

Genetic Studies for Shattering Tolerance in Soybean

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By

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2011

CERTIFICATE-I

This is to certify that the thesis entitled, "**Genetic Studies for Shattering Tolerance in Soybean**" submitted in partial fulfillment of the requirement of degree of **MASTER OF SCIENCE IN AGRICULTURE (Plant Breeding and Genetics)** for the Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur is a record of the bonafide research work carried out by **Ku. Neelmani Bara** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (Certificate awarded *etc.*) or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigation has been acknowledged by him.

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CERTIFICATE-II

This is to certify that the thesis entitled, "**Genetic Studies for Shattering Tolerance in Soybean**" submitted by **Ku. Neelmani Bara** to the Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE IN DEPARTMENT OF PLANT BREEDING AND GENETICS**, has been, after evaluation, approved by the External Examiner and by the Student's Advisory Committee after an oral examination on the same.

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Place: Jabalpur

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Date:

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] belongs to family *Leguminosae*, subfamily *Papilionoideae* tribe *Phaseoleae* (Ingham, 1990). and subtribe *Glycininea* with the genome size of 1100 Mb (Walling *et al.*2006). The genus *Glycine* is divided into two subgenera *Glycine* and *Soja*. The subgenus *Soja* includes the cultivated soybean *Glycine max* and wild soybean *Glycine soja*. Both the species are annual and diploid with chromosome number $2n=40$.

Soybean has been originated in North and Central China (Hymowitz,1970); emerged as a domestic crop around 11th century B.C. in the eastern half of Northern China; migrated to Southern China, Korea, Japan, and South East Asia probably between 11th to 13th century B.C. (Hymowitz, 1970).

It is a major leguminous crop at global level that contains high quantity (40%) of quality protein (glycine, tryptophan and lysine) and about 20 % high quality oil.

Globally (EPI, 2009) 220.9 million tonnes of soybean is produced from 101 million ha area with a productivity of 2470 Kg/ha.

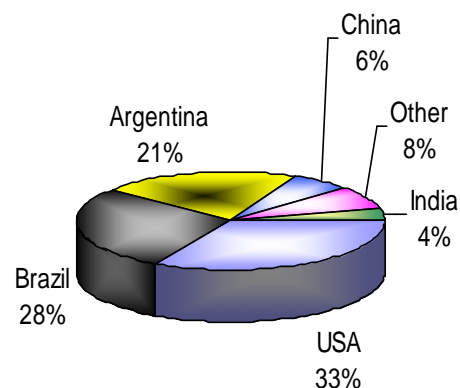


Fig 1.1 Contribution (%) of countries in soybean production at global level

The three major countries producing soybean are USA (90 million tonnes from 31 million ha, with 2900 Kg/ha productivity), Brazil (62 million tonnes from 22.5 million ha, with 2750 Kg/ha productivity) and Argentina (52.5 million tonnes from 18.55 million ha, with 2750 Kg/ha productivity) (Fig 1.1). India ranked fifth in the world with 9.7 million tonnes production from 9.67 million ha with the productivity of 1000.3 kg/ha (Fig 1.2).

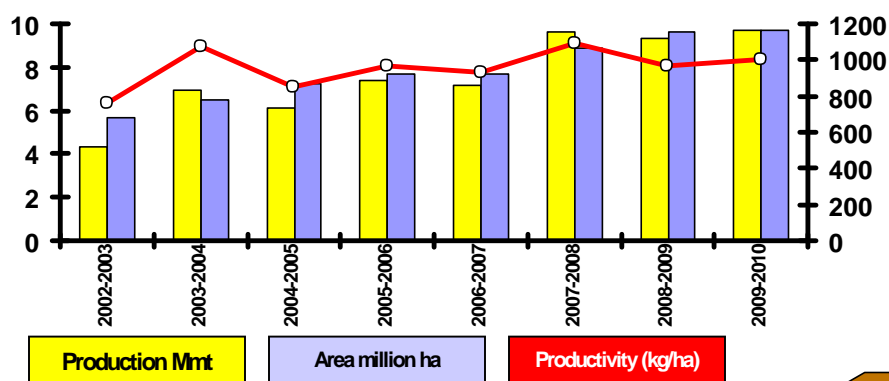


Fig 1.2 Area, production and productivity of soybean in India

Madhya Pradesh contributes 61% (5.5 million tones) of total production of soybean in the country from 5.3 million ha area with productivity of 1030.7 kg/ha followed by Maharashtra and Rajasthan (Fig 1.3).

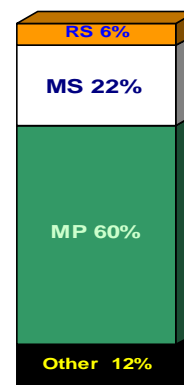


Fig 1.3 Contribution (%) of states in soybean production at national level

Basically soybean was cultivated in temperate region after 1940s, its production started to expand in tropical and sub-tropical regions (Franca and Henning, 1994). The warmer and humid climate of these areas pose many production problems such as infestation of pests and diseases, pod shattering and reduced seed viability (Franca and Henning, 1994).

Delayed harvest due to management or unfavorable weather conditions or recent unusual climatic fluctuations and the widespread use of combine harvesters are increasing the importance of breeding cultivars resistant to pod dehiscence.

Pod shattering is a specific characteristic observed not only in soybean but also in *Brassica species* (Meaken and Roberts, 1990; Child *et al.*, 2003), sesame (Langham and Wiemers, 2002), other pulse crops (Weeden *et al.*, 2002). It may cause 50-100% loss in soybean (IITA, 1986) depending upon the time of

harvesting, environmental condition and genetic endowment of the variety (Tiwari and Bhatnagar, 1988).

Enhancement in shattering tolerance may promote productivity, harvesting of uniformly ripe seeds, efficiency of seed recovery and improved oil extraction; adjustment in harvesting and threshing time; reduction in cost of production and problem of volunteer plants (Morgan *et al.*, 1998).

Hence study on identification of the factors affecting pod shattering and their genetics are essential to reduce the losses to a greater extent. The present study in soybean was conducted with the following objectives:

1. To study the genetic variability of attributes associated with pod shattering
2. To assess varietal difference and methods of measuring pod shattering
3. To work out the association coefficient of observed traits with pod shattering
4. To work out the direct and indirect effects of observed traits with pod shattering
5. To suggest an ideotype to describe tolerance to pod shattering

REVIEW OF LITERATURE

Soybean is the most important oil seed crop of India. Pod shattering is a major cause of yield loss in mechanical harvesting of soybean. The extent of yield loss due to pod shattering in soybean may range from 34 to 100 percent depending upon delayed harvesting after maturity, the environmental conditions during harvesting and genotype (IITA, 1986; Tiwari and Bhatnagar, 1991).

2.1 The Mechanism of shattering

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. 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 (Picart and Morgan, 1984). The dehiscence zone cells separate along the line of the middle lamella, following degradation of the pectin by polygalacturonase and subsequent breakdown of the dehiscence zone cell walls. The pods open as a result of the application of external forces supplied by contact with other pods, racemes or harvesting machinery which separate the vascular connections across the dehiscence zone from pod wall to the replum (Peterson *et al.* 1996).

2.2 Factors affecting shattering

Morphological architecture of plant, anatomical structures of the pod, chemical composition of the pod wall, genetic constitution of the variety and environmental conditions at maturity determine the degree of pod shattering (Gulluoglu *et al.*, 2006). The development of an ideotype which describes a crop more resistant to seed loss at harvest and maintains high performance depends upon morphological characteristics of the whole plant and raceme as well as those of single pods and their association with each other (Thurling, 1991).

2.2.1 Morphological architecture of plant: Pod shattering occurs mainly due to the natural movement of the canopy that results in knocking of pods against each other and with stems or branches. Magnitude of shattering depends on pod attributes such as angles, length and width and plant architecture such as plant height and stem stiffness (Loof and Jhonson, 1970; Thompson and Hughes, 1986). In Japan, varieties with determinate growth habit were reported susceptible while indeterminate as resistant to pod shattering (Tsuchiya ,1987).

A significant negative correlation of shattering with 100 seed weight and direct negative effect of days to maturity and grain yield on pod shattering have been reported by Tiwari and Bhatnagar (1990).

2.2.2 Anatomical structures of pod: Soybean pods contain seed in the central cavity enclosed by a single carpel with dorsal and ventral sutures along the length of the pod, where the pod opens at maturity (Christiansen *et al.*, 2002). The thickness and length of the bundle cap on the dorsal side of the pod and thickness of the pod were negatively and significantly correlated with the degree of pod shattering and pod thickness (Tiwari and Bhatia, 1995). Varietal differences in pod shattering was reported by Tiwari and Bhatia,(1995) with presence of clefts in the tissues above the inner schlerenchyma in susceptible genotypes especially below the bundles whereas, no such clefts were observed in resistant varieties.

Closer examination of the top of the bundle cap reveals that the two halves of the structure do not meet where the suture begins, but are delimited by two different kinds of cells. Microscopic examination of cross sections of dorsal and ventral sutures of soybean pods at two different stages of maturity revealed that the dehiscence zone of soybean pods is functionally equivalent to the dehiscence zone known from crucifers (Christiansen *et al.*, 2002).

2.2.3 Chemical composition of pod wall: Enzymatic assays demonstrated the peaked accumulation and activity of endo-1, 4- -glucanases and endopolygalacturonase in the dehiscence zone during maturation, which is presumably involved in the breakdown of the middle lamella prior to dehiscence (Christiansen *et al.*, 2002).

Hydrolytic enzymes cellulose and polygalacturonase, and oxidoreductase enzymes peroxidase and polyphenol oxidase have contrast activity at the shattering and non-shattering zones in soybean. The continuous increase of cellulose activity at the shattering zone of a susceptible variety indicates the involvement and role of this enzyme in the pod shattering process. The activity of cellulose enzyme was shifted from the non-shattering zone to the shattering zone in susceptible variety and vice versa in resistant variety (Agrawal *et al.*, 2002). High quantity of Heat Shock Protein HSP72-73 is synthesized in shattering resistant cultivars (Lu *et al.*, 1998).

2.2.4 Genetic constitution of the variety : Genetic constitution of the variety plays an important role on the overall expression of pod shattering. Several genetic studies conducted to understand the genetic control of pod shattering in soybeans have generated inconsistent data.

Shattering resistance has been introduced into leading cultivars in some regions including North America (Bailey *et al.*, 1997) where soybean cultivation has long been carried out on a large scale, while other regions still face the problem of pod dehiscence (Bhatnagar and Karmakar, 1995; Jiang *et al.*, 1991; Tiwari and Bhatnagar 1991; Tukamuhabwa *et al.*, 2002).

Crosses between domesticated cultivars and wild types in terms of shattering did not show any significant variation (Caviness, 1969). Analysis of pod shattering carried out by Tiwari and Bhatnagar (1992) in F₁ populations revealed contradictory observations, where some crosses showed dominance of susceptibility while other showed partial dominance for resistance.

Discrete classes of shattering scores in segregating populations of soybean involving a susceptible wild relative *Glycine soja* and two resistant cultivars have been reported by Carpenter and Fehr,(1986).They observed shattering frequency decrease in with each generation of backcrossing and concluded that the shattering trait could be eliminated by three to four backcrosses, indicating that only a few genes were involved. The varieties derived from crosses between Determinate (Japanese) and indeterminate growth habit (Chinese) were classified into resistant or moderately resistant (Tsuchiya, 1987).

Pod shattering has been reported as highly heritable trait with 98.8% (Tiwari and Bhatnagar,1991), 90% (Caviness,1969) and 93% (Tsuchiya,1987) broad sense heritability estimate which is a measure of additive variance. Pod shattering in soybean is highly heritable with narrow sense heritability of 0.79 and it is not influenced by maternal effects (Tukamuhabwa *et al.*, 2000).

Involvement of four (Caviness, 1963), several genes (Misra *et al.*, 1980), one to two (Tsuchiya, 1986), six to 14 (Akpan, 1988) and two genes (Tukamuhabwa *et al.*, 2000) for shattering in soybean have been reported Bailey *et al.*, (1997) reported control of one major quantitative locus, and a few minor QTLs on control pod shattering in soybean.

Pod shattering in soybean is partially dominant over resistance and its inheritance of pod shattering is non-allelic resulting in classical dominant epistasis Tukamuhabwa *et al.*,(2000). While Agrawal *et al.*,(2003) reported that segregation of pod shattering is highly complex with quantitative response in the cross of susceptible and resistant varieties.

The major QTL for pod shattering, designated as qPDH1, was found to be located between simple sequence repeat (SSR) markers, Sat_093 and Sat_366 (Funatsuki *et al.*, 2008). Moreover, the shattering resistance allele at qPDH1 proved useful in various genetic backgrounds at multiple locations (Funatsuki *et al.*, 2008). Analysis of the relationship between degree of pod dehiscence and graphical genotype of soybean lines confined the location of qPDH1 to a 134-kb

region on chromosome 16 (formerly linkage group J), where ten putative genes were predicted to be present (Suzuki *et al.*, 2010).

Environmental conditions at maturity: 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).

2.4 Variability for pod shattering and sources of tolerance

Significant genotypic differences among soybean cultivars and lines for shattering resistance have been reported (Caviness 1965; Tsuchiya 1986; Helms 1994; Romkaew and Umezaki 2006). Varietal differences for pod shattering behavior in China have been reported by (Peng *et al.*, 1991) with shattering as soon as from maturity to 6-12 days after maturity. Pod dehiscence is relatively uncommon in modern North American soybean cultivars, but is often observed when unimproved germplasm or the wild species, *G. soja* Siebold & Zucc, are used as parents to introgress useful genes or to develop genetically diverse breeding populations (Bailey *et al.*, 1997).

The first report on pod shattering study in India was published from National Research Centre for soybean (Tiwari and Bhatnagar, 1988) in which 25 varieties were grouped into five categories i.e., resistance (no pod shattering), tolerant (<25% pod shattering), moderate (26 to 50% pod shattering), high susceptible (51 to 75% pod shattering) and very high susceptible (more than 75% of pod shattering).

Tsuchiya and Sunanda (1977) screened 52 representative genotypes from Japan, USA and China and reported that genotypes from Japan were highly susceptible, whereas, genotypes from USA and China possessed more resistance to pod shattering. Three sources of germplasm i.e., a genetic resource from Thailand, an accession from North America and the third one from China

were reported useful for pod-shattering resistance in soybean breeding (Tsuchiya, 1986, 1987).

Genotypes Bragg, Himsoy-1520 (Tiwari and Bhatnagar, 1988), VLS-1, Pusa-22, PK-416 (Tiwari and Bhatnagar, 1990), JS15-15, JS-1608 and JS-1625 (Upadhyaya and Paradkar, 1991) have been reported as pod shattering resistant. Whereas Jain *et al.*, (1989) observed that yellow seeded genotypes show less pod shattering losses than the black seeded genotypes.

Tiwari and Bhatnagar,(1992) tested consistency in resisting pod shattering in different environments at field level by using Finlay and Wilkinson's,(1963) regression coefficient as well as the Eberhart and Russell's,(1966) deviation parameter. They considered Bragg (1.85%) and JS-71-05 (3.24%) with the lowest shattering mean values as pod shattering resistant, whereas Punjab-1 (45.53%) as highly susceptible.

The varieties have been classified at (NRC Soybean, Indore 1992) as pod shattering resistant (Bragg, VLS-1 and Pusa-22), tolerant (PK-416, JS-71-05 and PK-472) and susceptible (JS-2, Punjab-1 and Monetta) based on laboratory method of screening.

High genotypic variance for pod shattering at maturity and low genotypic variance at 10 days after maturity was reported by (Vimala Devi,1993). The optimum genotypic variance with clear difference between the genotypes was evident on fifth day after maturity. She recorded minimum pod shattering in Pusa-40 and maximum in Monetta. She screened genotypes both in laboratory and field conditions and found laboratory method better due to no influence of environment.

2.5 Various techniques of assessing pod shattering

To incorporate resistance against pod shattering, evaluation is the initial process of crop breeding programme. Therefore, review on germplasm evaluation with reference to pod shattering behavior and different screening techniques being used is presented here

2.5.1 Field-screening method: Assessments of pod shattering rely mainly upon visual observations of the crop in the field or upon hand tests of pods with delayed harvesting (Caviness, 1963; Tiwari and Bhatnagar, 1993; Helmes, 1994).

