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Molecular breeding for charcoal rot resistance in soybean I. Screening and mapping population development

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Abstract

Charcoal rot caused by *Macrophomina phaseolina* is a major yield reducing disease in the soybean growing countries across the world. Its effect is more pronounced in crops under stress, biotic or abiotic. Changing global climatic conditions particularly occurrence of frequent drought or drought-like situations are making soybean more vulnerable to this disease. Improper screening methods rendered conventional breeding approaches unproductive. Identification of molecular marker(s) linked to the charcoal rot resistance gene would greatly facilitate screening and thus accelerate the development of new cultivars. A core set of 100 diverse genotypes were subjected to screening for resistance under paper towel methods. No genotypes were immune; 7 germplasm lines appeared to be resistant. F_1 hybrids were produced by crossing resistant and highly susceptible genotypes. Parental polymorphism and purity of the F_1 hybrids was established using SSR markers. Advancement has been made to develop mapping population to map QTL for charcoal rot resistance in soybean.

Key Words: Charcoal rot, soybean, *Macrophomina phaseolina*, SSR marker

Introduction

Soybean (*Glycine max* L.) is the world's foremost provider of protein (40%) and oil (20%). Soybean is not only used for human consumption, but also used to produce low-cost, high protein feed ingredient. It also finds wider application in industry to produce number of products and services for human uses and comforts. Production of soybean is highly challenged by abiotic and biotic stresses. Charcoal rot caused by *Macrophomina phaseolina* is the second largest yield reducing disease after brown spot (*Septoria glycines*) in the soybean growing countries across the globe [1]. The fungus causes a general root rot in soybean, infecting the roots and lower stems. It was named from the fact that infected tissues look as if they have been dipped in charcoal dust [2].

M. phaseolina infects an extremely wide range of hosts, including sorghum, soybean, cucurbits and various weed species. Its effect is more pronounced in crops under stress, biotic or abiotic. It is drawing more attention from the breeders in present time because of changing global climatic conditions. In fact, because of occurrence of frequent drought or drought-like situations is making soybean more vulnerable to this disease. Effect of the disease is not confined to the field alone, but it can affect the beans in storage conditions, as well. It can also reduce the seed germination badly [3]. Therefore, disease resistance to charcoal rot must be improved and incorporated into selected genotype to minimize yield loss. However, breeding for charcoal rot resistance through conventional technique did not met with much success. It is time consuming, laborious and largely ineffective. Hence, identification of molecular marker(s) linked to the charcoal rot resistance gene would greatly facilitate screening of breeding materials and thus accelerate the process of development of resistant cultivars. Here, advancement made in this respect at Indian Agricultural Research Institute, New Delhi is discussed.

Materials and methods

A core set of 100 diverse genotypes was extracted from the germplasm collection maintained at Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi (Table 1). The genotypes differed in various agro-morphological traits including maturity duration, seed colour, seed shape as well as resistance to

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charcoal rot reactions. After initial screening, only 20 genotypes were selected for molecular breeding (Table 1).

Screening of genotypes for charcoal rot resistance

The genotypes were subjected to screening for charcoal rot resistance in paper towel as per Nene et al [4]. For this purpose, pure culture of the fungus was grown in PDA medium, incubated for 5 days at 25ºC. The fungal mats were removed and macerated in 100ml sterile distilled water in which 5 days old seedling of test lines were dipped. 20-20 seedlings of each test line were kept in blotter paper and incubated at 25ºC for ten days and were subjected for scoring. In each lot, one susceptible genotype was included to ascertain proper inoculation. The genotypes were observed periodically and watered occasionally. After 10 days of inoculation, scoring was done following 0-9 scale.

Table 1. List of selected genotypes and their reaction to infection by *M phaseolina* in paper towel screening

S.No.	Genotypes	Disease reaction
1	DS9712	R
2	DS9814	R
3	JS335	R
4	PK564	R
5	EC439618	R
6	EC439619	R
7	DS61	R
8	BR11	MR
9	DS-201-A	MR
10	NRC67	MR
11	NRC37	MR
12	NRC7	MR
13	Himso1602	MS
14	EC93751	MS
15	G2602	S
16	G2631	S
17	PS1398	S
18	MACS985	HS
19	TS40	HS
20	SH40	HS

R: Resistant; MR: Moderately Resistant; MS: Moderately Susceptible; S: Susceptible; HS: Highly Susceptible

Parental polymorphism study

Parental polymorphism between resistant and susceptible genotypes was studied using SSR markers. Fifty SSR markers covering the entire genome of soybean (at least two markers per chromosome) were selected. The sequence of the SSR markers and the PCR profile was used as per soybase (www.soybase.org). The PCR products were resolved on 3% metaphor and scored.

Hybrid purity testing of F_1 plants

Few sets of resistant and highly susceptible plants were crossed in different combinations. F_1 seeds were harvested and grown in National Phytotron Facilitiy, IARI, New Delhi. DNA was extracted from tender leaves of the F_1 plants and was subjected to PCR amplification using polymorphic markers. True F_1 hybrid plants to produce heterozygous bands while selfed plants to produce bands like the maternal plants. Seeds only from the true hybrid plants were harvested to advance the generation.

Results and discussion

Charcoal rot is a disease that appears in hot and dry weather when soil temperatures are 80-95°F (27-35°C) for 2 to 3 weeks. The disease has been an endemic problem in relatively dry or drought-like situations. The fungus is highly variable and can infect more than 500 crops and weed species. Its infection occurs in the spring when soil moisture is high; however symptoms of charcoal rot develop in the hottest, driest part of the growing season.

