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Research Article

Inheritance of pod shattering in soybean [*Glycine max* (L.) Merrill]

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Abstract
Three parents viz., NRC7, EC 241780 and Kalitur were crossed in cyclic manner during *Kharif* 2012 with the objectives to study the inheritance of pod shattering in soybean. Among these NRC 7 and others were resistant and susceptible to pod shattering respectively. Six generations (P_1, P_2, F_1, F_2, B_1 and B_2) were obtained by inter-mating three diverse parents. Pod shattering screening was done as per oven dry method. Inheritance of pod shattering was found to be governed by partial dominance of susceptibility over the resistance. Two major genes with inhibitory epistasis were involved in inheritance of pod shattering in soybean as evidenced from F_2 ratio (13:3) and confirmed by test cross ratio (3:1) in resistance x susceptible and susceptible x resistance crosses.

Keyword
Soybean, pod shattering, inheritance, inhibitory epistasis

Introduction

Soybean [*Glycine max* (L.) Merrill] is rapidly expanding partly due to its high nutritional value as food for both humans and livestock and as an important industrial crop. It is considered as a “Golden bean” due to its dual qualities *viz;* high protein (40%) and oil (18 to 20%) content. It is grown on approximately 250 million ha throughout the world (FAOSTAT 2012). The United States, Brazil, Argentina, India and Paraguay are the top five countries in the world with respect to area and production of soybean. In India, it occupies an area of 10.18 million ha with a production of 12.28 million tonnes, which accounts for a productivity of 1207 kg/ha during 2011-2012 (Anonymous, 2012).

Pod shattering refers to the opening of mature pods along the dorsal or ventral sutures and dispersal of seed as the crop reaches maturity, as well as during harvesting. The extent of yield loss due to pod shattering in soybean [Glycine max (L.) Merrill] may range from 34 to 99 per cent depending upon delayed harvesting after maturity, the environmental conditions during harvesting and genotype (Tiwari and Bhatnagar, 1991). Fully mature pods of soybean are extremely sensitive to opening, resulting in seed loss. This can take place in susceptible varieties prior to harvest due to disturbance of the canopy by wind or during harvesting as the harvesting equipment moves through the crop during dry weather conditions, leading to seed losses of 50-100% (IITA, 1986). Though this trait is important for the adaptation of the wild species to natural environments as a mechanism for seed dispersal, it leads to a significant yield loss in soybean production, if found in cultivated forms. This loss of seed not only has a drastic effect on yield but also results in the emergence of the crop as a weed in the subsequent growing season.

Within the crop canopy, before and during harvest, much pod shattering occurs because of the natural movement of the canopy which results in pods knocking against each other or against the stems and branches. With majority of agriculture operations depending on human labour, the untimely and delayed harvesting result in increased pod shattering. Pod shattering is aggravated if there is rain followed by dry weather, low humidity, high temperature, rapid temperature changes, wetting and drying (Agrawal *et al.* 2002). Among the causes mentioned above, the genotype of the variety plays an important role on the overall expression of pod shattering. Shattering takes place following dehydration of the pod wall and separation of the cells in a dehiscence zone which is situated in sutures between the lignified pod wall edge and a replum containing vascular tissue. Agrawal *et al.* (2003) reported that segregation of pod shattering was highly complex in F_2 generation and showed quantitative response in the cross of susceptible and resistant varieties and concluded that success of any conventional breeding program aimed at pod shattering resistance depends upon the desirable segregates. Increased shatter resistance will promote natural maturing of uniformly ripe seeds with improved oil extraction characteristics. Production costs, efficiency of seed recovery and quality of oil would all be improved by increased shatter resistance (Morgan *et al.* 1998).

The knowledge of inheritance of pod shattering provides useful tool for selection of suitable parents and segregating populations for developing shattering resistant progeny which is also challenging task to breeder due to complex nature of inheritance of the character. With this background, the present study was under taken.

