GENETIC ANALYSIS FOR RESISTANCE TO RUST IN SOYBEAN [*Glycine max* (L.) Merrill]

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CERTIFICATE

This is to certify that the thesis entitled **"GENETIC ANALYSIS FOR RESISTANCE TO RUST IN SOYBEAN [*Glycine max* (L.) Merrill]"** submitted by **Mr. SURESHA, P. G.** for the degree of **DOCTOR OF PHILOSOPHY** in **GENETICS AND PLANT BREEDING** of College of Agriculture, University of Agricultural Sciences, Dharwad is a record of bonafide research work done by him during the period of his study in this University, under my guidance and supervision and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

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C O N T E N T S

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LIST OF ABBREVIATIONS

Sl. No.	Abbreviations	Description
1.	^{0}C	Degree celsius
2.	ASR	Asian soybean rust
3.	AFLP	Amplified fragment length polymorphism
4.	Chr.	Chromosome
5.	cm	Centimetre
6.	cM	Centimorgan
7.	CTAB	Cetyl trimethyl ammonium bromide
8.	DNA	Deoxyribonucleic acid
9.	dNTPs	Deoxynucleotide triphosphates
10.	EDTA	Ethylene diamine tetra acetic acid
11.	FAO	Food and agriculture organisation
12.	FP	Forward primer
13.	g	Gram
14.	ha	Hectare
15.	*i.e.*	That is
17.	M	Molar
16.	Mb	Megabytes
18.	min	Minutes
19.	ml	Milli litre
20.	mM	Milli molar

Contd.…..

Sl. No.	Abbreviations	Description
21.	ng	Nano gram
25.	NILs	Near Isogenic Lines
22.	NTP	Nucleotide triphosphate
23.	nm	Nanometre
24.	PCR	Polymerase chain reaction
25.	pMol	Picomolar
26.	RB	Reddish brown
27.	RP	Reverse primer
28.	rpm	Revolution per minute
29.	SSR	Simple Sequence Repeats
30.	TAE	Tris acetate EDTA
31.	*Taq*	*Thermus aquaticus*
32.	UV	Ultra violet
33.	V	Volts
34.	*viz.*,	Namely
35.	µl	Microlitre

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1. INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] owes worldwide reputation by virtue of its high quality protein and low cholesterol edible oil. It has an average protein content of 40 per cent and is protein rich source than any of the common vegetable or animal food sources. Soybean seeds also contain about 20% oil on a dry matter basis of which 85 per cent is unsaturated and cholesterol free. In view of potentiality and wide range of agricultural, industrial and medicinal values, soybean is rightly described as “nature’s unique gift” to mankind and also known as a ‘miracle crop’. The area under soybean cultivation expanded significantly as a result of its nutritive, economic importance and diverse domestic usage. It is also a prime source of vegetable oil in the international market.

Currently, soybean ranks first as an oilseed crop both in area and production in India. Over a decade, area under soybean increasing consistently besides its spread to new areas all over India from 3000 ha in 1969 to 10.80 million ha during 2018-19. Soybean production has increased from 2.49 million tonnes in 1991-92 to 12.10 million tonnes during 2018-19. This corresponds to a growth rate of 15-20 per cent per annum which obviously is one of the highest for any crop in recent past and resulted in radical improvement of rural economy.

At present, soybean occupies an area of 127.19 million hectares producing 364.33 million tonnes with the productivity of 2864 kg per hectare in the world (Anon., 2018). In India, it occupies an area of 10.80 million hectares with the production of 12.10 million tonnes and productivity of 1120 kg/ha (Anon., 2018). In India, major soybean producing states are Madhya Pradesh, Maharashtra, Rajasthan, Karnataka and Telangana.

In Karnataka, soybean is grown over an area of 3.40 lakh hectares with a production of 3.40 lakh million tonnes and productivity of about 1000 kg per hectare (Anon., 2018). The crop is cultivated in northern transitional zone (rainfed conditions) and Ghataprabha, Krishna and Malaprabha project areas (irrigated conditions). In recent years, Belgaum, Bidar, Dharwad, Haveri, Bagalkot and Kalaburgi are the major soybean growing districts in Karnataka.