2.5.2 The desiccator method: Pods are subjected to desiccation inside a desiccator and duration required to shattering is recorded (Metcalf *et al.*, 1957; Caviness, 1965).

2.5.3 The oven-dry method: Pods are subjected to oven-drying at fixed temperature for a specified period before counting the number of shattered pods (Tsuchiya and Sunada, 1977; Tiwari and Bhatnagar, 1997, Tukamuhabwa *et al.*, 2002).

2.5.4 The mechanical cracking method: It is a laboratory procedure used to measure the mechanical properties of the individual pod for shattering (Kwon *et al.*, 1991; Davies and Bruce, 1997; Morgan *et al.*, 2000; Timothy *et al.*, 2003).

2.5.5 A random impact test (RIT): This procedure was evolved by Bruce *et al.*, (2002) to enable the rapid comparison of susceptibility to shattering in samples of fully mature pods from individual plants in a similar manner to those that occur in the crop canopy during harvest.

MATERIAL AND METHODS

3.1 Experimental site : The investigation was carried out during Kharif, 2010 at the Seed Breeding Farm, JNKVV, Jabalpur. The laboratory work was conducted in the Seed Technology Research Laboratory, Department of Plant Breeding and Genetic, JNKVV, Jabalpur and Department of Post Harvest Processing and Food Engineering, College of Agriculture Engineering, JNKVV, Jabalpur.

3.2 Climate and Weather: Jabalpur has a semi-humid and sub-tropical climate. It is situated at 23.9° N latitude and 78.58° E longitude at an altitude of 411.87m above the mean sea level. The main features of the region are hot and dry summers and cool winters with some occasional showers in winter. The average rainfall of the area is about 1400mm, which is mostly received during the months of July to September. The minimum and maximum temperature varies between 6°C-8°C in January to 40°C - 45°C in the month of May-June. During the crop period, the climatic condition was optimum for plant growth (Appendix-I).

3.3 Soil : The soil of the experiment field was heavy black, clayey, uniform in its topography and free from waterlogged condition. The details of experiment are given in Appendix II.

3.4 Experimental Material: The experimental was conducted on 69 genotypes(Appendix III) provided by All India Coordinated Soybean Improvement Project, Department of Plant Breeding and Genetics, JNKVV, Jabalpur.

3.5 Observation recorded : Following observations were recorded at field and in the laboratory

3.5.1 Observations recorded at field level : In the field all the 69 lines were cultivated in five replications consisting of three rows of 5m length each at 30cm row to row distance during *Kharif* 2010.

3.5.1.1 Days to flowering: Time from sowing in days to the day on which flowering initiated in 50% of the plants was recorded as days to 50% flowering.

3.5.1.2 Number of nodes on main branch : Number of nodes on the main branch is counted from ten randomly selected healthy plants at the time of maturity.

3.5.1.3 Plant height (cm) : Height of ten randomly selected, well developed healthy plants of each genotype in every treatment were measured at the time of maturity with the help of measuring scale.

3.5.1.4 Days to maturity : Number of days from sowing to the harvestable maturity i.e., 95% pods turn yellow was recorded as days to maturity.

3.5.1.5 Number of pods per plant : Average number of pods per plant was recorded at maturity based on observations made on 10 randomly selected well developed healthy plants.

3.5.1.6 Seed yield/plant (g): Seed yield of ten randomly selected healthy plants was recorded after sun drying at 10-15% moisture level in gram and the average value is reported.

3.5.1.7 Hundred seed weight: The weight of hundred randomly selected well developed healthy and dry (10-12%moisture) seeds was recorded and reported in grams.

3.5.1.8 Volumetric weight of seed: Volume of the seed was measured by displacement method with the help of measuring cylinder.

3.5.1.9 Presence of hairs on pod: Presence of hairs on fully matured well developed three seeded green pods was recorded visually.

3.5.1.10 Colour of hair: Colour of hairs on fully matured well developed three seeded green pods was recorded visually.

3.5.1.11 Presence of four seeded pod: The genotypes were visually observed for the presence or absence of four seeded pod.

3.5.2 Observation recorded in laboratory :

3.5.2.1 Weight of pod (g) : Weight of ten matured and healthy three seeded pods was measured in gram at 10-15% moisture content.

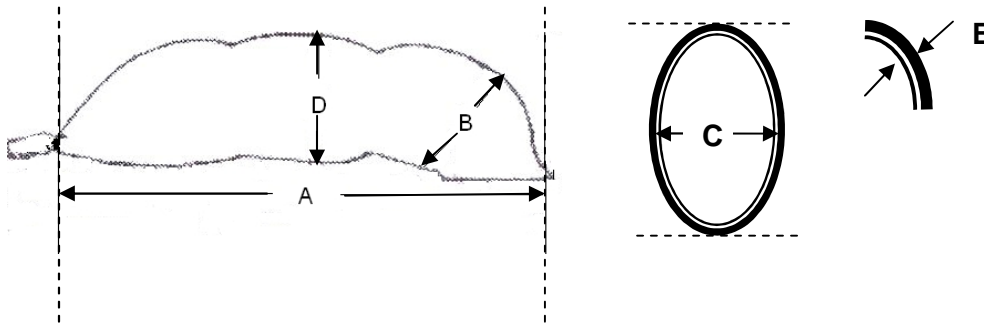
3.5.2.2 Length of pod (mm) A : Length of ten matured and healthy three seeded pods was measured with the help of Digital Vernier Caliper.

3.5.2.3 Width of pod (mm) B : Width of ten matured and healthy three seeded pods was measured with the help of Digital Vernier Caliper.

3.5.2.4 Length width ratio: (A/B) The ratio of length with the width of pod was calculated as length width ratio of pod.

3.5.2.5 Width length ratio (B/A) : The ratio of width with the length of pod was calculated as width length ratio of pod.

3.5.2.6 Width at mid part of pod (mm) D : Width of ten matured and healthy three seeded pods at the mid part of the pod was measured with the help of Digital Vernier Caliper (Tsuchiya, 1987).



3.5.2.7 Degree of curvature: (D/A) The ratio of length with the width of pod at mid position was calculated as degree of curvature.

3.5.2.8 Pod wall thickness (mm) E : Pod wall thickness of ten matured and healthy three seeded pods was measured with the help of Digital Venire Caliper.

3.5.2.9 Pod thickness (mm) C : [B-(Ex2)] Pod thickness was measured by subtracting twice thickness of pod wall from width of pod.

3.5.2.10 Thickness of pod and width ratio (C/B) : The ratio of thickness of pod and width of pod was calculated as thickness of pod and pod width ratio.

3.5.2.11 Weight of periphery region of pod (g) : The periphery region of the ten fully developed, healthy, three seeded green pods were removed with the help of sharp razor and weighed in gram after drying at $40\pm 2^{\circ}\text{C}$ for 3 hrs in oven.

3.5.2.12 Seed pod ratio : The ratio between weight of ten fully developed, healthy, three seeded pods (g) with weight of seed from these pods (g) was calculated as seed pod ratio.

3.5.2.13 Moisture of pod wall (%) : The weight of ten yellow coloured fully developed, healthy three seeded pods was weighed in gram before drying as Fresh weight and after drying at $40\pm 2^{\circ}\text{C}$ for 3hrs in an oven as dry weight. The moisture of pod wall in percentage was calculated by

$$\text{Moisture of pod wall (\%)} = \frac{\text{Fresh weight of pod} - \text{Dry weight of pod}}{\text{Fresh weight of pod}} \times 100$$

3.5.2.14 Relative water content of pod wall (%) : 0.5g pod wall (Fresh weight) from fully developed, healthy yellow coloured pod was soaked in 50ml distilled water for 24hrs. The weight of the pod wall after 24 hr of soaking was recorded as turgid weight. It was dried at $40\pm 2^{\circ}\text{C}$ for 3hrs in an oven and the weight was recorded as dry weight

$$\text{Relative water content of pod wall (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

3.6 Screening for shattering resistance

3.6.1 At field level Following observations were recorded on 10 competitive well developed healthy plants from the middle row during *Kharif* 2010.

3.6.1.1 Days to incipient shattering : Number of days from maturity till the day when the pod shattering starts on ten randomly selected plants.

3.6.1.2 Position of first pod dehiscence: The position of the first pod shattered on the plant was measured with the help of scale in cm and classified its position as in upper, middle or lower part.

3.6.1.3 Height of lowest pod shattered (cm) : The height of the shattered pod located nearest to ground surface was measured in cm from the ground surface on ten randomly selected plants and the average value is recorded as height of lowest pod shattered.

3.6.1.4 Degree of shattering (%) : The degree of shattering was calculated with total number of pods/ plant and number of pods shattered at five days interval i.e., on 0, 5, 10, 15 and 20 days after maturity on 10 competitive well developed healthy plants from the middle row.

3.6.2 Pod wall membrane permeability: 0.5g pod wall from fully developed, healthy and yellow coloured randomly selected pod was soaked in 10ml distilled water. After 24hrs of soaking the electrical conductivity was measured with the help of electrical conductivity meter to record pod wall membrane permeability.

3.6.3 Oven dry method : Pods of ten randomly selected healthy plants harvested at maturity were oven dried for 3hrs at 60°C. The number of pods shattered after prescribed period was recorded and shattering percentage was calculated with the help of total number of pod (Funatsuki *et al.* 2006).

3.6.4 Pod Strength Test : It was tested by Texture Analyser with 3 mm cylindrical probe (Plate 1) under the guidance of Professor, Post Harvest Processing CAE, Jabalpur. The pod at physiological maturity with 10-15% moisture content was put over the test platform of Texture Analyser to test the surface hardness. Texture Analyser is a microprocessor controlled texture analysing system, which can be interfaced to a wide range of peripherals, including PC-type computers. Texture analyser measures force, distance and time, thus providing two independent variables (distance and time). Based on two dimensional product analysis, forces is measured against set distances and distances are measured to achieve set forces as per specification given in Table 3.1.

Table: 3.1 Specification of Texture Analyser

Test mode: Measure force in compression	Test option	: Return to start
Specification of probe: 3 mm cylindrical probe	Pretest speed	: 5 mm/sec
Test speed : 2 mm/sec	Post test speed	: 10 mm/sec
Distance moved : 5 mm	Trigger type	: Auto
Trigger force : 25 g	Data Acquisition rate:	200 pps
Accessory : Heavy-duty platform		

The data and data plot were acquired and drawn automatically by the Texture Analyser through the PC attached with the system and the data obtained were used for screening of genotypes.

3.7 Statistical analysis:

The data recorded were subjected to the following statistical analysis.

3.7.1 Mean: It was calculated by using the formula:

$$\text{Mean } (\bar{X}) = \frac{\text{-----}}{N}$$

Where, $\sum x$ = Sum of all observations, N = Number of observations

3.7.2 Arrangement of data in RCBD:

Treatment	Replications					Total
	I	II	III	-	r	
1	Y_{11}	Y_{12}	Y_{13}	-	Y_{1r}	T_1
2	Y_{21}	Y_{22}	Y_{23}	-	Y_{2r}	T_2
-	-	-	-	-	-	-
t.	Y_{t1}	Y_{t2}	Y_{t3}	-	Y_{tr}	T_t
Total	R_1	R_2	R_3	-	R_r	GT

Model => $Y_{ij} = \mu + t_i + r_j + e_{ij}$

Where,

Y_{ij} = Phenotypic performance of j^{th} genotype in i^{th} block

μ = General mean

t_i = i^{th} treatment effect

r_j = j^{th} replication effect

e_{ij} = Random error, which is supposed to be identically and independently distributed with mean zero and variance σ^2 .

To work out the standard error for comparison of means, an ANOVA table was prepared for Randomized Complete Block Design.

3.7.3 Analysis of variance: The data were statistically analyzed on the basis of method described by Panse and Sukhatme (1967) to work out existing variance in different traits.

Skeleton of Analysis of Variance for Randomized Complete Block design

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F value
Replication	(r-1)	RSS	RMS	RMS / EMS
Treatment	(t-1)	TSS	TMS	TMS / EMS
Error	(r-1) (t-1)	ESS	EMS	
Total	(rt - 1)			

r	=	Number of replications	ESS	=	Error sum of square
t	=	Number of treatments	RMS	=	Replication mean sum of square
df	=	Degree of freedom	TMS	=	Treatment mean sum of square
RSS	=	Replication sum of square	EMS	=	Error mean sum of square
TSS	=	Treatment sum of square			

A significant value of F-test indicates that the test entries differ significantly among themselves, which requires computing the critical difference (CD).

$$\text{Coefficient of variation (CV)} = \frac{\sqrt{2 \text{ EMS}}}{\text{G.M.}} \times 100$$

$$\text{Standard Error of difference SE (d)} = \frac{\sqrt{2 \text{ EMS}}}{r}$$

$$\text{Critical difference (CD)} = t_{(0.01)} \times \text{S.Em}_{(d)}$$

Where,

G.M. = General mean; $t_{(0.01)}$ = t-value of 1% probability level, R = Replications

3.7.4 Genetic variability: Genotypic, environmental and phenotypic variances (expressed in percentage) were calculated by the formulae of Burton (1952).

$$\text{Genotypic variance } (\sigma^2_g) = \frac{\text{TMS} - \text{EMS}}{r}$$

$$\text{Environmental variance } (\sigma^2_e) = \text{EMS}$$

$$\text{Phenotypic variance } (\sigma^2_p) = 2\sigma^2_g + \sigma^2_e$$

Where,

TMS = Treatment mean sum of square; EMS = Error mean sum of square; r = replications

3.7.5 Coefficient of variation

The coefficients of variation at genotypic and phenotypic levels were calculated as per the formula proposed by (Burton 1952).

$$3.7.5.1 \text{ Genotypic coefficient of variation (GCV\%)} = \frac{\sqrt{2g}}{\bar{X}} \times 100$$

$$3.7.5.2 \text{ Phenotypic coefficient of variation (PCV\%)} = \frac{\sqrt{2P}}{\bar{X}} \times 100$$

3.7.6 Heritability estimates (h^2_b): It was calculated in broad sense by using the formula proposed by Hanson *et al.* (1956).

$$\text{Heritability (} h^2 \text{)} = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

3.7.7 Genetic advance (GA): Genetic advance was calculated by the formula suggested by Johnson *et al.* (1955).

$$GA = h \times \sigma_p \times K$$

Where,

GA = Genetic advance; σ_p = Phenotypic standard deviation;
 h = Heritability; K = Selection intensity at 1% level of selection *i.e.*, 3.40.

3.7.8 Estimation of correlation coefficients: All correlation coefficients were calculated at genotypic and phenotypic levels using the formula suggested by (Miller *et al.* 1958).

$$r_{x_i x_j} = \frac{\text{Cov}(x_i, x_j)}{\sqrt{\text{Var.}(x_i) \text{Var.}(x_j)}}$$

$$r(x, y) = \frac{\text{Cov}(x_i, y_j)}{\sqrt{\text{Var.}(x_i) \text{Var.}(y_j)}}$$

Where,

$r(x, y)$ = Correlation coefficient between characters x_i and y_j
 $\text{Cov}(x_i, x_j)$ = Covariance of characters x_i and y_j
 $\text{Var. } x_i$ = Variance of character x_i
 $\text{Var. } y_j$ = Variance of character y_j

Genotypic and phenotypic correlation coefficients were compared by substituting the corresponding variance and covariance for all possible characters.

$$\text{Genotypic } r(x_i, y_j) = \frac{\text{Genotypic Cov}(x_i, y_j)}{\sqrt{\sigma_g^2(x_i) \sigma_g^2(y_j)}}$$

$$\text{Phenotypic } r(x_i, y_j) = \frac{\text{Phenotypic Cov}(x_i, y_j)}{\sqrt{\sigma_p^2(x_i) \sigma_p^2(y_j)}}$$

Where,

$\sigma_g^2(x_i)$ = Genotypic variance of character x_i
 $\sigma_g^2(y_i)$ = Genotypic variance of character y_i
 $\sigma_p^2(x_i)$ = Phenotypic variance of character x_i
 $\sigma_p^2(y_i)$ = Phenotypic variance of character y_i

Testing of correlation for significance: To test the significance of correlation, t-value was computed and compared with the tabulated value of 't' at (n-2) degree of freedom at 5 per cent and 1 per cent level.

$$t_e = \frac{r}{\sqrt{1 - r^2}} \quad \text{follows t-distribution with } n - 2 \text{ degree of freedom.}$$

Where,

t_e = Calculated value of 't'
 r = Estimated value of correlation coefficient
 n = Number of observations.

