JSM 285 and GP 465 were found disease free against the test pathotypes. However, 10 lines viz., Himso 1685, DSb 37, Hardee, VP 1164, UPSL 77, MACS 1566, CAT 183, CAT 193, AMS 39 2-2 and VLS 11 were found to be highly resistant.

In India several researchers had identified resistant sources of soybean against frogeye leaf spot disease under field natural epiphytotic conditions. Joshi *et al*. (1989) screened 37 cultivars against FLS of soybean and reported resistance in 17 cultivars. Chandra *et al*. (1995) screened 19 genotypes of soybean against FLS in Meghalaya under natural epiphytotic conditions and reported that genotypes viz; JS 75-46, JS 80-21, PK 262 and PK 472 were resistant against FLS. Mishra *et al*. (2021) identified resistant sources against FLS of soybean by using Percent Disease Index (PDI) where eleven genotypes were found highly resistant against FLS as PDI ranged between 1.9 to 10.9 per cent. In the present study attempt was made to verify the field resistance under artificial inoculation with diverse pathotypes.

Table 1. Disease reaction of soybean genotypes into different categories based on PDI under field conditions

Sr. No.	Disease Reaction with PDI	Number of genotypes	Name of soybean genotypes
1	AR (0.0)	5	JSM 285, CAT 195, GP 465, Himso1685, Harder
2	HR(0.01-11.11)	20	EC 241780, JSM 245, JSM 227, SL 525, ASb 51, AUKS 218, VLS 11, ASb 51, MACS 1566, EC 48157, PS 1052, RSC-1052, UPSL 422, AMS MBS-18, TNAU-5-55, ASb 50, UPSM 479, ASb 51, JS 89-24, EC 251-383
3	MR(11.12-33.33)	74	PS 1641, RVS 2011-32, NRC 163, JS 22-01, Himso 1690, EC 77883, JS 20-89, EC 391181, EC 350664, EC 14117, JSM 245, MACS 7102, PK 431337, TGX 293-41E, DSb 33, DSb 19, DSb 21, DSb 34, DSb 37, EC 241778, SKF 6029, VP 1164, AGS 747, AMS 39-2-2, EC 393222, JS 20-481, JS 91-52, PK 1038, NRC 37, NRC 2006M, NRC 2007-1-3, TGX 899-813, UPSM 57, VLS 11, VLS 89, JS 20-55, JS 20-96, JS 72-280, TS 46, EC 456549, EC 114573, JS 20-48, TNAU 20049, UPSL 470, EC 241771, EC 393222, EC 241780, H 330, Hardee, UPSL 742, T5 53, , NRC 34, PI 2043, EC 241771, NRC 163, PS 1572, PK 726, UPSM 780, KDS 1099, DSb 37, DSb 32, UGM 77, SAL 72, EC 457214, AGS 163B, EC 172576, JSM 22, UPSL 77, CAT 1911, CAT 1042, CAT 183,CAT 183, CAT 01, AVRAL 508, CAT 1873, MACS 1566
4	MS(33.34-55-55)	70	MAUS 717, PS 1642, AMS 20-19, DS 1326, SL 1234, EC 457074, EC 457198, JSM 222, GP 434, MACS 303, MACS 171, PK 25, RKS 54, SL 738, UGM 75, Z-22(20-146), Z-17 23-108, AGS 164, CAT 228, Himso1690, JS 20-29, JS 21-25, JS 335, MAUS 128, MLT 75. PS 93108, NRC 149, TGX 702 4-8, UPSL 72, JS 20-34, JS 20-116, JS 20-84, JS 20-60, JS 20-27, JS 20-53, JS 20-69, JS 9305, MACS NRC1576, MACS 58, PK 472, AGS 114, CAT 411A, PS 16-11, EC 456527, EC 1619, EC 251516, NRC 138, PK 262, PS 1556, UPSL 415, VLS 58, Bragg, JS 20-72, RVSM 2011-77, PS 1336, TS 53 , JS 20-69, MAUS 712, Himso 1689, JS 95-60, VLS 99, AGS 367,CAT 527, CAT 282, CAT 244, CAT 1227, PKT 01, JS 20-116
5	S(55.56-77.77)	28	MACS 1655, DS 1318, VLS 59, KDS 1097, VLS 89, NRC 149, RVSM 2011-77, EC 381884, SL(PE)1, Z-19 14-11, MACS 303, JS 20-77, JS 20-36, KDS 921, KHSB 2, VLS 63, Monetta, Palam Soya, NRC168, GP 496, Hara Soya, NRC 59, NRC 136, JS 20-86, Shivalik, NRC 2320, NRC 142, CAT 491, EC 350664
6	HS(77.78-100.00)	3	NRC 154, Him Soya, Punjab-1