The important constraints for cultivation of soybean in India are outbreak of diseases and insect pests. Diseases play a major role in yield reduction. Among the diseases of soybean, rust caused by *Phakospora pachyrhizi* Syd. is one of the devastating foliar disease of soybean.Soybean rust has spread around the globe causing extensive damage to soybean crop throughout the Southern hemisphere. Apparently it is able to travel great distances via wind-borne spores. Also known as Asian rust, this fungal infection can defoliate soybean fields rapidly, often resulting in severe and sometimes total loss (Stewart *et al.*, 2005).

Soybean rust was first reported from Japan during 1902 and later from different soybean growing areas of the world. Soybean rust is a foliar disease that causes significant yield losses (Bromfield, 1984). In Taiwan, up to 80% yield losses in soybean were reported in experimental plots. The first soybean rust outbreak in South America was observed in 2001 (Yorinori *et al.*, 2005).

Soybean rust was first reported in the continental U.S. in Louisiana in 2004 (Schneider *et al.*, 2005). One possible explanation for the entry of soybean rust into the continental U.S. is that *P. pachyrhizi* urediniospores moved from South America with Hurricane Ivan (Isard *et al.*, 2005). This was followed by a number of outbreaks in various states in the southeast region of the U.S. (Christiano and Scherm, 2007). After the initial soybean rust outbreak in the continental U.S. in 2004, the importance of finding more sources of resistance increased in the U.S. since it is the leading soybean producer worldwide.

In India, rust was first noticed at Pantnagar in September 1970 subsequently in Kalyani (West Bengal) and in low hills of Uttar Pradesh. The severity of disease may range from 10 to 100 per cent (Sarbhay and Pal, 1997) depending upon locality, season and cultivar. The disease appeared suddenly in epiphytotic form during *Kharif* 1994/95 and caused substantial yield losses particularly in parts of Karnataka, Maharashtra and Madhya Pradesh (Anahosur *et al.*, 1995). Now, it has become a major constraint for the soybean production particularly in northern Karnataka and southern parts of Maharashtra.

Soybean rust reduces yield through premature defoliation, decreasing the number of filled pods and by reducing the weight of seeds per plant. It also lowers the

quality of seed produced. The severity of loss and the particular components of yield affected depend primarily on the time of disease onset and the intensity of disease at particular growth stages of the crop (Bromfield, 1984). When early infection and unfavourable environmental conditions exist, yield losses of 50-60% can be experienced (Kloppers, 2002).Currently, the primary form of control is based on the use of fungicides of different classes and action modes (Miles *et al.*, 2007). The continuous use of these chemical fungicides may pose problem of development of resistance to pathogen. Therefore, the development of high yielding rust resistant varieties is of prime importance.

This is partial control because the fungus has several races with multiple virulence alleles which suggest that the soybean rust pathogen has high genetic variability. Therefore, the incorporation of durable soybean rust resistance into agronomically desirable and high yielding varieties is important in soybean breeding programmes.

A key requirement in breeding effort is the screening of plants for resistance to rust pathogen to identify cultivars that are likely to withstand infection. Field screening has been a routine procedure for evaluating soybean genotypes for rust resistance to local rust isolates. However, field screening can be carried out only once a year in most of the locations which are season dependent and can be affected by environmental conditions such as temperature, humidity and the simultaneous presence of other pathogens. As alternative to field screening, green house screening has been used in number of studies. The success of green house screening depends on seedling age, inoculum density, virulence, quantity, inoculation technique, pre and post inoculation environmental conditions.

The heritability of resistance to soybean rust, however, is not well documented in literature. Falconer (1989) reported that narrow sense heritability can be achieved by parent-offspring regression if parental values are means of both parents. Brim and Hanson (1961) and Fehr (1987) suggested use of expected mean squares from analysis of variance of progenies of interest, to estimate heritability. Lavett (1993) reported that narrow sense heritability is of much importance to plant breeders since low estimates indicate that only a small fraction of trait of interest will be reflected in the next

generation, whereas larger estimates indicate that the character will respond to selection easily. Griffiths *et al.* (1997) attributed the low narrow sense h^2estimates to the small amount of additive variance compared to dominance interaction. Estimating these genetic parameters will give breeders a picture of which selection methods to be employed to ensure higher genetic transfers from parents to offspring.