3.7.9 Path coefficient analysis: It is a simple standardized partial coefficient method helpful to detect the direct and indirect effects of the independent

variable on dependent variable. It permits separation of correlation into components of direct and indirect effects.

The method of path coefficient was developed by (Wright 1921) and modified by (Dewey and Lu 1959). The following set of simultaneous equations were formed and used for estimation of direct and indirect effects.

$$r_{iy} = p_{iy} + r_{12}p_{2y} + r_{13}p_{3y} + \dots + r_{iy}p_{iy}$$

$$r_{2y} = r_{2y} p_{iy} + p_{2y} + r_{23}p_{3y} + \dots + r_{21y}p_{1y}$$

$$r_{ky} = r_{ki} + r_{ki} p_{2y} + r_{13}p_{3y} + \dots + r_{2iy}p_{iy}$$

$$r_{xky} = r_{xki} p_{iy} + r_{zk2} p_{2y} + r_{xk3} p_{3y} + \dots + p_{ky}$$

r_{xky} => Coefficient of correlation between the independent character.

p_{iy} to p_{ky} = Direct effects of character 1 to k on dependent character y.

Direct effect: The direct effects were calculated as follows -

$$P_{Ky} = \sum_{i=1}^k C_{Ki} r_{Ky}$$

Indirect effect: Indirect effect of any independent traits on the dependent one via other independent traits was computed by multiplying the direct effect (P_{ky}) of that independent variable with the corresponding correlation coefficient as follows:

$$K^{\text{th}} \text{ trait via } (n-1) = r_k (n-1) P (n-1) Y$$

Residual effect: Residual effect was obtained as per the formula given below:

$$R = \frac{1}{1 - \sum d_i r_{ji}}$$

Where, d_i = Direct effect of character

r_{ij} = Correlation coefficient of i^{th} character with j^{th} character.

Result

The present investigation was carried out on 69 genotypes of soybean to screen expression for shattering and identify shattering tolerant genotypes. To have a clear picture of variability in genotypes, the genetic parameters of variability were studied. Correlation analysis was performed to find the degree of relationship between characters. Path analysis was conducted to determine the direct and indirect effect of various independent on dependent one.

The result of the present investigation are describe under the following heads

- 4.1 Analysis of variance for the observed traits
- 4.2 Mean performance of soybean genotypes
- 4.3 Parameters of genetic variability of the observed traits
- 4.4 Association of observed morphological, phenological traits, pod characters and screening method with shattering tolerance.
- 4.5 Analysis of direct and indirect effect of observed morphological, phonological and pod traits on pod shattering.
- 4.6 Association of quality traits of pod i.e., pod pubescence and presence of four seeded pods with pod shattering.
- 4.7 Classification of genotypes for shattering tolerance based on different screening methods.
- 4.8 Classification of soybean genotypes based on the location of first shattered pod on the plant.
- 4.9 Classification of soybean genotypes based on the height of the lowest pod shattered.
- 4.10 Classification of soybean genotypes based on the days to incipient shattering.

4.1 Analysis of variance of observed traits

Analysis of variance: It refers to the observable differences in individuals for a particular trait. To know extent of variation of observed characters among the 69 genotypes of soybean, analysis of variance was performed in Table 4.1.

Analysis of variance showed a significant variation for all the observed morphological, phenological and pod characteristics. Maximum variance was observed in plant height and lowest for weight of periphery region.

Significant variability was observed for the observed morphological and phenological traits viz., plant height, pods per plant, days to maturity, days to 50% flowering, seed yield per plant and nodes/ plant..

Analysis of variance showed less than 1 variance among the characteristics observed on pod viz., B/A, volumetric weight of pod, D/A, weight of seed / pod, weight of pod, seed pod ratio, C/B, thickness of pod wall, weight of periphery region and A/B. Variance was in between 1-10 for thickness of Pod, width at mid part of pod, width of pod, moisture of pod wall (%) and 100 seed weight. Whereas, variance was high for RWC%, pod wall and Length of pod .

Among the methods of screening for shattering tolerance maximum variability was observed for the Force applied on texture analyser followed by heat treatment and field screening on 20th day after maturity. It was low for membrane thermo-stability test.

Table 4.1 Analysis of variance for observed characteristics in soybean

Sources of Variation	Replication	Treatment	Error
Degree of Freedom	2	68	136
<i>Mean Sum of Squares</i>			
Morphological and phenological traits			
Days to 50% flowering	00.294680	029.3529**	00.3486
Days to maturity	04.004830	095.6317**	00.4411
Plant height	296.84540	717.6817**	39.8797
Nodes per plant	09.144928	010.2003**	01.0370
Pods per plant	01.743961	670.5947**	47.5969
Seed yield per plant	00.178580	026.6934**	01.4462
Pod characteristics			
Weight of pod	0.000183	0.01333**	0.000337
Weight of seed / pod	0.000487	00.0069**	0.000250
Seed pod ratio	0.000094	00.0243**	0.000108
100 seed weight	0.016301	08.8393**	0.034844
Volumetric wt. of pod	0.000003	00.0023**	0.000002
Wt. of periphery region	0.000001	00.1164**	0.000012
Length of Pod (A)	0.381488	46.9638**	0.972070
Width of Pod (B)	0.012938	02.2960**	0.009239
Thickness of Pod (C)	0.017002	01.3479**	0.004679
Thickness of pod wall (E)	0.001193	00.0432**	0.000317
Width at mid part of pod (D)	0.003673	01.5571**	0.003252
A/B	0.000016	00.7296**	0.006965
D/A	0.002667	0.00402**	0.002973
B/A	0.000006	00.0012**	0.000013
C/B	0.000386	00.0284**	0.000102
RWC% pod wall	0.001145	29.9333**	0.002597
Moisture of pod wall %	0.000149	03.2104**	0.000179
Screening technology			
Membrane thermostability	0.000002	0.00026**	0.000007
Screening technology for shattering tolerance			
On 20 th day after maturity	3.475648	562.813**	11.633067
Heat treatment	0.021664	0849.58**	0.049733
Texture Analyzer			
Force	1209126.901	110984.01**	10567.0568
Distance	000.026970	0000.028**	00.025556
Time	000.091261	000.1146**	00.075460

** Significant at 5% level * Significant at 1% level

4.2 Mean performance of observed traits

Mean performance and range of observed traits achieved by population of soybean genotypes are presented in the Table 4.2. Performance of individual characteristic is presented herewith

4.2.1 Morphological and phenological traits

Days to 50% flowering: The mean time required for 50% flowering was 36.66 days with constant minimum limit of 24 and maximum 44 days. Variety JS 95-60 required less than 30 days; 57 genotypes 31 – 40 days and 11 genotypes more than 40 days for days to 50% flowering. JS 95-60 required minimum days (24) for 50% flowering followed by PS 1092 (31), NRC 12 and JS 93-05 (33), whereas maximum days for 50% flowering was required by Punjab 1 (42) followed by NRC 7 and PK 472 (41).

Days to maturity: Days to maturity ranged from 84-104 days. The mean time required for maturity was 95 days. Maximum time (104) was required for maturity in JS 97-52 and MACS 450 followed by PS 1029 (103). The earliest maturity (84) was observed in JS 95-60 and JSM 131 followed by JS 93-05 (90).

Plant Height: It ranged from 38.33-125 cm with mean plant height of 67.48 cm. Maximum plant height was recorded in genotype JS 76-205 (125) followed by JS 92-22 (101) and JS 92-12 (98.33). Minimum plant height was recorded in genotype JS 2 (38.33) followed by JSM 154 (42) and JSM 214 (42.66).

Nodes per plant: Mean nodes per plant ranged from 7-14 nodes with average 10 nodes per plant. Maximum number of nodes per plant was observed in MACS 58 NRC 37 (13) followed by MACS 450 (12). Whereas, minimum number of nodes per plant recorded in JSM 117 (07) followed by PS 1024 and VLS 47 (8).

Pods per plant: Number of pod per plant ranged from 17-93 with mean of 46.44. Genotype JS 97-52 possessed the maximum number of pods per plant (93) followed by JSM 195 (90.66) and JS 76-205 (80.66). The minimum number of pods per plant was recorded in genotypes PS1341 and JSM 20 (17).

Seed yield per plant: It ranged from 1.72-23.39g with mean performance of 6.63g. Maximum seed yield per plant was recorded by genotype JS 97-52 (23.39) followed by JSM 174 (12.61) and JSM 131 (10.81). The lowest seed yield per plant was recorded for MACS 58 (1.72) followed by VLS 47 (2.46) and MAUS 61 (3.10).

4.2.2 Pod characteristics

Weight of pod: It ranged from 0.256-0.556g with the mean of 0.4064g. The maximum weight of pod was recorded in genotype JS 95-60 (0.556) followed by JSM 45 (0.533) and JSM 240 (0.4233). The minimum weight of pod was recorded in NRC 2 (0.256) followed by JSM 202 (0.2867) and MAUS 61 (0.3067).

Weight of seed/pod: It ranged from 0.1400-0.4067g with mean of 0.250. The maximum weight of seed of pod was recorded in JS 95-60 (0.4067) followed by JSM 45 (0.333) and JSM 240 (0.323). Whereas, minimum weight of seed/pod was recorded in NRC 2 (0.1733) followed by JSM 202(0.1432) and JSM 195 (0.1867).

Seed pod ratio: It ranged from 0.1707 - 0.8187g with mean 0.1887g. Maximum seed pod ratio was recorded by JS 93-05 (0.8187) followed by JSM 207 (0.7717) and JS 95-60 (0.7260). The minimum seed pod ratio was recorded by JS 76-205 (0.1707) followed by JS 2 (0.4493) and JSM 202 (0.4880).

100 seed weight: It ranged from 4.2 – 15.366g with mean 8.918g. The maximum hundred seed weight was recorded by the genotype NRC 12 (15.366) followed by JSM 127 (13.566) and JSM 170 (12.166). The minimum hundred seed weight was recorded by the genotype MAUS 61 (4.2) followed by JSM 202 (5.333) and JSM 256 (5.766).

Volumetric weight of pod: Volumetric weight of pod ranged from 4.77 to 4.90g with a mean 4.85. Maximum volumetric weight of seed was recorded by NRC 37(4.90) followed by JSM 207 and JSM 170 (4.89). The minimum volumetric weight of seed was recorded by the genotype Harasoya (4.77) followed by NRC 12 (4.81) and NRC 2 (4.80).

Weight of periphery region: It ranged from 0.1186-1.3573 with a mean of 0.2068g. Maximum weight of periphery region in genotype JSM 135 (1.3573) followed by JSM 131 (1.2883) and SL 295 (0.2741). The minimum Wt. of periphery region was recorded in genotype TAMS 98-21 (0.1186) followed by JSM 154 (0.1387) and JSM 152 (0.1392).

Length of Pod (A): Length of pod ranged from 36.44-53.93 with a mean of 43.562mm. Maximum length of pod was recorded by genotype NRC 12 (53.92) followed by JSM 184 (51.76) and Harasoya (51.006). The minimum length of pod was recorded by genotype MAUS 61-2 (36.44) followed by PK 472 (37.325) and MAUS 71(36.4627).

Width of Pod (B): It ranged from 4.656-10.151mm with a mean of 8.6407mm. Maximum width of pod was recorded by genotype Harasoya (10.1513) followed by NRC 12 (10.056) and PK 472 (7.743). The minimum width of Pod was recorded by genotype MAUS 81 (4.656) followed by JSM 155 (7.474) and JS 76-205 (7.631).

Thickness of Pod (C): It ranged from 3.034-6.3487mm with a mean of 4.7353mm. Maximum thickness of pod was recorded by genotype TAMS 98-21 (6.3487) followed by TAMS 38 (6.336) and Harasoya (6.2567). The minimum thickness of pod was recorded by genotype JSM 229 (3.034) followed by JS 2 (3.4527) and JSM 170 (3.6233).

Thickness of pod wall (E): It ranged from 0.1153-0.7233mm with a mean of 0.3439mm. Maximum thickness was recorded in Harasoya (0.7232) followed by

PS 1225 (0.533) and PS 1241(0.5123). The minimum thickness was recorded by genotype JSM 207 (0.1153) followed by JSM 195 (0.1187) and JS 95-60 (0.1453).

Table 4.2 Mean performance for observed traits on soybean genotypes

Observation	Mean	Range	SE	CD (5%)
Morphological and phenological traits				
Days to 50% flowering	36.6667	24 - 44	0.4821	0.9533
Days to maturity	94.3478	84 – 104	0.5423	1.0724
Plant height	67.4831	38.33 – 125	5.1562	10.1967
Nodes per plant	10.3478	7.002 – 14.33	0.8333	1.6479
Pods per plant	46.4444	17.00 – 93.00	5.6331	11.1397
Seed yield per plant	6.6337	1.7267 – 27.0467	0.9819	1.9418
Pod characteristics				
Weight of pod	0.4064	0.2567 - 0.5567	0.0150	0.0296
Weight of seed/ pod	0.2500	0.1400 – 0.3667	0.0036	0.0100
Seed pod ratio	0.6069	0.1707 – 0.1887	0.0085	0.0168
100 seed weight	8.9189	4.200 – 15.4333	0.1524	0.3014
Volumetric wt. of pod	4.8509	4.7773 – 4.9013	0.0017	0.0034
Wt. of periphery region	0.2068	0.1186 – 1.3573	0.0028	0.0055
Length of Pod (A)	43.5626	36.44 - 51.76	0.8050	1.5920
Width of Pod (B)	8.6407	7.4740 – 10.1513	0.0785	0.1552
Thickness of Pod (C)	4.7353	3.0340 – 6.3487	0.0559	0.1105
Thickness of pod wall (E)	0.3439	0.1153 – 0.5333	0.0145	0.0202
Width at mid part of pod (D)	8.5903	7.2320 – 10.3680	0.0466	0.0921
A/B	5.0258	3.8560 – 6.6610	0.0537	0.1062
D/A	0.2016	0.1590 – 0.4627	0.0445	0.0881
B/A	0.2002	0.1500 – 0.2587	0.0030	0.0059
C/B	0.5410	0.1307- 0.8127	0.0084	0.0166
RWC% pod wall	14.9282	9.0323 – 20.3567	0.0410	0.0819
Moisture of pod wall %	17.1693	14.9567 – 18.8333	0.0142	0.0283
Screening technology for shattering tolerance				
Membrane thermostability	1.4735	1.4577 -1.4887	0.0012	0.0024
Shattering percentage				
On 20th day after maturity	29.1124	7.6730 - 67.0533	2.7848	5.5072
Heat treatment	36.1619	18.323-89.8333	0.1821	0.3601
Texture Analyzer				
Force	600.25	245.40 – 1013.6	83.93	167.57
Distance	0.2504	0.0717 – 0.6233	0.1305	0.2606
Time	0.4810	0.1367 – 1.2400	0.2243	0.4478

Width at mid part of pod (D): It ranged from 7.2320-10.3680mm with a mean of 8.5903mm. Maximum width at mid part of pod was recorded by genotype JS 2 (10.3680) followed by NRC 12 (9.7207). The minimum width at mid part of pod wall was recorded by genotype JS 76-205 (7.232) followed by PS 1241 (7.443) and PS 1347 (7.4727).

A/B: It ranged from 3.856-6.661mm with a mean of 5.0258mm. Maximum A/B was recorded by genotype PS JSM 240 (6.661) followed by PS 1241 (5.6753) and PS 1092 (5.6210). The minimum A/B was recorded by genotype PK 472 (3.856) followed by MAUS 61 (3.944) and MAUS 47 (3.9423).

D/A: It ranged from 0.1587-0.4627mm with a mean of 0.2016mm. Maximum D/A was recorded by genotype MACS 58 (0.4627) followed by MAUS 61-2 (0.2233) and PK 472 (0.2423). The minimum D/A was recorded by genotype PS 1241 (0.1587) followed by PS 1347 (0.1567).