Disease reaction

In the present study, genotypes were subjected to *in vitro* screening following the protol described above [4]. Here, no genotypes were found to be immune; however, 7 genotypes appeared to be resistant and rest were moderately resistant through highly susceptible (Table 1). Non-appearance of any immune genotypes indicated seriousness of the disease. Intensities of the disease were found to be increasing in recent years with frequent occurrence of drought/ drought-like situations in soybean growing areas of our country, which resulted in great economic losses to the soybean growers [5]. Moreover, non-availability of immune genotypes indicated the urgent need of searching gene for resistance in the wild type soybean including *G soja*. It has also been observed that the expression of the disease reaction is continuous i.e. it started from highly susceptible through moderately resistant to highly

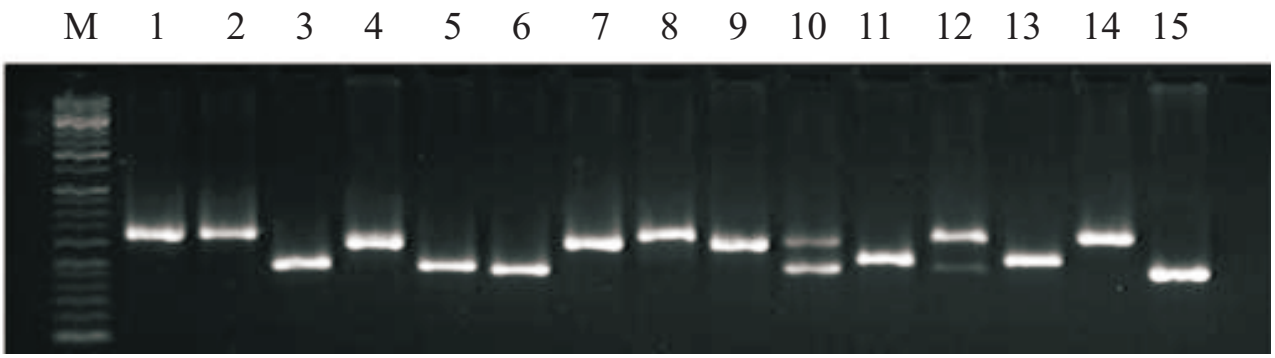

Fig. 1. SSR marker (satt301) showing polymorphism in parental population. M: Marker, 1: SH40, 2: DS9814, 3: TS40, 4: DS9712, 5Macs985, 6: JS335, 7: PK444, 8: DS2101, 9: Pusa16, 10: EC1021, 11: DS178, 12: EC44303, 13: L690, 14: EC439618, 15: NRC67

Fig. 2. F_1 plants (centre) with parental genotypes

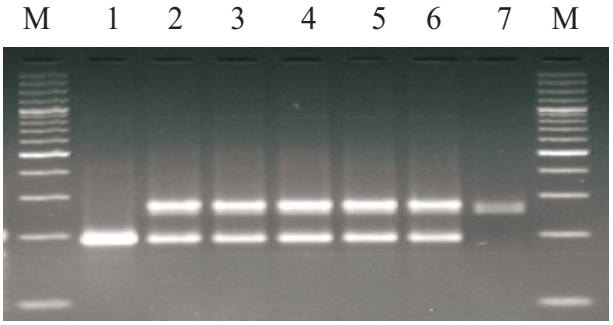

Fig. 3. F_1purity testing with polymorphic SSR markers. M: Marker; 1: Macs985; 2-6: F_1 plants, 7: DS9814

resistant. It might indicate involvement of more than one locus in controlling the resistance of the disease. Therefore, QTL approach might be the appropriate approach to breed soybean for charcoal rot resistance.

Polymorphism study

In this study, polymorphism among the parental population was found to be nearly 50%. The number of alleles per marker was found to range from 2 to 4, only. It reflected the poor variability among the soybean genotypes used in the study (Fig. 1). It is said that the

genetic base of Indian soybean is narrow. It can be attributed to the fact that soybean is not native to India and has been introduced from China and USA. All the breeding efforts made in India used those introduced germplasm and hence genetic base remained narrow. It is therefore highly needed to involve wild type genotype in breeding to introgress newer allele into Indian genotypes. The wild type allele with positive effect may not only confer resistance to biotic and abiotic stresses but can enhance yield as well.

Purity testing of F_1 hybrids and development of mapping populations

Identification of true hybrid plant is an important task in any breeding program. Conventionally, morphological differences between the putative hybrid plant and the parents are considered to identify the F_1 hybrid. However, result of such observation is often misleading. Use of co-dominant molecular markers can offer solution to this problem. Here, the DNA from the F_1 plants was subjected to PCR amplification along with the parents using polymorphic SSR markers. True F_1 plants (Fig. 2) produced heterozygous bands while selved plants produced bands like the maternal plants (Fig. 3). Thus true F_1 hybrid plants were identified and seeds were harvested. This technique not only ensured accuracy in selecting true hybrid plants but also saved time, space and money. The seeds of F_1 hybrids have now been used to develop mapping population for mapping the QTL for charcoal rot resistance in soybean

Charcoal rot resistance breeding is more relevant in the changing climatic conditions. The process of development of mapping population and identification of linked molecular markers is in progress. Already two mapping populations with more than 150 plants each have been developed and its characterization is going on. It is hoped that soon the QTL for charcoal rot resistance in soybean will be mapped and linked marker(s) will be identified. For this matter, development of RIL will be the right approach as it would permit multilocational trials with replications. As the impact of climate change has already been witnessed in many plants and animals species, hence it is felt necessary to expedite breeding for charcoal rot resistance with efficiency. It is expected that the materials developed in this study through effective screening and marker-assisted generation advancements would facilitate breeding of soybean for developing varieties resistant to charcoal rot disease.

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