Breeding for soybean rust resistance has been in progress for many years in Asia (Hartman *et al.*, 1992) and more recently in USA (Miles *et al.*, 2006) and Africa (Kawuki *et al.*, 2004; Twizeyimana *et al.*, 2007). As a result specific resistance, partial resistance and tolerance against soybean rust have been identified (Hartman *et al.*, 2005). For example, Six single dominant genes for specific resistance to *P. pachyrhizi* have been identified in different cultivars as *Rpp1*, *Rpp2*, *Rpp3*, *Rpp4* (PI 459025) (Hartwig 1986), *Rpp5* (Garcia *et al.*, 2008) and *Rpp6* (Shuxian *et al.*, 2012). In addition, three single recessive genes *(rpp2*, *rpp3* and *rpp5)* controlling soybean rust was recently identified by Calvo *et al.* (2008), Ray *et al.* (2011) and Gowtham *et al.*, (2018). Despite the discovery of different resistance mechanisms, there were no commercial varieties with universally acceptable levels of resistance to rust up to 2012. This is because specific genes are resistant to some *P. pachyrhizi* isolates but ineffective against other isolates. This is due to the presence of races of *P. pachyrhizi* with virulence against the genes involved in monogenic resistance (Hartman *et al.*, 2005). Therefore it is necessary to verify the effectiveness of these genes against local isolates before they are utilized in breeding programmes. In addition, local varieties and advanced lines may possess resistance to some isolates that need to be verified for proper utilization in the breeding programmes.

Another constraint in breeding for rust resistance in soybean is lack of information regarding the genetic mechanisms controlling the inheritance of rust resistance. Previous genetic studies on the inheritance of rust resistance have reported variable findings on the type of gene action and mode of inheritance among different sources (Garcia *et al.*, 2008). A few genetic studies have been conducted with the goal of understanding the genetics of rust resistance. Some studies have shown that rust resistance is qualitatively inherited and largely controlled by single dominant genes. For instance, Bromfield and Hartwig (1980) determined the inheritance of rust resistance in two F_2 populations with PI 230970 and PI 230971 as the resistant parents. Their

analysis of these F_2s showed that their rust resistance was dominant and qualitatively (simply) inherited. Other studies have reported partial to complete dominance action in the inheritance of rust resistance (Garcia *et al.*, 2008; Ray *et al.*, 2009).

Some studies reported that resistance to rust is predominantly controlled by additive gene action (Maphosa *et al.*, 2012; Ribeiro *et al.*, 2007), while others reported partial and complete dominant gene action (Laperuta *et al.*, 2008), and more recently epistatic gene action was detected (Garcia *et al.*, 2008; Laperuta *et al.*, 2008). These studies have provided useful genetic information to the plant breeders but it is applicable to specific germplasm and range of tested environments. Therefore, further genetic studies may be useful to identify sources of resistance that are applicable to different environments.

The genetic diversity is a key component of any agricultural production system. The material from diverse geographical origin of the crop species can help to ensure conservation of co-adapted gene complexes (Brown, 1978; Frankel and Souel, 1981; Frankel, 1984; Frankel *et al.*, 1995). The importance of genetic diversity in plant breeding is obvious from the results obtained in different crops (Smartt, 1990; Ghafoor *et al.*, 2001; Upadhyaya *et al.*, 2002)

The observations for the last 25 years with respect to production trend revealed that increase in soybean yield per hectare has made only modest advances in the United States and other countries. However, the improvement is meager in India i.e. having around 1000 kg/ha for the last two decades. This is ascribed to narrow genetic base of soybean cultivars having in susceptibility to biotic and abiotic stresses resulting in yield stagnation.

Success of the breeding programme is largely depends on the extents of genetic variability present in the population for evolving promising and desired genotype. A detailed study of extent of variability in different characters associated with the yield and the knowledge of their heritability in relation to the contribution towards the yield is the prime requisite for an efficient plant breeding programme.