B/A: It ranged from 0.1500-0.2587 mm with a mean of 0.2002mm. Maximum B/A was recorded by genotype PK 472 (0.287) followed by MAUS 71-2 (0.2533) and JS 97-52 (0.2133). The minimum B/A was recorded by genotype JSM 245 (0.1500) followed by PS 1092 (0.1777) and JSM 127 (0.1737).

C/B: C/B ratio ranges from 0.1307-0.8127 with a mean of 0.5410. The maximum C/B ratio was recorded in genotype JS 76-205 (0.8127) followed by JS 92-22 (.6343) and JS 92-12 (.6313). The minimum C/B was recorded by genotype JSM 248 (0.1307) followed by JS-2 (0.3457) and JSM 152 (0.4213).

Relative water content percent pod wall: It ranged from 9.0323-20.3567 with a mean of 14.928. Percent maximum relative water content was recorded by genotype JS 76-205 (20.3567) followed by PS 1347 and JS 92-12 (19.24). The minimum relative water content percent was recorded by genotype NRC 7 (9.0323) followed by Shivalik (9.1867) and NRC 37 (9.8143).

Moisture percent of pod wall: Moisture of pod wall ranged from 14.6067-19.873, with a mean of 17.169 percent. The maximum moisture in pod wall was recorded in genotype NRC 2 (19.873) followed by JS 93-03 (18.833) and SL 295 (18.550), whereas, minimum moisture of pod wall was recorded by genotype Harasoya (14.9567) followed by JSM 131 (14.6067) and PS 1092 (15.4233).

4.2.3 Screening for shattering tolerance

4.2.3.1 Membrane thermo-stability : Mean membrane thermo-stability was 1.4735 μ S with a range of 1.4577 to 1.4887 μ S. Genotypes were classified in three groups based on membrane thermo-stability i.e., high (>1.48 μ S) as tolerant; medium (1.6-1.7 μ S) as moderately tolerant and low (<1.5 μ S) as sensitive. In all 13 genotypes were classified as tolerant, six as sensitive and rest as moderately tolerant. Genotype Harasoya, JS 93-50, JS 95-60, JS 335, MAUS 61-2 and other genotypes were classified as tolerant. Whereas, JS 97-52, JSM 131, JSM 37, NRC 2, Pb 1 and TAMS 98-21 as sensitive. (Table 4.3).

Table 4.3 Classification of soybean genotypes for shattering based on screening by membrane thermo-stability

Tolerant >1.48 μ S	Moderately tolerant 1.6-1.7 μ S	Sensitive <1.5 μ S
Harasoya, JS 93-05, JS 95-60, JS 335, JSM 170, JSM 256, MACS 58, MAUS 61-2, MAUS 81, NRC 12, PK 416, PK 472, VLS 47	JS 2, JS 76-205, JS 92-12, JS 92-22, JSM 3, JSM 5, JSM 7, JSM 20, JSM 45, JSM 104, JSM 117, JSM 120A, JSM 120B, JSM 127, JSM 135, JSM 152, JSM 154, JSM 155, JSM 170, JSM 184, JSM 189, JSM 195, JSM 200, JSM 202, JSM 203, JSM 205, JSM 207, JSM 214, JSM 227, JSM 228, JSM 229, JSM 239, JSM 240, JSM 245, JSM 248, MACS 450, MAUS 47, MAUS 61, MAUS 71, NRC 7, NRC 37, PS1024, PS 1042, PS 1092, PS 1225, PS1241PS 1347, TAMS 38, SHIVALIK	JS 97-52, JSM 131, JSM 37, NRC 2, Pb1, TAMS 98-21

4.2.3.2 Screening for shattering tolerance at field level:

Screening on the day of physical maturity: The mean shattering was 0.553% with a range from 0% to 6.93% on the day of harvestable maturity. Genotypes were classified in three groups (Table 4.4) based on shattering percentage on the day of maturity viz., no shattering (51 genotypes); 0.1 – 3 % shattering (15 genotypes) and > 3% shattering (3 genotypes). Maximum percent of shattering was observed in genotype) JSM 131 (6.93) followed by NRC 2 (5.25%), JSM 45 (4.66) and MACS 450 (2.44).

Table 4.4 Classification of soybean genotypes for shattering based on screening at field level on the day of physical maturity

Shattering of genotypes at day of maturity	Genotypes	
	Number	Name
0%	51	MACS 450, JS 92-22, JS92-12, JS 93-05, JS335, MACS 58, MAUS 61-2, MAUS 71, MAUS 81, NRC 7, NRC 37, MAUS 61, NRC 12, PK 416, PK 472, PS 1024, PS 1042, PS 1029, PS 1092, PS1225, PS1241, PS 1347, TAM5 98-21, VLS 47, JSM 3, JSM 7, JSM 20, JSM 45, JSM104, JSM 117, JSM 120A, JSM 120B, JSM 127, JSM 152, JSM154, JSM155, JSM170, JSM184, JSM189, JSM195, JSM200, JSM203, JSM214, JSM227, JSM228, JSM239, JSM240, JSM245, JSM256
0.1 – 3%	15	Harasoya, JS 76-205, JS 95-60, JS 97-52, MAUS 47, SL 295, TAM5 38, JS 2, JSM 5, JSM 135, JSM202, JSM248, JSM250, JSM207, JSM229, Pb1, Shivalik,
>3%	3	NRC 2, JSM 131, JSM 37

Screening on 5th day after maturity: The mean shattering was 2.99% with a range from 0% to 12.7%. Genotypes were classified in three groups (Table 4.5) based on shattering percentage on the day of maturity viz., no shattering (29 genotypes); 0.1 – 5% shattering (27 genotypes) and > 5% shattering (13

genotypes). Maximum shattering was observed in genotype Shivalik (12.7) followed by NRC 2 (12.32), JSM 45 (11.1), JSM 131 (11.65).

Table 4.5 Classification of soybean genotypes for shattering based on screening at field level on 5th day after maturity

Tolerant 0%	Moderately tolerant 0.1-5 %	Sensitive >5%
MACS 450, MACS 58, MAUS 61-2, NRC 7, NRC 37, JS 93-05, JS 97-52, JS335, JS 92-12, JS 95-60, MAUS 71, MAUS 81, NRC 12, PK 416, PK 472, PS 1024, PS 1029, PS1241, PS 1347, VLS 47, JSM 7, JSM104, JSM 117, JSM 120B, JSM 127, JSM154, JSM155, JSM170, JSM189, JSM195, JSM228, JSM240, JSM229, JSM245	Harasoya, JS 76-205, MAUS 61, PS 1092, PS1225, SL 295, TAMS 98-21, JS 92-22, JSM 3, JSM 5, JSM 20, JSM 120A, JSM184, JSM202, JSM200, JSM203, JSM227, JSM248, JSM250, JSM207, JSM239, JSM256	MAUS 47, Shivalik, NRC 2, JS 2, JSM37, JSM 45, JSM 131, JSM 135, JSM 152, JSM214, Pb1, PS 1042, TAMS 38

Screening on 10th day after maturity: The mean shattering was 10.42% with a range from 0% to 25.85%. Genotypes were classified in three groups (Table 4.6) based on shattering percentage on the day of maturity viz., no shattering (10 genotypes); 0.1 – 5% shattering (49 genotypes) and > 5% shattering (10 genotypes). Maximum shattering was observed in genotype JSM 131 (25.85) followed by JS2 (25.7), Shivalik (17.6) and JS 97-52 (16.6%).

Table 4.6 Classification of soybean genotypes for shattering based on screening at field level on 10th day after maturity

Tolerant 0%	Moderately tolerant 0.1-15 %	Sensitive >15%
VLS 47, JS92-12, JSM104, JSM 117, JSM154, JSM155, JSM170, JSM189, JSM229, JSM240	Harasoya, JS 93-05, JS 95-60, JS335, SL 295, TAMS 38, JS 2, JSM 5, JSM202, JSM248, JSM250, JSM207, MACS 58, MACS 450, MAUS 61-2, MAUS 71, MAUS 81, NRC 12, NRC 37, PK 416, PK 472, PS 1024, PS 1042, PS 1029, PS 1092, PS1225, PS1241, PS 1347, TAMS 98-21, JSM 3, JSM 7, JSM 20, JSM 37, JSM 120B, JSM 127, JSM 152, JSM184, JSM195, JSM200, JSM203, JSM214, JSM227, JSM228, JSM245, JSM239, NRC 7, JS 92-22, MAUS 61	JS 76-205, JS 97-52, MAUS 47, NRC 2, Shivalik, JSM 131, JSM 45, JSM 120A, JSM 135, Pb1

Screening on 20th day after maturity: Mean shattering on 20th day after maturity was 29.71 percent with range from 7.67 to 67.053 percent. Genotypes were classified in three groups (Table 4.7) based on shattering percentage 20 days after maturity viz., less than 15% shattering as tolerant (6 genotypes); 15-35% shattering as moderately tolerant (57 genotypes) and more than 35% shattering as sensitive (06) genotypes. Minimum shattering was observed in genotype JSM 170 (7.67%) followed by VLS 47 (7.38%) and MAUS 61-2 (9.55%), whereas highest shattering was recorded in the genotype JSM 131 (67.05%) followed by JSM 37 (52.11%).

Table 4.7 Classification of soybean genotypes for shattering based on screening at field level on 20th day after maturity

Tolerant <15%	Moderately tolerant 15%– 35 %	Sensitive >35%
JS 335, JSM 170, MAUS 61-2, PK416, PK472, VLS 47	Harasoya, JS76-205, JS 93-05, JS 95-60, JS 97-52, JS 92-12, JSM 5, JSM 3, JSM 7, JSM 20, JSM 37, JSM 45, JSM 104, JSM 117, JSM 120A, JSM 120B, JSM 127, JSM 135, JSM 152 JSM154, JSM 155,, JSM 184, JSM189, JSM195, JSM 200, JSM 202, JSM 203, JSM 214, JSM 205, JSM 207, JSM 228, JSM227, JSM229, JSM239, JSM 240, JSM 245, JSM248, JSM 256, MACS 450, MAUS 47, MACS 58, MAUS 61, MAUS 71, MAUS 81, NRC 7, NRC 12, NRC 37, PS 1042, PS 1024, PS 1029, PS1092, PS1225, PS1241, PS1347,SL 295, TAMS 38, TAMS 98-21	SHIVALIK, NRC2, JS2, Pb1, JSM 37, JSM 131

4.2.3.3 Screening by heat treatment: Mean shattering percentage by heat treatment was 36.16% with a range from 18.32 to 89.83%. Genotypes were classified in three groups (Table 4.8) based on shattering percentage by heat treatment viz., less than 30% shattering as tolerant (03 genotypes); 30-60% shattering as moderately tolerant (54genotypes) and more than 60% shattering as sensitive (12) genotypes. Minimum shattering percentage was observed in MAUS 61(18.32%), PS1029 (24.6%). Whereas, it was highest in JSM 131 (89.83%) followed by JSM 117 (69.62%)

Table 4.8 Classification of soybean genotypes for shattering tolerance based on screening by heat treatment

Tolerant <30%	Moderately tolerant 30%- 60%	Sensitive >60%
JS 335, JSM 170, MAUS 61-2	Harasoya, JS92-12, JS92-22, JS 93-05, JS 95-60, JSM 5, JSM 7, JSM 20, JSM120B, JSM117, JSM45, JSM104, JSM120A, JSM127, JSM135, JSM 152, JSM154, JSM155, JSM184, JSM 189, JSM195, JSM 200, JSM 202, JSM203, JSM205, JSM207, JSM214, JSM227, JSM228, JSM229, JSM239, JSM240, JSM245, JSM256, MACS58, MACS61, MACS450, MAUS47, MAUS71, MAUS81 NRC 7, NRC12, NRC37, PK416, PK472, PS1029, PS1092, PS1042, PS1225, PS1241, PS1347, SHIVALIK, TAMS38, VLS47	JS2, JS76-205, JS97-52, JSM 37, JSM117, JSM 131, JSM 248, PS1024, NRC 2, Pb1, SL295, TAMS98-21

4.2.3.4 Screening by texture analyser : Mean force applied for screening of shattering tolerance by texture analyser was 600 (g) with range of 245 to 1013g. Genotypes were classified in three groups (Table 4.9) based on the force applied to break the pod from suture *viz.*, more than 800g force as tolerant (12 genotypes): 500-800 g as moderately tolerant (58 genotypes) and less than 500g as sensitive (9 genotypes). Minimum force for shattering was required in JSM 131 (245g) followed by JS 97-52 (346g) whereas maximum force was required in MAUS 47 (1013g) followed by NRC 12 (924g).

Table 4.9 Classification of soybean genotypes for expression of pod shattering based on force applied by texture analyser

Tolerant >800g	Moderately tolerant 500-800g	Sensitive < 500g
JSM 170, JSM 104, JSM 154, JSM 170, JSM 184, JSM207, JSM 214, JSM 227, MAUS 47, Harasoya, NRC 12, NRC 37	JS 92-12, JS 92-22, JS 93-05, JS 97-60, JS 335, JSM 3, JSM 5, JSM 7, JSM 20, JSM 45, JSM 117, JSM 120A, JSM 120B, JSM 127, JSM 135, JSM152, JSM155, JSM 189, JSM195, JSM200, JSM 202, JSM 203, JSM205,, JSM228, JSM229, JSM239, JSM240, JSM245, JSM248, JSM256, PS1024, PS1042, MACS58, MACS450, MAUS61, MAUS61-2, MAUS71, MAUS81 NRC2, NRC 7, PK416, PK472, PS1029, PS1092, PS 1225, PS1241, PS1347, SL295, TAMS38, TAMS98-21	JS76-205, JS97 52, JSM 37, Shivalik, JS2, PI 1, PS 1024 JSM131, VLS 47

4.3 Parameters of genetic variability

4.3.1 Coefficient of variation: To get a more clear picture concerning the variability in genotypes, variance was calculated at phenotypic and genotypic levels for each observed traits (Table 4.10).

Morphological and phenological traits Phenotypic variation was higher in magnitude than genotypic variation for all the observed traits (Table 4.7). Highest phenotypic variation was recorded for seed yield per plant (139.51), followed by pods per plant (38.11), plant height (26.20) and nodes per plant (22.60). However lowest phenotypic variation was recorded for days to 50% flowering (10.11) and days to maturity (7.629).

Genotypic variation was highest for seed yield per plant (44.924), followed by pods per plant (31.028), plant height (22.274) and nodes per plant (16.889). However genotypic variation was lowest for days to maturity (5.984) followed by days to 50% flowering (8.531).

Pod characteristics Highest phenotypic variation was recorded for weight of periphery region (102.78) followed by thickness of pod wall (35.59), weight of seed/ pod (24.85) and Relative Water Content (%) of pod wall (23.02). However, lowest phenotypic variation was recorded for volumetric weight of seed (0.63) followed by moisture % of pod wall (6.69), width at mid part of pod wall (8.60) and length of pod (9.876).

Highest genotypic variation was recorded for weight of periphery region (95.279) followed by thickness of pod wall (34.807) , RWC (%) of pod wall (21.159) and hundred seed weight (19.538). Whereas, lowest genotypic variation was recorded for volumetric weight of seed (0.571) followed by moisture percent of pod wall (6.025) and width at mid part of pod wall (8.378).

Screening technology for shattering tolerance Highest phenotypic and genotypic variations were recorded for shattering on 20th day after maturity (111.22; 93.450) followed by heat treatment (90.78; 81.695). The phenotypic and genotypic variations were lowest for membrane thermo-stability (0.73; 0.643).

4.3.2 Heritability in broad sense

Morphological and phenological traits Heritability in broad sense was highest for plant height (85.0), followed by days to flowering (84.3), pods per plant (81.4). However, lowest heritability in broad sense was recorded for seed yield per plant (32.2).

Pod characteristics Heritability in broad sense was highest for thickness of pod wall (97.8) followed by width at mid part of pod wall (97.4), pod length: width ratio (97.1) and pod width: length ratio (96.8). However lowest heritability in broad sense was recorded for weight of pod (78.2) followed by weight of seed / pod (78.2) and seed pod ratio (88.7).

