Genetics of rust resistance in soybean [*Glycine max* (L.) Merrill]

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Abstract

Inheritance of rust resistance in soybean [*Glycine max* (L.) Merrill] was studied in nine crosses involving 2 susceptible and 5 resistant genotypes. The crosses were made in three triangles keeping one parent common in all the 3 triangles. Seeds of all the generations *viz.*, P_1, P_2, F_1, F_2 and F_3 were divided into two sets, one of which was used in field screening and other for controlled condition study in greenhouse. Results obtained from both the environments are similar. The F_2 segregation analysis in all the six susceptible × resistant cross combinations revealed that rust resistance is governed by a single dominant gene. In Bragg × MACS 13, a cross of both susceptible parents revealed that, there is no complementation. In one of the 2 resistant × resistant cross combinations TS 98-21 × EC 389160, there are two different genes imparting resistance. Whereas in the cross, PK 1029 × EC 389165, there was no segregation for rust reaction in any of the generations which reveals presence of the same gene for resistance in both the parental lines. In all these crosses the F_2 results were confirmed by studying the F_3 progenies.

Key words: Soybean, rust, inheritance, rust resistance, genetics.

Introduction

Soybean [*Glycine max* (L.) Merrill] is second major oilseed crop of India. The productivity of soybean in India remained constant around one t/ha, which is about half of US average (2.27 t/ha). The low productivity of soybean in India is due to many constraints, among them one of the major constraint is soybean rust caused by the fungus *Phakopsora pachyrhizi* Sydow. Soybean rust is the most devastating disease causing significant yield losses [1-3]. In India, soybean rust occurs in the high productive zone of the soybean cultivation, i.e. southern part of Maharashtra and north Karnataka and North-eastern regions of India. Various control measures recommended for soybean rust are not so effective and are costly due to high prices of the fungicides.

Under such circumstances, utilization of existing resistant germplasm lines is an alternative. Unfortunately, in the soybean germplasm not a single line is available with immunity to rust. Some lines are found resistant to rust, but with narrow adaptability. Therefore, there is a need to evaluate and exploit existing germplasm and develop soybean cultivars with high degree of resistance in combination with better agronomic characters. For developing disease resistant genotypes, better understanding of genetic behavior of rust resistance is required. In India, on the basis of field screening and also screening under controlled conditions several germplasm lines were identified as resistant to rust [4-6]. Globally, four different genes have been identified imparting specific resistance to soybean rust races [7, 8]. These genes are present in four different soybean genotypes. There is no report on mode of inheritance of rust resistance in India. Single dominant gene for resistance in UPSS-3 reported on the basis of monogenic inheritance of resistance to rust [9]. Bromfield and Hartwig [10] observed single dominant gene responsible for rust resistance in two genotypes, PI 230970 and PI 230971. They found that the resistant genotypes develop brown lesions on leaves with no or a few sporulating pustules. Similar results were also reported in the cultivar PI 200492 [11].

Hartwig and Bromfield [7] identified three different dominant genes for soybean rust resistance. These genes are specific to different races and present on different loci and in different soybean genotypes. The gene symbols have been assigned to the genotypes *viz.*, *Rpp1* in PI 200492, *Rpp2* in PI 230970 and *Rpp3* in PI 462312 (Ankur). *Rpp2* has been found resistant to both the races of Taiwan and Indian races, while *Rpp3* is resistant to only Indian race. A fourth major dominant gene conferring resistance against two Taiwan races and one Indian race was reported. This gene was assigned the symbol *Rpp4* and present in the

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genotype PI 459025. This genotype also gave classical 3:1 ratio of resistant to susceptible when crossed with a susceptible cultivar. Therefore, an experiment was undertaken to study inheritance of rust resistance in soybean lines.

Materials and methods

On the basis of screening for rust reaction, seven soybean genotypes (Table 1) including five resistant viz., Ankur, PK 1029, TS 98-21, EC 389160 and EC 389165 and two susceptible lines, Bragg and MACS 13. Nine crosses were made in three triangles keeping Bragg as a common parent in all the triangles during rainy season of 2000 at Agharkar Research Institute, Pune. These included four different types of combinations, i.e., (1) susceptible × susceptible, (2) resistant × susceptible, (3) susceptible × resistant and (4) resistant × resistant. Half of the F_1 seeds were sown during summer 2001 season to advance the generation and F_2 seeds were harvested from the confirmed F_1 plants. The harvested seeds of parents and subsequent generations were divided into 2 sets. During 2001 rainy season, one set of F_1 and F_2 generations along with the parents were grown at the field of The Ugar Sugar Works Ltd., Ugarkhurd, Karnataka, which is a hot spot area of soybean rust. Another set of seeds was stored at −18°C in moisture free conditions for greenhouse study.