The extent of association assessed through correlation coefficient among the characters and resolving of such correlations into direct and indirect effects by path

coefficient analysis developed by Wright (1921) will provide breeders with insight into selection criteria to be adopted in achieving desired yield improvement. However, inheritance of quantitative characters is often influenced by variation in other characters which may be due to pleiotropy or genetic linkage (Harland, 1939). Hence, knowledge of association between yield and its components obtainable through estimation of genotypic and phenotypic correlations helps a great deal to formulate selection.

Study of inter characters association amongst the major quantitative traits greatly helps to work out the relationship among different characters thereby adopting appropriate selection index. Variability present in genetics resource, since time immemorial has always played the key role in the development of desirable lines and ultimately the yield improvement. Further, estimation of heritability and genetic advance expected after selection indicate the possibility and extent to which improvement is possible through selection.

Even though more than 110 varieties have been released in India, none of them were resistant to soybean rust. In Karnataka too, the popular cultivars namely JS 335, JS 93-05 and DSb 1 were highly susceptible to rust resulting in 30-80 % yield loss depending on the severity of disease. Among them, JS 335 was the most popular variety in this region and more than 80 per cent of the area was covered by this variety (monoculture) which is highly susceptible for rust. Though, several fungicides were found to be effective in managing the disease, but were not found economical and also cause environmental pollution and health hazards. Under these circumstances, the best strategy would be breeding for resistant cultivars or incorporating resistance into popular susceptible cultivars. Keeping this in view, a long term breeding programme was initiated at the University of Agricultural Sciences, Dharwad to develop rust resistant genotypes using conventional plant breeding approach.

After rigorous screening of more than 2000 germplasm lines two lines *viz.*, EC 241778 and EC 241780 were identified as rust resistant at hot spots *viz.*, Dharwad and Ugarkhurd (Belagavi District) during 2002-05. Immediately these two lines were utilized in hybridization programme with agronomically superior but rust susceptible varieties *viz.*, JS 335, JS 93-05 and DSb 1. This lead to the development and release of first ever highly rust resistant and high yielding variety DSb 21 (Basavaraja *et al.*, 2012).

Keeping these points in view, the investigation was carried out with the following objectives.

Objectives of Investigation

1. Evaluation of exotic germplasm lines for identification of new sources for resistance to rust
2. Studies on genetic diversity in exotic germplasm lines
3. Studies on inheritance pattern of rust
4. Study on the nature and extent of variability generated in the segregating populations with respect to yield and its component traits
5. Validation of molecular markers linked to rust resistance

2. REVIEW OF LITERATURE

Soybean [*Glycine max* (L.) Merrill] is a legume crop that grows in tropical, subtropical and temperate climate. It has 40 chromosomes (2n = 40) and is a self-fertile species with less than one per cent out-crossing. Soybean was domesticated during 11^{th} century BC around northeast of China. It is believed that it might have been introduced to Africa in the 19^{th} century by Chinese traders along the east-coast of Africa.

Several factors account for low productivity among them climatic conditions, differences in rainfall pattern, outbreak of diseases and pests are important. Diseases play a major role in yield reduction. About 100 plus pathogens are known to affect soybean crop, of which sixty six fungi, six bacteria, eight viruses and seven nematodes are involved. The world loss of more than seven million tonnes of soybean is due to diseases alone (Sinclair, 1988). These diseases cause considerable yield losses.

Soybean rust caused by the fungus *Phakopsora pachyrhizi* Syd. is the most aggressive soybean disease and can result in losses of 10% to 90% of the crop (Hartman *et al.*, 1999). Currently, the primary form of control is based on the use of fungicides of different classes and action modes (Miles *et al.*, 2007). This is partially because the fungus has several races with multiple virulence alleles which suggest that the soybean rust pathogen has high genetic variability. Therefore, the incorporation of durable soybean rust resistance into agronomically desirable and high yielding varieties is important in soybean breeding programmes.

Even though there are many options for the management of these diseases such as cultural, chemical and biological methods; host plant resistance is the best, because of its eco-friendly nature and cost effectiveness. In the host plant resistance, multiple disease resistance is more important and desirable too, as they reduce losses caused by more than one disease. Identification of multiple disease resistant sources is also important as they can be utilized in breeding for multiple disease resistance.