Screening technology for shattering tolerance Heritability in broad sense was highest for heat treatment (89.98) followed by membrane thermo-stability (87.63). However lowest heritability in broad sense was recorded for 20th day after maturity (84.02).

Table 4.10 Genetic Parameters of variation for observed traits in soybean

Observation	CV		h ²	GA	GA as % of mean
	PCV	GCV			
Morphological and phenological traits					
Days to 50% flowering	10.11	8.531	84.3	06.44	017.57
Days to maturity	7.629	5.984	78.5	11.63	012.32
Plant height	26.20	22.274	85.0	28.54	042.30
Nodes per plant	22.60	16.889	74.7	03.11	030.06
Pods per plant	38.11	31.028	81.4	26.77	057.65
Seed yield per plant	139.51	44.924	32.2	03.48	052.53
Pod characteristics					
Weight of pod	20.16	15.766	78.2	00.13	032.17
Weight of seed / pod	24.85	19.483	78.4	00.10	039.81
Seed pod ratio	16.70	14.820	88.7	00.18	030.33
100 seed weight	22.00	19.538	88.8	03.58	040.24
Volumetric wt. of pod	0.63	0.571	89.8	00.05	001.17
Wt. of periphery region	102.78	95.279	92.7	00.40	196.24
Length of Pod (A)	9.876	8.988	91.0	07.82	017.95
Width of Pod (B)	11.12	10.104	90.8	01.78	020.69
Thickness of Pod (C)	15.78	14.205	90.0	01.37	028.95
Thickness of pod wall (E)	35.59	34.8074	97.8	00.24	070.92
Width at mid part of pod (D)	8.60	8.378	97.4	01.47	017.20
A/B	10.05	9.7659	97.1	00.99	019.83
D/A	10.23	9.265	90.5	00.01	006.18
B/A	10.31	9.986	96.8	00.04	020.24
C/B	19.54	17.959	91.9	00.19	036.79
RWC% pod wall	23.02	21.159	91.9	06.50	043.58
Moisture of pod wall %	6.69	6.025	89.9	02.13	012.41
Screening technology for shattering tolerance					
Membrane thermo-stability	0.73	0.643	87.63	00.01	001.32
<i>Shattering percentage</i>					
on 20 th day after maturity	111.22	93.450	84.02	29.97	156.83
Heat treatment	90.78	81.695	89.98	39.61	151.41

4.3.3 Genetic advance

Morphological and phenological traits The highest amount of genetic advance was observed for plant height (28.54) followed by pods per plant (26.77), days to maturity (11.63). Whereas lowest amount of genetic advance was observed for nodes per plant (3.11) followed by seed yield per plant (3.48).

Pod characteristics The highest amount of genetic advance was observed for length of pod (7.82) followed by relative water content (%) of pod wall (6.50) and hundred seed weight (3.58). Whereas, lowest amount of genetic advance was observed for width at mid part of pod: length of pod (0.01) followed by width of pod: length of pod (0.04) and volumetric weight of seed (0.05).

Screening technology for shattering tolerance The highest amount of genetic advance was observed for heat treatment (39.61) followed by 20th day after maturity (29.97). Whereas, It was lowest for membrane thermo-stability (0.01).

4.4 Association analysis

Correlation analysis is a statistical tool that provides the degree and direction of relationship between two variables. Association analysis was performed among all the observed morphological and phenological traits and characteristics of pods. Association of observed pod traits were also worked out with pod shattering percentage screen by different methods and among different methods adopted for screening of soybean genotypes for shattering tolerance.

4.4.1 Association analysis among observed morphological and phenological traits of soybean

The association analysis (Table 4.11) among the observed morphological and phenological traits revealed significant positive association of seed yield per plant with number of pods per plant (0.527) and number of nodes per plant (0.289).

Out of these two traits, number of pods per plant had significant positive association with number of nodes per plant (0.464) and plant height (0.278).

Table 4.11 Association analysis among observed morphological and phenological traits in soybean

	Days to maturity	Plant height	Node/ plant	Pod/ plant	Seed yield/ plant
Days to 50% flowering	0.5138**	0.3254**	0.1294	0.2221**	-0.0456
Days to maturity		0.2797**	-0.3297**	-0.0553	-0.0372
Plant height			0.2729**	0.2783**	0.0588
Node/ plant				0.4614**	0.2897**
Pod/ plant					0.5271**

***Significant at 1% level*

4.4.2 Association of shattering with morphological and phenological traits

All the Phenological and morphological traits had non -significant association with shattering percentage on zero, five and ten days after maturity. Days to 50% lowering, days to maturity and seed yield/plant had significant negative association with shattering percentage observed on 20th day after maturity (-0.1987, -0.3625 and, -0.1955, respectively) and by heat treatment (-0.1780, -0.2062 and -0.2825* respectively), Whereas, Distance and time applied on texture analyser for screening showed significant negative association with days to maturity (Table 4.12). Other associations were non- significant.

Table 4.12 Association analysis of morphological and phenological with shattering percentage

Method		Days to 50% flowering	Days to maturity	Plant height	Nodes per plant	Pods per plant	Seed yield per plant
Days after maturity	00	0.0543	0.02620	-0.0987	0.0423	-0.0347	0.0872
	05	0.0714	0.0695	-0.0902	-0.0470	0.0179	-0.0989
	10	-0.1478	-0.2468*	-0.1267	-0.0089	0.0549	0.1799*
	20	-0.1987*	-0.1780*	-0.1163	-0.0303	-0.0878	-0.1955**
Heat treatment		-0.3625*	-0.2062*	-0.1433	-0.1185	-0.2426*	-0.2825*
Texture analyser	Force	-0.2981	-0.0568	-0.0053	0.0681	0.1310	-0.1105
	Distance	-0.0336	-0.1872*	0.1010	0.1052	0.0766	0.1041
	Time	-0.0423	-0.1742*	0.0995	0.1051	0.1164	0.1168

***Significant at 5% level*

4.4.3 Association analysis among pod characteristics in soybean

The association analysis among pod characteristics observed in soybean (Table 4.13) revealed significant positive association of pod weight with one hundred seed weight (0.4305), pod length (0.4251), pod thickness (0.3426), pod thickness and width ratio (0.2713), volumetric weight of seed (0.1405), weight of periphery region (0.1623), and width of pod at mid part (0.1687). Whereas, it was significant and negative with pod width and length ratio (-0.3615) and (-0.1997).

Seed pod ratio had significant positive association with moisture percent of pod wall (0.2862) and relative water content percent (0.1653). Whereas, its association was significant negative with pod thickness (-0.1596), pod wall thickness (-0.2119) and pod thickness : width ratio (-0.2149).

One hundred seed weight had significant positive association with weight of periphery region of pod (0.1631), pod length (0.3910), width at mid part of pod (0.1617), pod length: width ratio (0.2447) and moisture percent of pod wall (0.1501). Its was significant negative with pod width :length ratio (-0.2558).

Volumetric weight of seed had significant positive association with moisture percent of pod wall (0.1605) and significant negative with pod wall thickness (-0.2291). Weight of periphery region of pod had significant negative association with pod wall thickness and volumetric weight of seed but positive with relative water content of pod wall (0.1535).

Significant positive association of pod length was observed with pod width (0.3856), pod wall thickness (0.2704), width at mid part of pod (0.4652) and pod length :width ratio (0.5524). However, its association with width at mid part of pod : pod length (-0.5837) and relative water content of pod wall (-0.2636) was significant and negative .Pod width had positive association with pod width at mid part (0.7314),pod width :length ratio (0.4027) and pod length. Whereas, it was

negatively associated with pod length: width ratio (-0.4124) and pod thickness : width ratio (-0.3621).

Association of pod thickness was significant and positive with thickness : width ratio (0.6526), relative water content (0.3292) and pod weight. However, its association was negative with moisture percent of pod wall (-0.3974) and seed pod ratio. Association of pod wall thickness was positive and significant with width of mid part of pod (0.1597), pod length : width ratio (0.1888) and pod length. However, its association was significant negative with pod width: length ratio (-0.1500), moisture percent of pod wall (-0.3955), seed pod ratio and volumetric weight of seed.

Pod width at mid part had significant negative association with pod length: width ratio (-0.2126), pod width length ratio (0.1971), pod thickness: width ratio (-0.3728) and relative water content (-0.4205). Pod length width ratio had significant negative association with pod width at mid part : pod length(-0.2648) and pod width length ratio (-0.1740), whereas, its association was significant and positive with pod thickness and width ratio.

Pod width at mid part: pod length had significant positive association with pod width: length ratio(0.2638). Pod width length ratio had significant positive association with pod thickness: pod width ratio (-0.2654). Whereas, pod thickness : width ratio

Whereas, number of nodes per plant had significant positive association with plant height (0.2729). Plant height had significant positive association with days to flowering (0.3254) and maturity (0.2797). Whereas, days to flowering had positive association with days to maturity (0.5138), significant negative association was observed only between number of nodes per plant and days to maturity (0.3297).had significant positive association with relative water content (0.9893).

Table 4.13

4.4.4 Association of observed pod traits with pod shattering percent recorded in different screening method

The association analysis of shattering percentage on 20th day after maturity (Table 4.14) was significant and positive with seed pod ratio (0.1832), one hundred seed weight (0.1586), weight of periphery region (0.3685), pod length (0.2128), pod width (0.3020) and width at mid part (0.2582). Whereas, significant and negative with volumetric weight of seed (-0.1726) and pod thickness: width ratio (-0.1461).

Association analysis of shattering percentage by heat treatment was significant and positive with seed pod ratio (0.1928), weight of periphery region (0.4306), pod width (0.3020), width at mid part of pod (0.1813) and volumetric weight of seed (0.1947). Whereas, significant and negative with pod weight (-0.0163), and pod thickness: width ratio (-0.1750).

Association analysis of force applied in texture analyzer was significant and positive with 100 seed weight (0.2113), volumetric weight of seed (0.1433), weight of periphery region (0.3278) and width at mid part of pod (0.1892). Whereas, significant and negative with pod thickness: width ratio (-0.2041).

4.4.5 Association analysis among different methods adopted for screening of soybean genotypes for shattering tolerance

Association analysis among different methods adopted for screening of soybean genotypes for shattering tolerance (Table 4.15) revealed that shattering percent on 20th day had significant positive association with shattering percent by heat treatment (0.5186), distance (0.1800) and time (0.1942) required in texture analyzer. Whereas, shattering percent by heat treatment had significant positive association with time (0.2948) required in texture analyzer. Force required in texture analyzer had significant positive association with distance (0.2511) required in texture analyzer. Whereas, distance required in texture analyzer had significant positive association with time (0.8695) required in texture analyzer.

Table 4.14 Association among pod traits with pod shattering percentage screened by different methods in soybean

Pod traits	20 th day after maturity	Shattering (%) by heat treatment	Force applied in texture analyzer
Pod weight	0.1106	-0.0163	0.0013
Seed pod ratio	0.1832**	0.1928**	0.0260
100 seed weight	0.1586*	0.0276	0.2113**
Volumetric weight of seed	0.1726*	0.1947*	-0.1433*
Weight of periphery region	0.3685**	0.4306**	0.3278**
Pod length (A)	0.2128**	0.1011	0.1348
Pod width (B)	0.3020**	0.1849**	0.2140**
Pod thickness (*C)	0.0019	0.0465	-0.0636
Pod wall thickness (E)	0.0345	-0.0147	0.0753
Width at mid part (D)	0.2582**	0.1813**	0.1892**
A/B	-0.0994	-0.0616	-0.0533
D/A	-0.0707	-0.0711	0.0254
B/A	0.0457	0.0523	0.0840
C/B	-0.1461*	-0.1750*	-0.2041**
RWC%	-0.1188	0.059	0.1001
Moisture % of pod wall	0.0598	-0.0171	0.1075

* Significant at 5% level **Significant at 1% level

Table 4.15 Association analyses among different methods adopted for screening of soybean genotypes for shattering tolerance

Methods	Heat treatment		Texture analyser	
	Shattering (%)	Force	Distance	Time
Shattering (%) on 20 th day after maturity	0.5186**	-0.1117	0.1800*	0.1942*
Shattering (%) in heat treatment		-0.0823	-0.0499	0.2948**
Force applied in texture analyser			0.2511**	0.0084
Distance				0.8695**

*Significant at 5% level ** Significant at 1% level

4.4.6 Association analysis of pod characteristics with screening of soybean genotypes with texture analyser

Association of force applied was significant and positive (Table 4.16) with one hundred seed weight, weight of periphery region, width of pod and width at mid part of pod. Whereas, significant and negative with pod thickness: width ratio.

Association of distance applied in texture analyzer was significant and positive with seed pod ratio and width at mid part. Association of time required in texture analyzer was significant and positive with seed pod ratio and width of pod at mid part.

Table 4.16 Association analysis of observed pods traits of soybean with texture analyzer for shattering tolerance

Pod characteristics	Force	Distance	Time
Wt. of pod	0.0013	0.0620	0.0209
Seed pod ratio	0.0260	0.1338*	0.1584*
100 seed weight	0.2113**	0.0652	0.0745
Volumetric weight of seed	-0.0433	0.0179	0.0937
weight of periphery region	0.3278**	0.0104	-0.0289
Length of pod (A)	0.1348*	0.0261	-0.0314
Width of pod (B)	0.2140**	0.0751	0.0519
Thickness of pod(C)	-0.0636	0.0815	-0.0274
Thickness of pod wall (E)	0.0753	0.0144	-0.0266
Width at mid part (D)	0.1892*	0.1607*	0.1471*
A/B	-0.0533	-0.0350	-0.0775
D/A	0.0254	0.0538	0.0712
B/A	0.0840	0.0183	0.0581
C/B	-0.2041**	0.0227	-0.0546
RWC% pod wall	0.1001	-0.0188	0.0201
Moisture of pod wall (%)	0.1075	0.0018	0.0428

**Significant at 1 % level

4.5 Path coefficient analysis

It is standardized partial regression coefficient, which splits the correlation into the measures of direct and indirect effects. It measures the direct and indirect contribution of various independent characters on dependent characters, for a replicated data. Path coefficients are worked out from all possible correlation coefficient among the various characteristics under study. Direct effect is the straightway effect of an independent character on a dependent one. Indirect effect is effect of an independent character on dependent one via other independent characters. Residual effect is the measure of the effect of other possible independent characters, which were not included in the study on the dependent character.

4.5.1 Direct and indirect effect of morphological and phenological traits on pod shattering after 20 days of maturity

Among the observed morphological and phenological traits (Table 4.17), only seed yield per plant showed direct positive effect (0.1657) on shattering percentage after 20 days of maturity. Whereas, number of pod per plant (-0.1942), number of nodes per plant (-0.0571), days to maturity (-0.1856), days to flowering (-0.0376) and plant height (-0.0297) had negative direct effect on pod shattering after 20 days of maturity. Magnitudes of all the indirect effects on pod shattering after 20th days of maturity were negligible. The value for residual effect was 0.4410.

Shattering percentage screened by heat treatment showed high magnitude of negative direct effect of days to 50% flowering (-0.2106), days to maturity (-0.2615), number of nodes/plant (-0.2495) and number of pod/plant (-0.3172). However seed yield/plant had positive direct effect on shattering percentage (0.2962). The value for residual effect was 0.3359. In general indirect effects were negligible.