Material and Methods

The present investigation was conducted at Post Graduate Institute Research Farm, Botany Farm, Central Campus, All India Network Research Project on Underutilized Crop, MPKV, Rahuri, during the period from 2012-13 and 2013-14. On the basis of susceptibility and resistance to pod shattering of soybean three genotypes were selected for present investigation. Out of three, a genotype NRC 7 was resistant to pod shattering and two genotypes (EC 241780 and Kalitur) were susceptible to pod shattering. These three genotypes were crossed in cyclic manner to produce three combinations for pod shattering traits *viz;* (1) susceptible x resistant (Kalitur x NRC 7) (2) resistant x susceptible (NRC 7 x EC 241780) and (3) susceptible x susceptible(EC 241780 x Kalitur). Crosses were effected in *Kharif* 2012 and early *summer* 2013 to produce the F_1 seeds. In early *summer* 2013 F_1s were sown and F_2s seeds were harvested. Backcrosses, B_1s and B_2s of three crosses were also made in *early summer* 2013.

The experiment was laid out in Randomized Block Design with three replications. The experimental material consisted of 18 treatments consisting three parents, $3F_1$s, $3F_2$s, $3B_1$s, $3B_2$s, of three crosses (NRC 7 x EC 241780, EC 241780 x Kalitur and Kalitur x NRC 7). The parents, F_1s, F_2s, and back crosses were randomized separately in each of the three replications. Sowing was done in rows of 3m length and 30 cm apart accommodating 30 plants at 10 cm distance in a row (medium soil). One row was assigned to P_1s, P_2s, F_1s, while two rows to each of the B_1s and B_2s and 10 rows to F_2s. This has permitted for raising of 30 plants in each of P_1s, P_2s, F_1s, 60 plants in B_1s and B_2s, and 300 plants in each of the F_2s, in each of the three replication for each cross. Fertilizer dose of 50 kg N and 75 Kg P_2O_5/ha for irrigated situation was applied at the time of sowing. The experiment was sown on 7th July 2013. The operations like thinning, weeding, hoeing, irrigation and plant protection were carried out regularly as per need and stage of crop growth.

Pod shattering screening was done as per oven dry method reported by Tiwari and Bhatnagar (1997) with little modification. The properly harvested 20 pods each of P_1s, P_2s, F_1s, F_2s, B_1s and B_2s generations were kept in brown paper bags and allowed to dry at room temperature for 15 days to equalize the moisture content of all pods. Then the bags were kept in hot air oven for 44°C (6 hrs in a day and ambient temperature at night) for 7 days. Percentage of shattering was recorded when more than 70% pods of susceptible parents were shattered and scored. Based on the scale by Bailey *et al.* (1997) and Mohammed (2010) a 1 - 3 scale was developed and phenotypic classes were assigned as follows:

1-3 digits scale	Phenotype	Shattering per cent
1	Resistant	0-10%
2	Intermediate	11 to 70 %
3	Susceptible	71 to 100 %

To study the mode of inheritance of pod shattering of soybean all generations of all three crosses which involves (1) susceptible x resistant, (2) resistant x susceptible and (3) susceptible x susceptible combinations were evaluated. Plants were classified in to resistant (R) and susceptible (S) on the basis of shattering scores.

Results and Discussion

Results of various resistant and susceptible plants observed in F_2 and backcross generations for all three crosses studied for pod shattering resistance are presented in Table 1. The genetics of soybean pod shattering resistant observed in the present finding presented below crosswise.

Cross I : NRC 7 x EC 241780: The F_1 generation of the cross of NRC 7 x EC 241780 produced intermediate plants which were very close to pod shattering susceptible parent (EC 241780). This indicated that the susceptibility was partially dominant over resistance for soybean pod shattering.

The segregating F_2 generation of the cross NRC 7 x EC 241780 was studied for 296 plants. Out of this, 233 (125 highly susceptible + 108 intermediate) pod shattering susceptible and 63 pod shattering resistant plants were observed in F_2. Intermediates were behaved as susceptible phenotypes hence the two phenotypic classes (resistant and susceptible) were made to fit into the chi-square test. The data fitted with 13:3 ratio indicated presence of inhibitory gene interaction for the inheritance of pod shattering in soybean. The test cross (B1) ratio also confirmed the presence of inhibitory gene interaction for the inheritance of pod shattering in soybean. In B_2 generation of the cross studied for 18 plants, all plants were shattering susceptible as it was cross between susceptible F_1 and susceptible parent.