Keeping in view the objectives of the present investigation, literature available has been reviewed and presented in chronological order in this chapter under following headings;

2.1 Evaluation of exotic germplasm lines for identification of new sources for resistance to soybean rust

2.2 Studies on genetic diversity in exotic germplasm lines

2.3 Studies on inheritance pattern of rust

2.4 Study on nature and extent of variability created in the segregating populations with respect to yield and its component traits

2.5 Validation of molecular markers linked to rust resistance

2.1 Evaluation of exotic germplasm lines for identification of new sources for resistance to soybean rust

Rust is the major problem in soybean growing areas of the world. So effective management practices need to be evolved for the management of this disease. Among them, the most cost effective method of managing rust disease is use of resistant cultivars. Screening of germplasm lines and utilization of resistant germplasm lines in breeding programme helps to develop the cultivars with resistance to Asian soybean rust.

For the first time soybean genotype PI 200492 was identified as rust resistant in Taiwan and it was used as resistant parent to breed Tainung 3 and Tainung 4 (Chang and Chan, 1968).

Lantican (1977) reported PI 200492 as the main source of rust resistance used in Taiwan to develop resistant cultivars K-3, T-3 and T-4. Cultivars reported as highly resistant in Australia and India it was found susceptible in Philippines. Cultivars which did show resistance when first introduced into Philippines *viz*., TK-5, Wayne, K-3, T-3, T-4 and PI series gradually lost their resistance.

Over 3300 lines were screened for rust resistance in India by Singh and Thapliyal (1977). The lines were classified into three groups, resistant: PI 200465, PI 200466, PI 200477, PI 400492 and PI 224268, moderately resistant: EC 11695 (UPSM-85), EC 50081 (UPSM-168), PI 200455, PI 200474, EC 36956 (UPSM-85), Ankur and

PK 71-39 and PI 8816 as susceptible. A number of high yielding rust resistant lines were developed such as PK 73-84, PK 73-94, PK 73109, PK73-148 and PK 73-156 but later they become susceptible.

Nine out of 1080 soybean lines were rated as moderately resistant in screening tests conducted in 1975 at AVRDC Taiwan. These lines were inoculated with rust isolates of Australia 72-1, India 73-1 and Philippine 77-1. Among them PI 230970 and PI 230971 were resistant to all the isolates (Bromfield and Hartwig, 1980).

Burdon and Marshall (1980) screened six Australian species of *Glycine viz. G. canescens, G. cladestina, G. falcate, G. latrobeana, G. tobacina* and *G. tomentella.* Out of six, they reported *G. tabacina* and *G. tomentella* as a potentially valuable source of resistant genes for soybean rust resistant breeding programme.

Patil and Basavaraja (1997) evaluated several soybean germplasm lines and varieties against rust under natural epiphytotic condition and reported that the lines EC 392530, EC 392538, EC 392539, EC 392541, EC 392548, SL 423, RSC 1, RSC 2, RSC 3, JS 80-21 and PK 1029 were moderately resistant.

Hundekar (1999) reported that S 22, WC 12 and 92-10 as rust resistant germplasm lines. Among the varieties PK 1162, PK 1029, JS 80-21 and PK1024 showed moderately rust resistant reaction with better yield.

A study conducted by Bag (2002) to identify the field resistance of 60 soybean lines against rust under rainfed conditions, indicated that only four lines JS 89-49, JS 80-20, PK 416 and JS (SH) 89-59 showed consistent resistance to *P. pachyrhizi,* whereas 13 lines showed moderate resistance.

Rahangdale and Raut (2002) screened 54 soybean genotypes under green house condition and they observed that none of the soybean lines tested were immune to rust but three lines *viz.*, EC 38916, EC 39320 and TS 9821 were found highly resistant.

Verma *et al.* (2004) evaluated 242 germplasm lines of soybean under natural epiphytotic conditions for resistance to rust and reported only one line SJ-1 as highly resistant, three lines *viz.*, JS-19, RPSP-728 and PK-838 as resistant, 16 lines as moderately resistant and rest as either susceptible or highly susceptible.

Hartman *et al.* (2004) compared the virulence of *P. pachyrhizi* isolates from Asia and Australia with the isolates from Africa. The most virulent isolate was Zimbabwe 01-1 of Africa in which no resistant or immune reactions were found.