Table 1. Details of the parents used in the present study

Parental genotypes	Pedigree	Source	Rust reaction
Bragg	Jackson × D 49-2491	USA	Susceptible
MACS 13	Hampton × EC 7034	ARI, Pune	Susceptible
Ankur	PI 462312	Pantnagar	Resistant
TS 98-21	Mutant of JS 80-21	BARC, Mumbai	Resistant
PK 1029	PK 262 × PK 317	Pantnagar	Resistant
EC 389160	-	USA	Resistant
EC 389165	-	USA	Resistant

F_2 generation populations were grown along with their respective parents and F_1s in single row each. The seeds were sown in 3m long rows with inter-plant and inter-row distance of 0.1m and 0.45m, respectively. This facilitated easy and proper screening. An infector row of the susceptible cultivar MACS 13 was sown after each 20 rows to ensure sufficient inoculation.

The disease appeared during the second week of August 2001, as ash to tan coloured pustules on the susceptible cultivars and later on spread to the entire field. For even spread of the disease an aqueous suspension of rust spores was sprayed on the experimental material. The observations on rust reaction

were taken during first week of September 2001. Plants were classified into 2 categories, i.e., resistant (R) and susceptible (S) on the basis of colour of rust pustules. The resistant plants develop small, gray pustules initially, which further transformed into dark brown, non-sporulating pustules; whereas tan coloured profusely sporulating pustules were developed on susceptible plants. Complete premature defoliation occurred in susceptible plants. Data were recorded on individual plant basis in all the generations, viz., parents, F_1 and F_2 generations. The first 50 plants of F_2 generation were harvested individually to get F_3 seeds. The F_3 progenies along with the parents were raised during 2002 rainy season at Ugarkhurd, Karnataka and data were recorded on an individual plant basis for rust reaction.

The second set of seeds of different generations was grown during 2001 winter season at ARI Pune for study under controlled conditions. Under controlled conditions, the cultivation practices given by Rahangdale and Raut [6] were followed. Individual plant was raised in a 250 ml plastic cup for 20 days under natural conditions. Then the F_2 population along with the parents and F_1 generation plants were transferred to green house for study of rust reaction. After 2 days, rust spores were inoculated in water with a hand sprayer on the entire population as per the procedure adopted by Singh and Thapliyal [12]. Observations were recorded on 15th day after rust inoculation on individual plant basis. The first 50 F_2 plants were then transplanted to earthen pots under bright sunlight to get F_3 seeds. The F_3 generation plants were also screened for rust reaction under the same conditions. Segregation of rust reaction was analysed by χ^2 (chi square test) to determine the goodness of fit of collected data with the expected genetic ratios.

Results and discussion

Inheritance of rust reaction was studied in all the nine crosses. Results of field study are presented in the Table 2 and that of greenhouse study in Table 3. In Bragg × MACS 13, both the parents were susceptible and the progenies of subsequent generations were also susceptible indicating non-complementation of the susceptibility.

In resistant × resistant combinations, in the cross PK 1029 × EC 389165 the F_1 and subsequent generations were resistant. No segregation for rust reaction was observed, which indicated presence of alleles of the same resistance gene in both the parents. Whereas, in the cross TS 98-21 × EC 389160, all the F_1 plants were also resistant, and the F_2 population segregated into 428 resistant and 37 susceptible plants under field and 415 resistant and 34 susceptible plants

Table 2. Inheritance of rust resistance under field conditions

Crosses/Generations	Bragg × MACS 13	Ankur × MACS 13	Ankur × Bragg	Bragg × EC 389160	Bragg × TS 98-21	TS 98-21 × EC 389160	Bragg × PK 1029	Bragg × EC 389165	PK 1029 × EC 389165
F_1	S	R	R	R	R	R	R	R	R
F_2 plants									
R	0	203	95	329	262	428	141	171	278
S	328	68	32	105	83	37	51	63	0
Total	328	271	127	434	345	465	192	234	278
χ^2	-	0.001	0.003	0.150	0.163	2.312	0.250	0.461	-
d.f.	-	1	1	1	1	1	1	1	-
Expt. Ratio	-	3:1	3:1	3:1	3:1	15:1	3:1	3:1	-
P	-	0.98-0.95	0.98-0.95	0.70-0.50	0.70-0.50	0.20-0.10	0.70-0.50	0.50-0.30	-
F_3 progenies									
R	0	14	16	13	14	24	15	10	48
Seg.	0	23	28	27	23	25	25	28	0
S	47	8	9	11	13	5	12	12	0
Total	47	45	53	51	50	54	52	50	48
χ^2	-	1.622	2.019	0.334	0.360	0.936	0.423	0.880	-
d.f.	-	2	2	2	2	2	2	-	-
Expt. Ratio	-	1:2.1	1:2:1	1:2:1	1:2:1	7:8:1	1:2:1	1:2:1	-
P	-	0.50-0.30	0.50-0.30	0.90-0.80	0.90-0.80	0.70-0.50	0.90-0.80	0.70-0.50	-