Cross II : EC 241780 x Kalitur : The F_1 generation of the cross of EC 241780 x Kalitur produced all shattering susceptible plants. Both the susceptible parent produced susceptible F_1. The segregating F_2 generation of the cross EC 241780 x Kalitur was studied for 274 plants. All F_2 plants were found as shattering susceptible. Among the backcrosses, B_1 generation was studied for 24 plants, which were shattering susceptible. In B_1 generation of the cross studied for 20 plants, all plants were shattering susceptible.

Cross III : Kalitur x NRC 7: The F_1 generation of the cross of Kalitur x NRC 7 produced intermediate plants but which were very close to pod shattering susceptible parent (Kalitur). This indicated that the susceptibility was partially dominant over resistance for soybean pod shattering. The segregating F_2 generation of the cross Kalitur x NRC 7 was studied for 289 plants. The observed ratio was closely fitted with the 13:3 ratio, which indicated presence of inhibitory (dominance and recessive epistasis) gene interaction for the inheritance of pod shattering in soybean. The test cross ratio confirmed the presence of inhibitory gene interaction for the inheritance of pod shattering in soybean. In B_1 generation of the cross, all 22 plants were shattering susceptible as it was cross between susceptible F_1 and susceptible parent.

The F_1s of the crosses NRC 7 x EC 241780 (R x S) and Kalitur x NRC 7 (S x R) produced intermediate plants but they were very close to shattering susceptible parents (EC 241780 and Kalitur). This indicated that the susceptibility was partially dominant over resistance for soybean pod shattering. The F_1 of both the susceptible parents (EC 241780 and Kalitur) produced all susceptible plants and there was no complementation of genes.

The data of F_2 generations of the crosses NRC 7 x EC 241780 and Kalitur x NRC 7 showed the expected ratio of 13 susceptible: 3 resistant. This indicated presence of two major genes along with inhibitory epistasis for the inheritance of pod shattering in soybean. The results are in agreement with Mohammed (2010) who has reported that pod shattering in soybean controlled by two major genes with complementary and inhibitory types of epistasis.

Among the backcrosses, the test crosses of these two crosses, showed expected ratio of 3 resistant :1 susceptible. The test cross ratio confirmed the presence of inhibitory gene interaction for the inheritance of pod shattering in soybean. In case of B_2, when F_1s were crossed with dominant (susceptible) parent all progenies were shattering susceptible.

The role of four major genes reported by Caviness (1963), several genes by Misra *et al*. (1980) only a few genes by Carpenter and Fehr (1986), one or two gene by Tsuchiya (1986) , while six to 12 genes Akpan (1988) reported to be involved in controlling susceptibility to shattering. Shattering character is highly heritable as reported by Tiwari and Bhatnagar (1991). Analysis of pod shattering in F_1 populations showed susceptibility being dominant in some crosses while other crosses showed partial dominance for resistance as reported by Tiwari and Bhatnagar (1992). Pod shattering in soybean is under control of two genes and is partially dominant over resistance and concluded that inheritance of pod shattering is non-allelic resulting in classical dominant epistasis and it is not influenced by maternal effects as reported by Tukamuhabwa (2000). Presence of non allelic interaction of genes with partial dominance for the shattering trait reported by Tukamuhabwa *et al.* (2002). A major QTL was repeatedly identified on the linkage group (LG) J (chromosome 16) by Bailey *et al.* (1997), Funatsuki *et al*. (2005, 2006), Yamada *et al.* (2009). This locus was designated as qPDH1 by Funatsuki *et al*. (2008). Only minor QTLs were identified; no major QTL was detected either on LG J, or on other LGs (Kang *et al.*, 2009).