Fifty eight soybean cultivars were screened by Kumar and Jha (2004). The experiment revealed that at the lower altitude, none of the cultivars tested showed resistant reaction and at the higher altitude, cultivars NRC 25 and Punjab 1 showed resistant reaction to rust infection.

Nuntapunt *et al.* (2004) reported new resistant lines *viz.*, CN 60-10 Kr 71 and MJ 9519-5 which were resistant to all the prevailing rust races in Thailand. They also recommended tolerant cultivars *viz.*, SJ 4, SJ 5, Chiongmai 60 (CM60) and Doikhan for rust endemic areas of Thailand.

Patil *et al.* (2004) reported EC 241778 and EC 241780 as resistant, six genotypes as moderately resistant (EC 325115, EC 251378, EC 389149, EC 432536, EC 241760 and EC 333917), 68 genotypes as susceptible and 906 genotypes as highly susceptible to rust caused by *Phakopsora pachyrhizi.*

Ramteke *et al.* (2004) conducted an experiment during the rainy season of 2002 and 2003 to screen 41 genotypes of soybean [*Glycine max* (L.) Merrill] against rust under field condition at rust hot spot area Ugarkhurd, Belgaum Dist, Karnataka and found none of the genotypes as resistant including seven differentials (PI 200492, PI 230970, PI 462312 (Ankur), PI 459025, PI 230971 and PI 459024) which were reported earlier as resistant.

Hartman *et al.* (2005) evaluated 16,000 soybean accessions for resistance to *P. pachyrhizi* in the USDA-ARS, FDWSRU Biosafety level 3 containment green houses. The germplasm evaluations were done on seedlings using a mixture of isolates from Africa, Asia and South America. Out of 16,000, fewer than 800 were identified as resistant to rust.

Miles *et al.* (2006) evaluated 16,000 soybean accessions in a two-tiered inoculation program using a mixture of four *P. pachyrhizi* isolates in Bio safety Level 3 containment greenhouse. In the first round of evaluation, 16,595 accessions were rated

for rust severity, of these, 3,215 accessions based on low visual rust severity or the presence of a red-brown reaction, were selected for a second round of evaluation. After second round of replicated evaluations of the 3,215 accessions, 805 accessions were selected for further evaluation, again based on low mean visual severity or the presence of a red-brown reaction. Some of these selected accessions had the potential to provide soybean rust resistance genes that may be useful for incorporation into commercial soybean cultivars.

Mahesha (2006) screened 204 genotypes against soybean rust. Only two genotypes *viz.*, EC 241778 and EC 241780 showed highly resistant reaction, 12 genotypes showed susceptible reaction and rest of them showed highly susceptible reaction to rust.

Patzoldt *et al.* (2007) created inter sub generic hybrids between *G. max* and *G. tomentella* which is having additional gene for resistance to soybean rust. These amphidiploid hybrid lines were further back crossed to *Glycine max*. Both fertile and sterile sets of progenies were screened at USDA-ARS and they found that these hybrid clones retained the rust resistance.

Twizeyimana *et al.* (2007) evaluated fourteen soybean accessions and breeding lines for rust resistance in growth chambers using detached leaves, under green house and field conditions. The results revealed that accessions PI 594538A, PI 417089A andUG-5 had very low levels of disease compared with the susceptible checks and all other genotypes.

Twenty five rust resistant accessions were screened by Okolo *et al.* (2008) in Nambulongue (central Uganda), in which only 10 accessions, G 33, G 8527, G 8587, GC 60020-8-7-18, GC 87016-11-B-2, GC 87021-26-B-1, SRE-D-14A, SRE-D-14B and SS 86045-23-2, showed rust symptoms at R6 stage in three seasons of testing. Soybean rust resistant genes Rpp3 and Rpp4 did not confer resistance at Nambulongue and only gene Rpp2 was found to be effective.