Table 2. Artificial screening of resistant soybean genotypes with different pathotypes (0-5 scale)

Sr. No.	Soybean lines	PCS-01	PCS-02	PCS-03	PCS-04	PCS-05	PCS-06	PCS-07	PCS-08	PCS-09	PCS-10
1.	DSb 37	0	0	0	0	0	1	0	0	1	0
2.	H 330	2	1	1	2	1	0	1	2	2	1
3.	Himso 1685	1	0	1	1	0	1	0	1	1	1
4.	Hardee	0	1	1	1	0	0	0	0	1	0
5.	VP 1164	1	0	0	0	0	0	0	0	0	1
6.	AMS 39-2-2	0	2	0	1	0	0	1	0	2	0
7.	EC 241771	0	3	1	2	4	3	1	2	0	0
8.	UPSL 77	0	0	0	0	0	0	0	0	0	1
9.	Harder	0	0	0	0	0	0	0	0	0	0
10.	EC 14117	1	0	0	0	0	0	0	0	0	0
11.	JSM 285	0	0	0	0	0	0	0	0	0	0
12.	TGX 293-41E	2	0	1	3	2	3	3	2	3	2
13.	AUKS 218	1	3	2	2	3	3	4	5	3	2
14.	EC 241778	2	0	1	2	1	0	2	2	2	2
15.	VLS 11	1	0	0	0	0	1	0	1	0	1
16.	MACS 1566	1	0	0	0	0	0	0	0	0	1
17.	SKF 6029	3	3	3	2	3	2	3	1	1	3
18.	EC 241780	1	0	2	0	1	2	3	0	1	2
19.	CAT 195	0	0	0	0	0	0	0	0	0	0
20.	DSb 34	3	3	4	3	2	3	3	1	2	3
21.	JS 20-89	3	0	3	3	0	0	0	0	1	3
22.	GP 465	0	0	0	0	0	0	0	0	0	0
23.	SL 525	2	1	3	2	1	2	1	2	1	3
24.	EC 393222	3	2	4	4	3	2	2	1	3	1
25.	JS 20-116	5	3	4	3	4	5	3	1	3	1
26.	JSM 245	3	1	0	3	0	1	2	3	0	1
27.	CAT 183	1	0	1	0	0	0	0	0	0	0
28.	AGS 747	5	3	3	5	3	2	3	2	3	2
29.	CAT 193	1	1	1	0	0	0	1	2	0	1
30.	Shivalik	5	4	2	5	5	4	5	3	5	3

The resistant lines identified based on both natural as well as artificial epiphytotic conditions can be utilized in future hybridization programme in order to introgress the resistance against frogeye leaf spot of soybean (Table 3).

Table 3. Resistant genotypes showing disease reaction under natural as well as artificial epiphytotic conditions

Sr. No.	Soybean genotype	Field Conditions	Artificial conditions	Overall Reaction
1.	Harder	AR	AR	AR
2.	JSM 285	AR	AR	AR
3.	CAT 195	AR	AR	AR
4.	GP 465	AR	AR	AR

AR= Absolute resistance or disease free

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Conflict of interest: The authors declare that there is no conflict of interest in this research paper.

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