Table 4.17 Direct and indirect effect of morphological and phenological traits on pod shattering of soybean screened 20 days after maturity and by heat treatment

Characters	Screening method	Days to 50% flowering	Days to maturity	Plant height	Nodes/ plant	Pod/ plant	Seed yield/ plant
Days to 50% flowering	20 days after maturity	-0.0376	-0.0193	-0.0122	-0.0049	-0.0048	0.0017
	Heat treatment	-0.2106	-0.1082	0.0885	-0.0272	-0.0468	0.0096
Days to maturity	20 days after maturity	-0.0953	-0.1856	-0.0519	0.0612	0.0103	0.0069
	Heat treatment	-0.1343	-0.2615	-0.0731	0.0862	0.0145	0.0097
Plant height	20 days after maturity	-0.0097	-0.0083	-0.0297	-0.0081	-0.0083	-0.0017
	Heat treatment	0.0316	0.0271	0.0970	0.0265	0.0270	0.0057
Nodes per plant	20 days after maturity	-0.0074	0.0188	-0.0156	-0.0571	-0.0263	-0.0165
	Heat treatment	-0.0323	0.0823	-0.681	-0.2495	-0.1151	-0.0723
Pod per plant	20 days after maturity	-0.0431	0.0107	-0.0541	-0.0896	-0.1942	-0.1024
	Heat treatment	-0.0705	0.0175	-0.883	-0.1464	-0.3172	-0.1672
Seed yield per plant	20 days after maturity	-0.0076	-0.0062	0.0097	0.0480	0.0873	0.1657
	Heat treatment	-0.0185	-0.0110	0.0174	0.0858	0.1561	0.2962

Residual effect= 20 days after maturity 0.4410 ; Heat treatment 0.3359

4.5.2 Direct and indirect effect of pod characteristics on pod shattering of soybean after 20 days of maturity

Among the observed pod characteristics, moisture percent of pod wall (0.4090) had highest positive direct effect on pod shattering of soybean after 20th days of maturity followed by weight of periphery region (0.3115), width of pod (0.1405) and seed pod ratio (0.1217). Whereas, pod length : width ratio (-0.9139) and pod width : length ratio (-0.8390) had negative direct effect on pod shattering of soybean after 20th days of maturity was recorded (Table 4.18).

Table 4.18

Pod thickness : width ratio (0.3893), thickness of pod (0.3292), weight of periphery region of pod (0.1535), weight of pod (0.1090), volumetric weight of seed (0.1145) and pod width : length ratio(0.1152) has positive indirect effect, whereas width at mid part of pod (-0.4205), seed pod ratio(-0.1653), length of pod (-0.1244), one hundred seed weight (-0.1140) and thickness of pod wall (-0.1030) had high indirect effect via relative water content percent on pod shattering of soybean after 20th days of maturity.

Width of pod (0.1405) had positive direct effect on pod shattering of soybean after 20th days of maturity with positive indirect effect via width at mid part of pod (0.1027). Whereas width at mid part of pod (0.1025) had positive direct effect on pod shattering of soybean after 20th days of maturity.

Pod length : width ratio had positive indirect effect via pod width : length ratio (0.890), width of pod (0.376), pod width at mid part : length ratio (0.2420) and pod width at mid part (0.1943), but negative indirect effect via length of pod (-0.5049), weight of pod (-0.3131), pod thickness : width ratio(-0.2435), one hundred seed weight (-0.2236) and thickness of pod (-0.1725). Thickness of pod wall had negative indirect effect via moisture percent of pod wall (-0.1332).

Pod width : length ratio had positive indirect effect via pod length :width ratio (0.8172), length of pod (0.4898), weight of pod (0.3033), pod thickness : width ratio (0.2227), one hundred seed weight (0.2146) and thickness of pod wall (0.1258). Whereas, it had negative indirect effect via width of pod (-0.3378), pod width at mid part : length ratio (-0.2213) and width at mid part of pod (-0.1654).

Pod thickness : width ratio had negative indirect effect via moisture percent of pod wall (-0.1659). Moisture percent of pod wall had positive indirect effect via seed pod ratio (0.1231). Whereas, it had negative indirect effect via pod thickness :width ratio (-0.1332), thickness of pod (-0.1707) and thickness of pod wall(-0.1701).

4.5.4 Direct and indirect effect of observed pod traits on shattering percentage of soybean screened by heat treatment

Among the observed pod characteristics, thickness of pod wall (0.8895) had highest positive direct effect on pod shattering of soybean screened by heat treatment followed by weight of periphery region (0.4513), pod length: width ratio (0.1750) and width at mid part of pod (0.1630). Whereas, pod thickness: width ratio (-0.3605) had negative direct effect (Table 4.19). The magnitude of the direct effects on pod shattering by the remaining observed pod characteristics were very low. The residual effect was 0.5909.

Moisture percent of pod wall had very low direct effect on shattering percentage but many traits had high indirect effect via it viz., positive indirect effect of seed pod ratio (0.2862), volumetric weight of seed (0.1695) and one hundred seed weight (0.1501) and negative indirect effect of thickness of pod wall (-0.3955), pod thickness :width ratio (-0.3097) and weight of pod (-0.1997).

Pod length had indirect negative effect via relative water content percent (-0.1471). Thickness of pod had indirect positive effect via pod thickness :width ratio (0.2542), relative water content percent (0.1837) and weight of pod (0.1458). Width at mid part of pod had indirect positive effect via width of pod (0.1192) and indirect negative effect via relative water content percent (-0.2347) Pod length: width ratio had indirect negative effect via pod width: length ratio (-0.1705).

Pod thickness :width ratio had indirect positive effect via relative water content percent (0.2173), width at mid part of pod (0.1344) and width of pod (0.1305), whereas, indirect negative effect via pod thickness (-0.2353).

Table 4.19

Relative water content percent had indirect negative effect via width at mid part of pod (-0.2107) and pod thickness :width ratio (-0.1578).

4.6 Association of pod pubescence with pod shattering

Out of 69 genotypes, 55 genotypes were pubescent and 14 were puberulent (Table 4.20). Among the pubescent genotypes 47 had tawn and 8 had grey pubescence. Out of 55 pubescent genotypes, 30 had tolerant, 20 had moderately tolerant and six had sensitive expression for shattering. Out of 47 tawn coloured pubescent genotypes 27 had tolerant, 17 moderately tolerant and three had sensitive expression for shattering. Among the eight grey pubescent genotypes three each had tolerant and moderately tolerant and two had sensitive expression for pod shattering. Whereas, among the 14 puberulent genotypes 11 had tolerant and three had moderately tolerant expression for shattering.

Table 4.20 Classification of soybean genotypes based on presence/ colour of pubescence and pod shattering on 20th day after maturity

Pod shattering	Pubescent		Puberulent
	Tawn	Grey	
	47	8	14
Tolerant (<10%)	27	3	11
	JS76-205, JS335, MACS58, MACS450, MAUS61, MAUS 61-2, MAUS71, MAUS81, PK416, PS1024, PS1092, PS1347, JSM7, JSM20, JSM104, JSM120B, JSM154, JSM155, JSM189, JSM195, JSM227, JSM228, JSM229, JSM239, JSM245, JSM248, JS2	PK472, PS 1225, VLS47	JSM256, JSM240, JSM 207, JSM200, JSM170, JSM135, PS1241, JS 92-22, JSM 3, JSM 104, JSM 117
Moderately tolerant	17	3	3
	Harasoya, JS97-52, MAUS47, NRC7, NRC12, NRC37, PS1042, PS1029, SL295 JS92-12, JSM120A, JSM127, JSM152, JSM184, JSM202, JSM203, JSM250	TAMS38, TAMS 98-21, JSM5	JSM214, JS93-05, JS95-60
Sensitive	3	2	Nil
	NRC2, Pb1, JSM131	Shivalik JSM37	

The minimum mean shattering percentage on 20th day after maturity (Table 4.21) was observed in puberulent genotypes (15.44%) followed by tawn coloured (18.5%) and maximum in gray coloured genotypes (20.85%). The screening by heat treatment revealed minimum shattering in tawn pubescent genotypes (22.78%), whereas it was 28% in genotypes representing either h grey pubescent or puberulent group.

Maximum force by texture analyser to screen the genotypes for shattering tolerance was required on tawn coloured (601 g) followed by puberulent (559g) and gray coloured group (508g).

Table 4.21 Performance of soybean genotypes for pod shattering classified based on presence and colour of pubescence

	Tawn		Grey		Puberulent	
Number of genotypes	47		8		14	
Shattering percent	Mean	Range	Mean	Range	Mean	Range
20 th day after shattering	18.475	1.6 - 67	20.85	1.38 - 52.11	15.44	0.67-32.46
By heat treatment	22.783	2.3 – 89.83	28.56	7.0 - 56.48	28.43	10.6 -69.62
Force	601.14	245.4 – 1013.6	508.53	406.8 – 955.8	559.6	519 - 637.4

4.7 Association of presence of four seeded pods with pod shattering

Out of 69 genotypes four seeded pods had been expressed in 12 genotypes (Table 4.22). The minimum mean shattering percentage on 20th day after maturity (Table 4.23) was recorded in the group of genotypes with presence of four seeded pods (15.58) and maximum in the group without four seeded pods (23.4). The screening by heat treatment revealed minimum shattering in the group with presence of four seeded pods (24.54) and maximum in the group without four seeded pods(27.58). Maximum force by texture analyser to screen the genotypes for shattering tolerance was required in the group with presence of four seeded pods (643.1) and minimum in the group with absence of four seeded pods(530.5).

Table 4.22 Classification of soybean genotypes based on presence of four seeded pods

Expression of four seeded pod	Genotypes	
	Number	Name
Absent	57	JS335, JS76-205, MACS58, MAUS61, MAUS61-2, MAUS71, MAUS81, PK416, PK472, PS1024, PS1092, PS1225, PS1241, PS1347, VLS47, JSM3, JSM5, JSM120A, JSM152, JSM184, JSM203, JSM 127, MACS 450, JSM 131 JSM2 05JSM7, JSM20, JSM37, JSM104, JSM117, JSM120B, JSM154, JSM155, JSM170, JSM189, JSM195, J SM227, JSM228, JSM 229, JSM240, JSM245, JSM248,SHIVALIK, NRC2, JS2, Pb1, Harasoya , JS 97-52, MAUS 47, NRC7, NRC12 NRC37, PS1042, PS1029, SL295, TAM338, TAM398-21,
Present	12	JS93-05, JS95-60, JS92-12, JSM 37, JSM45, JSM135, JSM200, JSM202, JSM207, JSM214 ,JSM 239, JSM256

Table 4.23 Performance of soybean genotypes for pod shattering based on presence and absence of four seeded pods

	Absent		Present	
	Mean	Range	Mean	Range
Shattering percent 20 th day after shattering	23.4	8.0 – 52.11	15.58	0.67 – 67.05
By heat treatment	27.58	10.6 – 56.4	24.54	3.44– 89.8
Force	530.5	434.1 – 641.4	643.1	245.4 – 1013.6

4.8 Classification of soybean genotypes based on the location of first shattered pod on the plant

Genotypes were classified based on the location of the first pod shattered as upper, middle or lower part of the plant (Table 4.24). Out of 69 genotypes under study in 65 genotypes location of the first shattered pod was in the upper part , while only two genotypes represent the position of first shattered pod in the middle (JSM 127 and JSM 239) and lower (MACS 450 and JSM 131) part of the plant.

Table 4.24 Classification of soybean genotypes based on the position of first shattered pod on the plant

Position of first shattered pod	Genotypes	
	Number	Name
Upper	65	JS76-205, JS335, MACS58, MAUS61, MAUS61-2, MAUS71, MAUS81, PK416, PK472, PS1024, PS1092, PS1225, PS1241, PS1347, VLS47, JSM3, JSM7, JSM20, JSM37, JSM104, JSM117, JSM120B, JSM154, JSM155, JSM170, JSM189, JSM195, JSM207, JSM227, JSM228, JSM229, JSM240, JSM245, JSM248, JSM256, SHIVALIK, NRC2, JS2, Pb1, JSM37, Harasoya, JS93-05, JS95-60, JS97-52, MAUS47, NRC7, NRC12, NRC37, PS1042, PS1029, SL295, TAM38, TAM38-21, JS92-12, JSM5, JSM45, JSM120A, JSM135, JSM152, JSM184, JSM200, JSM202, JSM203, JSM214, JSM205
Middle	2	JSM 127, JSM 239
Lower	2	MACS 450, JSM 131

4.9 Classification of soybean genotypes based on the days to incipient of shattering

Days to incipient shattering ranged from 90 – 114 days with mean 102 days. The genotypes were classified (Table 4.25) based on days to incipient of shattering as early, (<100 days), medium (101 -110 days) and late (> 110 days).

Table 4.25 Classification of soybean genotypes based on the days to incipient of shattering

Classification based on the days to incipient of shattering		Genotypes	
		Number	Name
Early	<100days	37	Harasoya, JS 93-05, JS 95-60, JS95-60, JS335, MAUS 47, MAUS 71 NRC 12, PS 1029, PS1225, PS1241, PS 1347, Shivalik, TAMS 38, NRC2, JS2, Pb1, JSM 3, JSM 5, JSM 37, JSM 45, JSM 104, JSM 117, JSM 120A, JSM 131, JSM 135, JSM 152, JSM 155, JSM 170, JSM 170, JSM 189, JSM 195, JSM 200, JSM 207, JSM 214, JSM 227, JSM 229, JSM 250
Medium	101-110 days	11	MAUS 81, NRC 37, PS 1024, PS 1042, PS 1092, SL 295, TAMS 98-21, JSM 20, JSM 120B, JSM 202, JSM 248
Late	>110 days	21	JS 76-205, JS 97-52, MACS 58, MACS 450, MAUS 61, MAUS 61-2, NRC 7, PK 416, PK 472, VLS 47, JS 92-22, JS92-12, JSM 7, JSM 127, JSM 154, JSM 184, JSM 228, JSM 229, JSM 240, JSM 245, JSM 256

Among the 69 genotypes under test, the days to incipient of shattering was early in 37 i.e., >100 days; in 11 genotypes it was medium (101-110 days) and in 21 genotypes it was late (>110days).

4.10 Classification of soybean genotypes based on the height of the lowest pod shattered

Height of lowest pod shattered ranged from 13.33 – 123.66cm with mean height of 62.69cm. The genotypes were classified based on height of the lowest pod in three groups i.e., height of the lowest shattered pod less than 40 cm, in between 40-80cm and more than 80cm. (Table 4.26) .

Table 4.26 Classification of soybean genotypes based on the height of the lowest pod shattered

height of the lowest pod shattered	Genotypes	
	Number	Name
<40 cm	3	MACS 450, JSM 131, JSM239
40 – 80cm	58	Harasoya, JS 93-05, JS 95-60, JS335, MAUS 47, MAUS 61, , MAUS 71, MAUS 81, NRC 12, PK 416, PK 472, , PS 1024, PS 1042, PS 1029, PS 1092, PS1225, PS1241, PS 1347, , Shivalik , SL 295, TAMS 38, TAMS 98-21, VLS 47, NRC2, Pb1, JSM 3, JSM 5, JSM 7, JSM 20, JSM 37, JSM 45, JSM104, JSM 117, JSM 120A, JSM 120B, JSM 127, JSM 135, JSM 152, JSM154, JSM155, JSM170, JSM184, JSM189, JSM195, JSM200, JSM202, JSM207, JSM214, JSM227, JSM228, JSM229, JSM240, JSM245, JSM248, JSM250, JSM256
> 80cm	8	JS 76-205, JS 97-52, MACS 58, MAUS 61-2, NRC 7, NRC 37, , JS 92-22, JS92-12

Among the 69 genotypes under test, in 58 the height of the lowest shattered pod was in between 40-80cm.days; in 03 genotypes it was less than 40cm and in 8 it was more than 80cm.

DISCUSSION

Soybean (*Glycine max.* L. Merrill.) a miracle crop containing about 40% protein and 20% oil plays a major role in the world food system. It produces about three times more protein than rice, wheat or maize on per hectare production basis. The soybean is also an excellent source of good quality unsaturated oil. Although, it is a leguminous crop but it has been placed in oil seed category due to moderate oil content. The versatile plant serves as a natural soil fertilizer by fixing about 50 kilograms of nitrogen per hectare.

The most important objective in soybean breeding is to develop varieties with high seed yield. This objective is achieved by maximizing regional adaptation and increasing the yield potential *per se* and through genetic control of negative factors i.e., protecting against yield losses from biotic and abiotic stresses. In recent years, the area and production has increased, although the national productivity is less than half as compared to the world average yield mainly due to biotic and abiotic stresses. Among the abiotic stresses, loss due to shattering is an inherited problem of soybean.

Yielding ability of a plant is improved either through direct selection or indirectly through associated component traits. Character like pod shattering is not considered as component associated with seed yield/ unit area. It may cause 50-100% loss in soybean (IITA, 1986) depending upon the time of harvesting, environmental condition and genetic endowment of the variety (Tiwari and Bhatnagar, 1988).