Twizeyimana *et al.* (2008) evaluated 178 soybean breeding lines for rust severity in the field in 2002 and 2003 at Yandev and Ibadan, Nigeria. Thirty-six lines with disease severity ≤ 3 (based on a 0 to 5 scale) were selected for a second round of

evaluation in 2004 at Ibadan and 11 breeding lines with disease severity ≤ 2 were further evaluated in third round for rust resistance at Ibadan in 2005 and 2006. These results indicate that some of the breeding lines (TG x 1835-10E, TG x 1895-50F and TG x 1903-3F) and accessions (PI 594538A, PI 417089A and UG-5) would be useful sources of soybean rust resistance genes for incorporation into high yielding and adapted cultivars.

Basavaraja *et al.* (2009) reported one line each from three crosses JS 335 x EC 241778, JS 335 x EC 241780 and JS 93-05 x EC 241780 as highly resistant against rust among the 180 advanced breeding lines evaluated under natural epiphytic conditions of rust in Karnataka.

Pham *et al.* (2009) screened 20 resistant soybeans entries and compared those entries after inoculation with *P. pachyrhizi* inoculum in Paraguay and Vietnam. The entries included two universal susceptible cultivars and four resistant source genes (Rpp1-4). Out of 20, 4 to 10 resistant entries were selected from the field trial in Paraguay and Vietnam. The isolate M 103 was the most susceptible and GC 84058-18-4 was the most resistant. The reaction patterns on these resistant entries to *P. pachyrhizi* isolates were different compared with the four soybean accessions with Rpp genes, indicating that they contain novel source of resistance. Among the *P. pachyrhizi* isolates, TW 72-1 from Taiwan and IN 73-1 from India exhibited most susceptible and resistance reactions respectively.

Khot *et al.* (2010) conducted field trial during three consecutive *Kharif* crop seasons (2000-01, 2001-02 and 2002-03) in a randomized block design with three replications to evaluate soybean genotypes for stable type of resistance reaction. The rate of soybean rust over a period of time was measured by area under disease progress curve (AUDPC) on ten genotypes of soybean inoculated with heavy load inoculum of *Phakopsora pachyrhizi.* The varieties DS 228 and DS 227 exhibited resistant reaction relative to other genotypes. The apparent infection rate of DS 228 was found very less compared to other genotypes.

Paul *et al.* (2010) screened three soybean germplasm lines viz., TG x 198776F, TG x 1987118F and TG x 1987129F which were resistant to *P. pachyrhizi.* These lines were derived from a tropical soybean rust-resistant cultivar UG 5 and a rust-susceptible,

high-yielding elite breeding line TG x 180531F. A total of 297 F_7 lines were evaluated for rust resistance under greenhouse conditions using whole inoculated plants. Based on assessments from these various tests, they concluded that these three lines have combinations of high levels of rust and bacterial pustule resistance, good agronomic traits and adaptable maturity.

Shivakumar *et al.* (2011) screened segregating populations (F_3) of two crosses involving two high yielding varieties JS 335 and JS 93-05 (both susceptible to rust) and one germplasm line EC 241780 (resistant to rust) under artificial inoculation. Six among 62 progeny lines (one progeny line from cross JS 335 x EC241780 and five from JS 9305 x EC 241780) exhibited resistance and 16 progenies (two from JS 335 x EC241780 and 14 from JS 9305 x EC241780) exhibited moderate resistance and rest showed susceptible and highly susceptible reactions.

Radhika (2012) screened segregating populations (F_2) of two crosses involving two high yielding varieties JS 335 and JS 93-05 (both susceptible to rust) and germplasm line EC 241780 (resistant to rust) under artificial inoculation. Twenty among 200 progeny lines (Twelve progeny lines from cross JS 335 x EC 241780 and eight from JS 93-05 x EC 241780) exhibited resistant or moderately resistant and rest showed susceptible to highly susceptible reactions.

Baiswar *et al.* (2012) screened twenty three varieties/lines including a susceptible check JS 335. Results revealed that only two lines NRC 80 and MAUS417 were moderately susceptible. Lines TS 5, Himso 1676 and MAUS 282 were highly susceptible and all other lines were found susceptible. No line or variety was in the moderately resistant or resistant category as all the lines exhibited Tan type lesions.

Sulistyo and Sumartini (2016) evaluated ten soybean genotypes consisting of eight lines and two varieties (Argomulyo and Grobogan) for resistance to rust disease. The eight lines tested were a progeny of a cross between offspring of IAC 100 (resistant to rust disease) with high yielding soybean varieties (Argomulyo and Grobogan), results showed that no soybean genotype classified as immune or resistant genotype to rust disease. The whole genotypes tested were categorized as moderately resistant genotype.