R = Resistant, S = Susceptible, Seg. = Segregating

Table 3. Inheritance of rust resistance under controlled conditions (*Greenhouse*)

Crosses/Generations	Bragg × MACS 13	Ankur × MACS 13	Ankur × Bragg	Bragg × EC 389160	Bragg × TS 98-21	TS 98-21 × EC 389160	Bragg × PK 1029	Bragg × EC 389165	PK 1029 × EC 389165
F_1s	S	R	R	R	R	R	R	R	R
F_2 Plants									
R	0	234	224	264	284	415	240	227	204
S	270	78	80	91	98	34	91	90	0
Total	270	312	304	355	382	449	331	317	204
χ^2	-	0.427	0.281	0.076	0.087	1.340	1.097	1.939	-
d.f.	-	1	1	1	1	1	1	1	-
Expt. Ratio	-	3:1	3:1	3:1	3:1	15:1	3:1	3:1	-
P	-	0.70-0.50	0.70-0.50	0.80-0.75	0.80-0.75	0.25-0.20	0.30-0.25	0.20-0.10	-
F_3 progenies									
R	0	14	15	12	14	19	15	11	50
Seg.	0	25	22	22	21	27	22	29	0
S	50	11	13	13	15	4	13	10	0
Total	50	50	50	47	50	50	50	50	50
χ^2	-	0.360	0.88	0.234	1.32	0.78	0.88	1.32	-
d.f.	-	2	2	2	2	2	2	2	-
Expt. Ratio	-	1:2:1	1:2:1	1:2:1	1:2:1	7:8:1	1:2:1	1:2:1	-
P	-	0.90-0.80	0.70-0.50	0.90-0.80	0.70-0.50	0.70-0.50	0.70-0.50	0.70-0.50	-

R = Resistant, S = Susceptible, Seg. = Segregating

under controlled conditions. The data showed 15:1 ratio with good fit. The results indicated presence of two different resistance genes in these genotypes. The F_3 family segregation also confirmed F_2 results, as the first 54 families under field conditions and 50 families under greenhouse segregated into 7:8:1 ratio (7 resistant: 8 segregating : 1 susceptible).

Hartwig and Bromfield [7] observed 10:5:1 ratio (10 immune : 5 resistant : 1 susceptible) in resistant × resistant combination. There are no reports of 15:1 ratio for rust resistance. The above report of immunity may be because of the different races were used in the study and incidentally those two genes might confer complete resistance in presence of each other. These

observations give us a clue that high resistance against many races can be induced by bringing many resistance genes in one genotype.

In six crosses, one of the parents was susceptible. In two combinations, i.e., Ankur × MACS 13 and Ankur × Bragg, resistant parent was used as female, while in 4 crosses susceptible parent was used as female. The F_1s of all these 6 crosses were resistant to rust in the field as well as greenhouse conditions. In all these combinations, F_2 populations segregated into 3:1 ratio (3 resistant : 1 susceptible) indicating single gene responsible for resistance to soybean rust with resistance being dominant over susceptibility. The F_3 families segregated in 1:2:1 ratio (1 resistant : 2 segregating : 1 susceptible) confirming the results observed in F_2 generation.

In the present study, the genotype Ankur (*Rpp3*), EC 389160, EC 389165, TS 98-21 and PK 1029 were resistant to soybean rust under greenhouse as well as field conditions. The genes present in later mentioned four soybean lines are not known, because no crosses were made involving these lines with the genotypes with known resistant genes.

Significant finding of present study is that, these genotypes have not been previously studied in details for rust resistance except, Ankur (PI 462312) and the duplicate dominance control of rust resistance in the cross TS 98-21 × EC 389160 is a new report. The resistant genotypes reported in the present study can be used as confirmed sources of resistance and utilized in the breeding program. These may play an important role in the development of elite rust resistant soybean lines. The genotypes, TS 98-21, PK 1029, EC 389160 and EC 389165 have to be studied for identification of the genes present in these lines by undertaking breeding program with differential genotypes having known rust resistance genes.

Acknowledgements

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