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. 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. The trait shows the crop is still in the process of domestication/ evolution.

Enhancement in shattering tolerance may promote productivity, harvesting of uniformly ripe seeds, efficiency of seed recovery and improved oil extraction; adjustment in harvesting and threshing time; reduction in cost of production and problem of volunteer plants (Morgan *et al.*, 1998). Hence study on identification of the factors affecting pod shattering and their genetics are essential to reduce the losses to a greater extent.

To concentrate on development of high yielding pod shattering tolerant varieties, germplasm evaluation for pod shattering tolerance is the pre requisite. Measurement of expression for pod shattering is difficult due to difference in maturity period among genotypes and pods within the plant and association of several morphological, physiological and environmental factors. Therefore, four methods were adopted for screening the genotypes viz., pod wall thermostability, shattering at high temperature, texture analyser and shattering at field level at differ intervals after maturity.

On the day of harvestable maturity, no pod shattering was observed in 74% genotypes, whereas the range of shattering on the day of harvestable maturity was 0-6.97%. The average losses enhance upto 3% on 5th day and 10% on 10th day after maturity (Fig 5.1). It can be minimize by timely harvesting of the crop. The genotypes were judged as tolerant based on shattering percentage on 20th day after maturity. The average shattering on 20th day after maturity was 30% with a range of 7.67 to 67.05%. Less than 15% (tolerant) and more than 35% (sensitive) shattering was observed in 9% genotypes. On 20th day minimum shattering was observed in genotype JSM 170 and maximum in JSM 131. These two genotypes can be used for developing mapping population to develop pod shattering tolerant genotypes with the help of Molecular Assisted Selection.

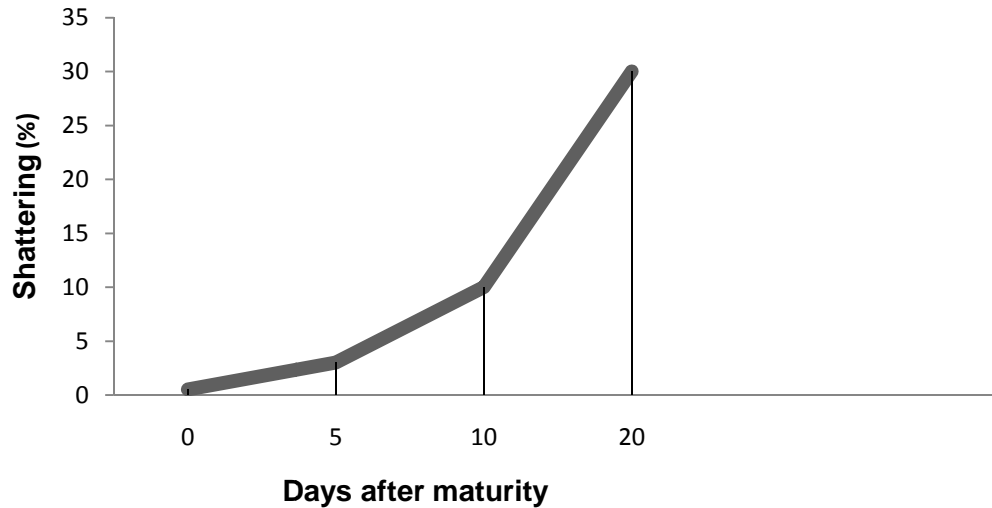


Fig 5.1 Enhancement of shattering percent with days after maturity

Comparing overall seed shattering patterns, pods were able to hold seeds for the first weeks, which showed limited shattering. The rate of seed shattering accelerated after 7 days, and enhances with the age of the mature pod.

With the age of plant, pod shattering is also influenced by high temperature at the time of maturity. Therefore, the genotypes were also screened by exposure of mature pod wall for thermo- stability and mature pod to high temperature (60°C). Thermo-stability test classified 13 genotypes as tolerant and six as sensitive. In addition to genotypes JS 335, JSM 170, MAUS 61-2, PK416, PK472 and VLS 47 classified as shattering tolerant 20th day after maturity Harasoya, JS 93-05, JS 95-60, JSM 256, MACS 58, MAUS 81 and NRC 12 were also classified as tolerant. Similarly with NRC 2, JS 2, Pb1, JSM 37 and JSM 131 genotypes classified as sensitive on 20th days after maturity JS 97-52 and TAMS 98-21 were also included by thermo-stability test.

Exposure of mature pod to 60°C recorded average 36% shattering with a range from 18.32 to 89.83%. It classified only JS 335, JSM 170 and MAUS 61-2 as tolerant with less than 30% shattering whereas 12 genotypes were classified as sensitive with more than 60% shattering. Minimum shattering percentage was observed in MAUS 61(18.32%), PS1029 (24.6%). Whereas, it was highest in JSM 131 (89.83%) followed by JSM 117 (69.62%). Total six genotypes were judged as tolerant to shattering on 20th day after maturity and 13 by thermo-stability, but the number reduced to three by heat treatment test. Similarly from 6 genotypes judged as sensitive at 20th day after maturity, the number reached to 12 by heat treatment. It shows that apart from the age of the pod, the environment after maturity with special reference to high temperature played important role in expression of shattering. This finding is in agreement with the report of Agrawal *et al.*, (2002) that pod shattering is aggravated if there is rain followed by dry weather, low humidity, high temperature, rapid temperature changes, wetting and drying.

Mean force applied for screening of shattering tolerance by texture analyser on the mature pod at harvestable maturity showed that was 600 (g) force with range of 245 to 1013g is required to break the pod at the suture. Genotypes required more than were 800g force are judged as tolerant (12 genotypes), whereas less than 500g as sensitive (9 genotypes). Minimum force for shattering was required in JSM 131 (245g) followed by JS 97-52 (346g) whereas maximum force was required in MAUS 47 (1013g) followed by NRC 12 (924g). These genotypes were also judged as tolerant and sensitive by other methods.

The texture analyser that compress pod suture to judge the genotype for expression of shattering is a simple method to perform and gave results indicative of machine harvest shatter levels. Such a standardized mechanical shatter index may prove a useful criterion in soybean breeding selection for harvest shatter resistance.

None of the genotype classified as tolerant fall under sensitive or vice versa in all the four tests. It shows that genotypes may be screened effectively by high temperature test or thermo-stability of pod wall or texture analyser for pod shattering with lesser time and minimum influence of environment. Minimum number of genotypes was classified as tolerant and sensitive by heat treatment (Fig.5.2), therefore is judged as the best method to test expression for shattering. Js 97-52 a popular variety of soybean in MP shoed moderately tolerant for pod shattering even after 20 days of maturity but by heat treatment it is classified as sensitive, it shows that under high terminal temperature the variety behaves as sensitive.

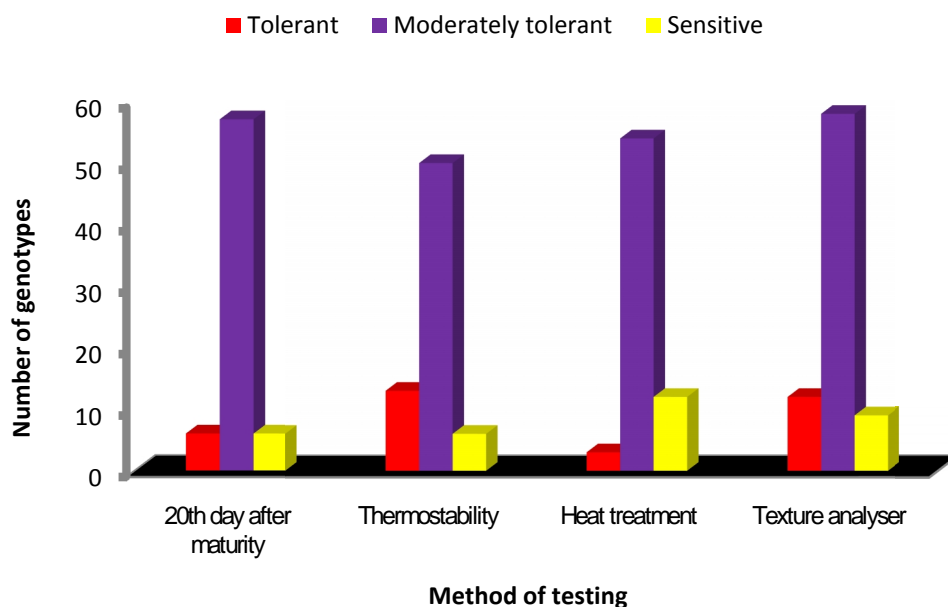


Fig 5.2 Classification of soybean genotypes based on screening technique applied for shattering tolerance

Variation refers to the observable or measurable difference for the observed trait. Significant variation exist among soybean genotypes for all the observed morphological, phenological and pod characteristics. Determination of genetic diversity and relationship among individuals and populations are important considerations for genetic conservation and utilization of plant genetic

resources. Present finding revealed that the significant genotypic variation for pod shattering tolerance, which is necessary to detect variation among genotypes and precludes the response of cultivar for delayed harvesting.

The existence of high amount of genetic variability in the material was also revealed by high genotypic coefficient of variation for most of the observed traits. Significant genotypic differences for shattering resistance are in agreement with the findings of Caviness (1965), Tsuchiya (1986), Helms (1994) and Romkaew and Umezaki (2006). High genotypic variance for pod shattering is in agreement with Vimala Devi(1993).

Heritability

High heritability (74-84%) among all the observed morphological and phenological traits at plant level viz., days to 50% flowering, days to maturity, plant height, nodes per plant and pods per plant with high genetic advance (12-57%) as percentage of mean advocate additive gene action. That anticipated high genetic gain from selection with meager effect of environment. But low heritability with high genetic advance as percentage of mean for seed yield per plant showed additive genetic control with environmentally sensitive selection gain.

High genetic advance as percentage of mean for weight of periphery region, thickness of pod wall, thickness of pod, weight of pod, and seed pod ratio attributed to additive gene effects and consequently a high genetic gain from selection would be anticipated with meager effect of environment. It shows that its phenotype reflects the genotype, assuming no environmental effects. This means that the selection for a particular genotype is accurate within the limits imposed by the environmental effects. Therefore, weight of periphery region, thickness of pod wall, thickness of pod, weight of pod, and seed pod ratio among the pod traits are more reliable for selection for improvement by, simple selection procedure.

Pod length, width and width at mid part, pod wall moisture and volumetric weight had high heritability with medium to low genetic advance indicating non additive gene action with more sensitivity to environment. Hence, selection for these traits will not be responsive. Wt. of periphery region, Thickness of pod wall, Weight of seed / pod, RWC% pod wall and 100 seed weight had high PCV with high GCV and high GA as percentage of mean indicating the possibilities of mass selection to be practiced.

Association analysis

Phenological characteristics with morphological traits of pods were given due emphasis as associated traits for pod shattering.

Days to 50%flowering and days to maturity had significant negative association with shattering percentage observed on 20th days after maturity and by heat treatment. It shows that to develop pod shattering tolerant genotypes, early flowering and maturity are the prerequisite. This result agree with reports of Tiwari and Bhatnagar, (1991) that pod shattering showed a significant negative correlation with days to maturity and seed yield. In the present investigation seed yield per plant also had negative significant association with shattering percentage at 20th day after maturity and heat treatment. The results suggest that, it is possible to breed a low shattering variety with early to medium maturity, higher number of pods/plant without significantly altering the seed yield performance.

The association analysis of seed yield/plant was non-significant with shattering percentage on zero and five day after maturity whereas it was negative and significant at 10th and 20th day after maturity. The association analysis of shattering percentage on 20th day after maturity was significant and positive with seed pod ratio, one hundred seed weight, weight of periphery region, pod length pod width and width at mid part. Whereas, significant and negative with volumetric weight of seed and pod thickness: width ratio. Correlation coefficients on the day of maturity showed that days to 50%

flowering, days to maturity, zero day after maturity, plant height, nodes/plant, pod/plat and seed yield/plant presented no serious problems for these characteristics while improvement for shattering tolerance.

Significant and positive association of shattering percentage with seed pod ratio, one hundred seed weight, weight of periphery region, pod length, pod width and width at mid part showed that with the increase of these factors shattering percentage will enhance after 20th day of maturity. Whereas, with the reduction of pod thickness: width ratio shattering percentage increases. It is in agreement with the report of Loof and Jhonson (1970) and Thompson and Hughes (1986) that magnitude of shattering depends on pod attributes such as angles, length and width and plant architecture.

Significant and positive association of shattering percentage with seed pod ratio, weight of periphery region, pod width, width at mid part of pod and volumetric weight of seed by heat treatment was recorded. Whereas, pod weight, and pod thickness: width ratio significant and negative.

Association analysis of force applied in texture analyzer was significant and positive with one hundred seed weight, weight of periphery region and width at mid part of pod. Whereas, significant and negative with volumetric weight of seed and pod thickness: width ratio. It is not in support of Morgan, *et al* (1998) who reported negative correlation between the force needed to break pod with number of seeds per pod in oilseed rape.

It indicates that genotype with small pod with less width and weight of periphery and low volume/weight of seed has low shattering percentage.

Path analysis

Among the observed morphological and phenological traits, seed yield per plant showed direct positive effect whereas, pod per plant and days to maturity had negative direct effect with low magnitudes of all the indirect effects on pod

shattering screened after 20 days of maturity and by heat treatment. It shows with the increase of seed yield, pod shattering percentage is enhanced directly.

Among the observed pod characteristics, moisture percent of pod wall, weight of periphery region, width of pod and seed pod ratio had positive direct effect, whereas, pod length : width ratio and pod width : length ratio had negative direct effect on pod shattering of soybean after 20th days of maturity. Whereas, screening by heat treatment showed positive direct effect of pod wall thickness and weight of periphery region, and negative direct effect of pod thickness: width ratio.

Moisture percent of pod wall had very low direct effect on shattering percentage but seed pod ratio, volumetric weight of seed and one hundred seed weight had positive and thickness of pod wall, pod thickness: width ratio and weight of pod had negative indirect effect via it. It shows importance of moisture in pod wall pod wall on expression of pod shattering.

Colour of hairs had non-significant association with pod shattering, however out of 14 puberulent genotypes 11 had tolerant and three had moderately tolerant expression for shattering. Shattering percentage and presence of four seeded pods had non-significant association.

In 94% genotypes location of the first shattered pod was in the upper part whereas in JSM 127 and JSM 239 it was in middle and MACS 450 and JSM 131 in lower part of the plant. In the present study days to incipient shattering ranged from 90 – 114 days with mean 102 days.

Ideotype

As per association and path coefficient analysis genotype with small pod with less width and weight of periphery and low volume/weight of seed is ideal for tolerance to pod shattering. Whereas mean performance of the genotypes classified as shattering sensitive and tolerance shows significant difference for

weight of periphery region of pod, and width at mid portion of pod (Table 5.1). These findings are in agreement with Tsuchiya (1987).

Table 5.1 Comparison of mean performance of pod characteristics between sensitive and tolerant genotypes for pod shattering screened on 20th day after maturity

Pod characteristics	Sensitive genotypes	Tolerant genotypes	Significance by t test
Weight. of periphery region(g)	01.256	00.867	5%
Length of Pod (mm)	49.840	42.600	5%
Width of Pod (mm)	08.630	09.240	ns
Thickness of Pod (mm)	04.860	05.120	ns
Thickness of pod wall (mm)	00.379	00.416	ns
Width at mid part of pod (mm)	08.875	06.964	5%

SUMMARY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

Pods of soybean are fragile at harvest maturity and seed loss can be a significant fraction of potential yield under high terminal temperature. In the event of late harvesting even in normal temperature, loss of up to 67% has been observed. Tolerance against shattering needs accurate identification of single genotype to provide resistance.

Resistance to pod shattering was found to be a pre-requisite for adoption of any variety by the farming communities, indicating that resistant varieties that can stand in the field for relatively longer periods after maturity without shattering must be developed.

The investigation was carried out during Kharif, 2010 at the Seed Breeding Farm, JNKVV, Jabalpur on 69 genotypes. Morphological, phenological and pod characteristics were observed for the investigation.