In inhibitory epistasis, one dominant allele (S^+) and recessive allele at another locus ($S^-S^-R^-R^-$) produced the same phenotypic effects. Thus $S^+S^+R^+R^+$/$S^+S^+R^+R^-$/$S^+S^+R^-R^-$/$S^+S^-R^-R^-$ and $S^-S^-R^-R^-$ produced one (susceptible) phenotype and $S^-S^-R^+R^-$/$S^-S^-R^+R^+$ produced another (resistant) phenotype. $S^+S^-R^+R^-$/$S^+S^-R^+R^+$ produced intermediate phenotype (Table 2 & 3). The effect of second gene is inhibited in presence of first dominant gene. Both recessive genes together produced susceptible phenotype like first dominant gene. Heterozygous conditions of both genes as well as heterozygous first and homozygous second gene produced intermediate effects. The present finding can be effectively used in soybean breeding programme to develop shattering resistant genotypes.

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Table 1. Inheritance of pod shattering resistance in soybean under controlled (Oven dry method) condition

Generations	Crosses		
	NRC 7 x EC 241780	EC 241780 x Kalitur	Kalitur x NRC 7
F_1	I (S)	S	I(S)
Observed F_2 plants			
S	125	274	131
I	108	0	113
Total S (S + I)	233	274	244
R	63	0	45
Total	296	274	289
χ^2	1.247 (N.S.)	----	1.844 (N.S.)
d. f.	1	----	1
Expected Ratio	13 : 3 (7S+6I:3R)	----	13 : 3 (7S+6I:3R)
Observed Ratio	12.59 : 3.41 (6.75S + 5.84I : 3.41)	----	13.51 : 2.49 (7.25S+6.26I : 2.49)
Backcrosses			
(Test Cross)	B_1	B_1	B_2
S	10	24	9
I	4	0	4
Total S (S + I)	14	24	13
R	6	0	5
Total	20	24	18
χ^2	0.266 (N.S.)	----	0.074 (N.S.)
d. f.	1	----	1
Expected Ratio	3 : 1	----	3 : 1
Observed Ratio	2.80 : 1.20	----	2.89 : 1.11
With dominant parent	B_2	B_2	B_1
S	9	20	11
I	9	0	11
Total S (S+I)	18	20	22
R	0	0	0
Total	18	20	22
Gene action	Gene interaction- inhibitory epistasis	----	Gene interaction- inhibitory epistasis

S= Susceptible; I = Intermediate; R= Resistant (χ^2 table value at 5% = 3.8414, at 1% = 6.6348)

Table 2. Relationship between phenotype and genotype in an F_2 population and Backcrosses showing classical inhibitory (dominant and recessive) epistasis for inheritance of pod shattering in soybean

F_2 Population				
Genotype	Frequency	Phenotype	Total	Ratio
$S^+S^+R^+R^+$	1/16	S		
$S^+S^+R^+R^-$	2/16	S		
$S^+S^+R^-R^-$	1/16	S	7 S	13 S
$S^+S^-R^-R^-$	2/16	S		
$S^-S^-R^-R^-$	1/16	S		
$S^+S^-R^+R^+$	2/16	I	6 I	(7 S + 6I =13 S)
$S^+S^-R^+R^-$	4/16	I		
$S^-S^-R^+R^+$	1/16	R	3 R	3 R
$S^-S^-R^+R^-$	2/16	R		
Backcross population				
$S^+S^-R^+R^-$	1/4	I	1 I	3 S (2 S + 1 I = 3S)
$S^+S^-R^-R^-$	1/4	S		
$S^-S^-R^-R^-$	1/4	S	2 S	
$S^-S^-R^+R^-$	1/4	R	1 R	1 R
B_1/B_2 cross with Dominant parent				
$S^+S^+R^+R^+$	1/4	S	2 S	
$S^+S^+R^+R^-$	1/4	S		All (4) susceptible (2S + 2I =4S)
$S^+S^-R^+R^+$	1/4	I	2 I	
$S^+S^-R^+R^-$	1/4	I		