To screen the pod shattering response three methods viz., thermal stability of pod wall, shattering after 20 days of maturity in filed condition, heat treatment on mature pod and force required to break the mature pod at the suture were adopted. Different methods used for screening for pod shattering tolerance had positive association. Laboratory method of pod shattering tolerance is not influenced by the environment and hence can be used as a tool for identification of pod shattering resistance genotypes.

Shattering percentage ranged from 0.6730 (JSM 170) to 67.05 (JSM 131) with mean of 19.11 percent. Genotypes JS 335, JSM 170, MAUS 61-2 were identified as pod shattering tolerant .

On the day of harvestable maturity, no pod shattering was observed in 74% genotypes, whereas the range of shattering on the day of harvestable maturity

was 0-6.97%. The average losses enhance upto 10% on 10th day after maturity can be minimize by timely harvesting of the crop. With the age of plant, pod shattering is also influenced by high temperature at the time of maturity.

Present finding revealed that the significant genotypic variation for pod shattering tolerance, which is necessary to detect variation among genotypes and precludes the response of cultivar for delayed harvesting.

High genetic advance as percentage of mean with high heritability was observed for weight of periphery region, thickness of pod wall, thickness of pod, weight of pod, and seed pod ratio.

Significant and positive association of shattering percentage with seed pod ratio, weight of periphery region, pod width, width at mid part of pod and volumetric weight of seed by heat treatment was recorded. Whereas, pod weight, and pod thickness: width ratio significant and negative.

Among the observed pod characteristics, moisture percent of pod wall, weight of periphery region, width of pod and seed pod ratio had positive direct effect, whereas, pod length : width ratio and pod width : length ratio had negative direct effect on pod shattering of soybean after 20th days of maturity. Whereas, screening by heat treatment showed positive direct effect of pod wall thickness and weight of periphery region, and negative direct effect of pod thickness: width ratio.

Colour of hairs had non significant association with pod shattering, however out of 14 puberulent genotypes 11 had tolerant expression. Non- significant association was established between shattering percentage and presence of four seeded pods. In most of the genotypes the first shattered pod was located in the upper part with incipient shattering ranged from 90 – 114 days with mean 102 days.

Conclusion

- Present study revealed considerable genetic variability among the genotypes of soybean for pod shattering and all the observed traits associated with pod shattering.
- Different methods used for screening for pod shattering tolerance had positive association. Laboratory method of pod shattering tolerance is not influenced by the environment and hence can be used as a tool for identification of pod shattering resistance genotypes.
- Although there was no direct relationship between shattering and final yield when seeds were harvested in time, but with the delay in harvesting it is negatively correlated.
- Soybean cultivated in MP has a critical shattering period, which occurs at about 10 days after maturity, but could be earlier depending on the weather conditions. Information from this study indicates that soybean should be harvested before 10 days after maturity.
- Shattering percentage ranged from 0.6730 (JSM 170) to 67.05 (JSM 131) with mean of 19.11 percent. Shattering tolerant genotypes JS 335, JSM 170, MAUS 61-2 could be used for cultivation and as donors of shattering resistance in breeding programmes to minimize yield losses.
- As per association and path coefficient analysis, genotype with small pod with less width and weight of periphery and low volume/weight of seed is ideal for tolerance to pod shattering.
- With the age of plant, pod shattering is also influenced by high temperature at the time of maturity.
- There is no association between colour of pubescence and pod shattering. However, puberulent genotypes showed tolerant expression.

Suggestion for further work

- More number of genotypes should be screened for tolerance to pod shattering.
- Mapping population for pod shattering may be developed by involving tolerant (JSM 170) and susceptible (JSM 131) genotype.
- Revalidation of molecular markers reported for pod shattering by field screening technique.

Table 4. 18 Direct and indirect effect of pod traits on pod shattering of soybean after 20 days of maturity

Characteristics	Weight of pod	Seed pod ratio	100 Seed weight	Volume tric weight of seed	Characteristics observed on pod										RWC%	Moisture % of pod wall
					Weight of Periphery region	Length (A)	Width (B)	Thickn ess of pod (C)	Thicknes s of pod wall (E)	Width at mid part (D)	A/B	D/A	B/A	C/B		
Pod weight	0.032	-0.009	0.014	0.004	0.005	0.013	0.003	0.012	0.002	0.005	0.011	-0.001	-0.011	0.008	0.109	0.011
Seed pod ratio	-0.003	0.121	0.007	0.004	0.012	-0.008	-0.002	-0.019	-0.025	-0.002	-0.004	0.003	0.006	-0.026	-0.165	0.001
100 seed wt	0.005	0.008	0.013	0.003	0.002	0.005	0.001	0.001	0.001	0.002	0.003	-0.001	-0.003	0.004	-0.114	-0.013
Vol. wt. of seed	-0.014	-0.004	0.002	-0.104	0.010	0.010	0.013	-0.002	0.023	-0.004	0.001	-0.008	-0.002	-0.003	0.114	0.070
Wt of periphery region	0.050	0.033	0.050	-0.030	0.311	0.016	0.025	0.033	0.010	-0.000	-0.011	-0.006	0.006	0.016	0.153	-0.015
Pod length (A)	0.030	-0.005	0.028	-0.007	0.003	0.072	0.027	0.006	0.019	0.033	0.039	-0.018	-0.042	-0.005	-0.263	0.014
Pod width (B)	0.014	-0.002	0.015	-0.018	0.011	0.054	0.140	0.017	0.012	0.102	-0.057	0.003	0.056	-0.050	-0.124	-0.078
Pod thickness (C)	-0.029	0.012	-0.009	-0.002	-0.008	-0.007	-0.009	-0.079	-0.004	0.002	0.003	0.004	-0.002	-0.051	0.329	0.053
Pod wall thickness (E)	0.001	-0.003	0.001	-0.003	0.005	0.003	0.001	0.001	0.014	0.002	0.002	0.001	-0.002	-0.009	-0.103	-0.133
Mid part width (D)	0.017	-0.002	0.017	0.004	-0.003	0.047	0.075	-0.003	0.016	0.102	-0.021	0.006	0.020	-0.038	-0.420	-0.055
A/B	-0.313	0.030	-0.226	0.011	0.035	-0.504	0.376	0.037	-0.172	0.194	-0.913	0.242	0.890	-0.243	-0.085	-0.0018
D/A	0.002	-0.002	0.006	-0.005	0.001	0.016	-0.001	0.003	-0.003	-0.004	0.016	-0.063	-0.016	0.004	0.027	-0.005
B/A	0.303	-0.045	0.214	-0.021	-0.017	0.489	-0.337	-0.023	0.125	-0.165	0.817	-0.221	-0.839	0.222	0.11	0.004
C/B	0.010	-0.082	0.001	0.001	0.002	-0.002	-0.013	0.024	-0.002	-0.014	0.010	-0.002	-0.011	0.038	0.38	-0.165
RWC%	-0.002	0.005	0.010	0.050	0.004	-0.005	-0.088	0.036	-0.001	0.021	0.0076	0.002	0.021	0.035	-0.315	-0.089
Mbisture % of pod wall	-0.086	0.123	0.064	0.072	-0.014	-0.033	-0.042	-0.170	-0.170	0.029	-0.037	0.035	0.011	-0.133	-0.024	0.409

Residual effect= 0.2398

Table 4.19 Direct and indirect effect of pod traits on shattering percentage of soybean screened by heat treatment

Characteristics	Weight of pod	Seed pod ratio	100 Seed weight	Volume tric weight of seed	Characteristics observed on pod										RWC%	Moisture % of pod wall
					Weight of Periphery region	Length (A)	Width (B)	Thickn ess of pod (C)	Thicknes s of pod wall (E)	Width at mid part (D)	A/B	D/A	B/A	C/B		
Pod weight	-0.179	0.004	-0.077	-0.025	-0.029	-0.076	-0.019	0.067	-0.015	-0.032	-0.064	0.057	0.064	-0.048	0.068	-0.199
Seed pod ratio	-0.007	0.064	0.008	0.002	0.006	-0.004	-0.001	-0.010	-0.017	-0.001	-0.022	0.021	0.035	-0.019	-0.092	0.2862
100 seed wt	-0.007	-0.001	-0.017	-0.004	-0.002	-0.006	-0.002	-0.002	-0.002	-0.003	-0.043	0.017	0.045	-0.005	-0.066	0.151
Vol. wt. of seed	0.015	-0.003	-0.002	-0.110	0.019	0.011	0.014	-0.003	0.025	-0.004	0.004	-0.009	-0.008	-0.008	0.069	0.169
Wt of periphery region	0.073	0.047	0.076	-0.044	0.451	0.024	0.037	0.048	0.018	-0.003	-0.017	-0.094	0.009	0.024	0.087	-0.039
Pod length (A)	-0.043	0.006	-0.039	0.010	-0.005	-0.101	-0.039	-0.009	-0.027	-0.047	-0.056	0.026	0.059	0.007	-0.147	-0.078
Pod width (B)	-0.007	0.001	-0.008	0.009	-0.006	-0.002	-0.006	-0.008	-0.006	-0.005	0.008	-0.002	0.007	0.005	-0.069	-0.098
Pod thickness (C)	0.145	0.062	0.045	0.010	0.042	0.035	0.047	0.889	0.008	-0.017	-0.016	-0.022	0.017	0.254	0.183	-0.397
Pod wall thickness (E)	-0.013	0.033	-0.005	0.039	-0.005	-0.042	-0.013	-0.007	-0.156	-0.025	-0.029	-0.006	0.023	0.009	-0.057	-0.395
Mid part width (D)	0.027	-0.008	0.027	0.006	-0.005	0.075	0.119	-0.005	0.026	0.163	-0.034	0.010	0.032	-0.068	-0.237	0.068
A/B	0.060	-0.005	0.048	-0.002	-0.006	0.096	0.072	-0.007	0.033	-0.037	0.175	-0.046	-0.175	0.046	-0.047	-0.087
D/A	-0.004	0.004	-0.001	0.001	-0.003	-0.003	0.003	-0.007	0.001	0.008	-0.032	0.012	0.032	-0.008	0.015	0.089
B/A	0.014	-0.007	0.019	-0.012	-0.001	0.029	-0.024	-0.004	0.007	-0.010	0.049	-0.014	-0.056	0.013	0.0643	0.027
C/B	-0.097	0.077	-0.011	-0.012	-0.019	0.028	0.135	-0.233	0.021	0.134	-0.096	0.023	0.097	-0.365	0.217	-0.309
RWC%	-0.011	-0.015	-0.017	-0.022	-0.046	-0.029	0.068	-0.029	0.051	-0.217	0.034	0.007	-0.076	-0.158	-0.119	-0.007
Moisture % of pod wall	0.007	-0.009	-0.002	-0.026	-0.007	0.064	-0.070	-0.051	0.016	0.035	0.017	0.004	0.034	0.008	-0.006	-0.026

Residual Effect=0.5909

Table 4.13: Association analysis among pod characteristics observed in soybean

	Seed pod ratio	100 Seed weight	Volumetric weight of seed	Pod characteristics										RWC%	Moisture % of pod wall
				Weight Periphery region	Length (A)	Width (B)	Pod thickness (C)	Pod wall thickness (E)	Width at mid part (D)	A/B	D/A	B/A	C/B		
Pod weight	-0.0269	0.4305**	0.1405*	0.1623*	0.4251**	0.1064	0.3744**	0.0836	0.1687*	0.3426**	-0.0316	-0.3615**	0.2713**	0.1090	-0.1997*
Seed pod ratio		0.0583	0.0355	0.1060	-0.0657	-0.0183	-0.1596*	-0.2119**	-0.0018	-0.0334	0.0320	0.0536	-0.2149**	0.1653*	0.2862**
100 seed weight.			0.0202	0.1631*	0.3910**	0.1120	0.1202	0.0099	0.1671*	0.2447**	-0.0983	-0.2558**	0.0280	-0.1140	0.1501*
Volumetric weight of seed				-0.0985	-0.1005	-0.1309	0.0272	-0.2291**	0.0413	-0.0128	0.0821	0.0253	0.0344	0.1145	0.1605*
Weight of periphery region					0.0527	0.0820	0.1078	0.0351	-0.0030	-0.0382	-0.0209	0.0208	0.0536	0.1535*	-0.0329
Pod length (A)						0.3856**	0.0901	0.2704**	0.4652**	0.5524**	-0.2614**	-0.5837**	-0.0770	-0.2636**	-0.0782
Pod width (B)							0.1214	0.0859	0.7314**	-0.4124**	0.0240	0.4027**	-0.3621**	-0.1244	-0.0998
Pod thickness (C)								0.0046	-0.0351	-0.0412	-0.0573	0.0276	0.6526**	0.3292**	-0.3974**
Pod wall thickness (E)									0.1597*	0.1888**	0.0041	-0.1500*	-0.0603	-0.1030	-0.3955**
Width at mid part (D)										-0.2126**	0.0642	0.1971**	-0.3728**	-0.4205**	0.0683
A/B											-0.2648**	-0.9740**	0.2665**	-0.0851	-0.0872
D/A												0.2638**	-0.0641	0.0278	0.0819
B/A													-0.2654**	0.1152	0.0273
C/B														0.3893**	-0.3097
RWC%															0.0598

**Significant at 1% level

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* *Original not seen*

Appendix -

Meteorological data during crop season

Kharif 2010-2011

Months	Temperature °C		Relative Humidity (%)		Sunshine (Hrs.)	Rainfall (mm)	Rainy days (number)
	Maximum	Minimum	Morning	Evening			
2010							
May	42.55	27.12	39.75	16.00	6.15	000.00	00
June	41.52	27.25	50.75	27.50	5.87	015.82	03
July	33.28	24.82	85.60	65.20	3.98	097.12	10
August	32.40	23.40	86.75	68.70	3.65	094.12	13
September	32.16	22.72	89.60	65.00	5.16	121.24	18
October	31.92	19.60	90.25	49.50	7.07	019.85	04
November	30.20	16.37	91.00	46.50	6.75	000.45	00
December	25.88	10.06	88.20	38.8	7.78	001.52	01
2011							
January	23.92	05.55	88.50	30.25	8.92	000.00	00
February	28.57	11.32	83.25	37.25	8.65	001.40	01

Experimental Details

1.	Season	:	<i>Kharif</i> (2010)
2.	Experimental site	:	Seed Breeding Farm , JNKVV, Jabalpur
3.	Soil texture	:	Clayey
4.	Soil type	:	Typic chromusturt
5.	Soil pH	:	7.5
6.	Status of nutrients (kg/ha)	:	N 20 ; P ₂ O ₅ 80
7.	Number of genotypes	:	69
8.	Number of rows	:	6
9.	Row to row distance	:	30cm
10.	Number of replications	:	3
11.	Design	:	RCBD
12.	Date of sowing	:	06.07.2010

List of soybean genotypes studied

1. Harasoya	21. PS 1029	41. JSM 154	59. JSM 120A
2. JS 76-205	22. PS 1092	42. JSM 155	60. JSM 120B
3. JS 93-05	23. PS 1225	43. JSM 170	61. JSM 127
4. JS 95-60	24. PS 1241	44. JSM 184	62. JSM 189
5. JS 97-52	25. PS 1347	45. JSM 227	63. JSM 195
6. JS 335	26. Shivalik	46. JSM 245	Mutant of NRC 37
7. MACS 58	27. SL 295	47. JSM 250	64. JSM 152
8. MACS 450	28. TAMS 38	48. JSM 256	65. JSM 228
9. MAUS 47	29. TAMS 98-21	Mutant of JS 93-05	66. JSM 229
10. MAUS 61	30. VLS 47	49. JSM 45	67. JSM 239
11. MAUS 61-2	31. NRC 2	50. JSM 135	68. JSM 240
12. MAUS 71	32. JS 2	51. JSM 200	69. JSM 248
13. MAUS 81	33. Pb 1	52. JSM 202	
14. NRC 7	34. JS 92-22	53. JSM 203	
15. NRC 12	35. JS 92-12	54. JSM 207	
16. NRC 37	36. JSM 5	55. JSM 214	
17. PK 416	37. JSM 20	Mutant of JS 335	
18. PK 472	38. JSM 37	56. JSM 3	
19. PS 1024	39. JSM 104	57. JSM 7	
20. PS 1042	40. JSM 131	58. JSM 117	

VITA

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