2.2 Studies on genetic diversity in exotic germplasm lines

The domestication of soybean [*Glycine max* (L.) Merrill] from its wild progenitor (*Glycine soja*) occurred in China (Chung and Singh, 2008). *Glycine soja* has smaller pods and seeds, viny and twining stems, and pronounced shattering at maturity. Hybridization between wild and cultivated species produced fertile progeny. Semi wild accessions can be distinguished from *G. max* and *G. soja* based on either phenotypic or genotypic data (Chen and Nelson, 2004). Major genetic bottlenecks occurred between wild and cultivated soybean species that were characterized with loss of genetic diversity. Using chloroplast microsatellites, eight haplotypes were found in cultivated soybean. However, using DNA sequences from 102 genes, Hyten *et al.* (2007) reported that diversity in the wild species was halved in the domestication process and that 81% of the rare alleles were lost. Exportation of soybean from centers of origin to the New World has therefore resulted in significant loss of genetic diversity which justifies the need to broaden the current soybean germplasm base in order to sustain and/or increase production.

Quantification of germplasm genetic diversity is very vital for efficient selection of parental lines for crossing and/or for germplasm conservation (Tatineni *et al.*, 1996). Plant breeding has played a part in shaping genetic diversity of crops. For instance, soybean genetic diversity was enhanced through genetic recombination along the process of varietal improvement to meet agricultural, social and economic needs. Some varieties were artificially selected by farmers to suit their needs while others were naturally selected in response to geographical, climatic and edaphic factors. However, studies have indicated that only a few accessions have contributed majority of genes in current cultivars, leading to low genetic diversity in soybean varieties which is a major constraint for genetic improvement.

The success of soybean breeding programme depends on degree of variability in germplasm, choice of parents and selection procedure (Dong *et al.*, 2004). Although soybean has a rich source of germplasm, narrow spectrum of variability is a problem to its breeding programme. This setback is worsened due to high level of self-pollination. Diversity in soybean serves as key for finding and incorporating new genes into elite soybean genotypes. Genetic distinction among genotypes are useful for planning future

breeding programme for yield, oil content, protein, pest and disease resistance improvement (Wang *et al.*, 2006). Understanding the amount and distribution of genetic difference within and among soybean genotypes is a key for predicting the degree of inheritance, variation and extent of heterosis that are crucial for breeding.

As we know, phenotypic traits are controlled by genes and affected by environment, but large number of accessions can adapt to environments. The phenotypic data has more polymorphism in genetic diversity and reveal genetic variation indirectly. On the contrary, the molecular data reveal genetic variation directly, but fewer markers have less polymorphism. It is very difficult to obtain molecular data for a large number of accessions that has enough polymorphism to show the genetic diversity of germplasm. So, the morphological traits are the suitable and practical tools for studying the genetic diversity on large number of accessions.

Variation in shape of plants has always been an important means of (1) distinguishing individuals; (2) controlling seed production; and (3) identifying the negative traits those effects on yield, the genetic diversity centres of annual wild soybean and the soybean lines resistance to pod shattering, drought, pests or disease (Malik *et al.,* 2006). The studies on soybean germplasm exhibited a wide range of phenotypic variation for pod number, seed number and plant yield. It also showed that soybean developing stages had close association with agronomic traits as well as yield and yield components.

Soybean genetic diversity can be evaluated by the differences in agronomic traits, morphological traits, pedigree information, isozymes and DNA markers (Sneller, 1994; Dong *et al.*, 2004; Wang *et al,* 2010). The polymorphism can also be observed at morphological, molecular and biochemical levels. The accuracy of genetic variation is determined by the method used. Compared with morphological variation, molecular polymorphism is generally considered to be independent of the environment (Gauthier *et al.*, 2002).

2.2.1 Genetic diversity in soybean germplasm based on morphological characters

Success of a crop breeding programme depends on the extent of variability present in the available germplasm, choice of the parents and the selection procedure.

Morphological traits or characters reflect not only on the genetic composition of a cultivar, but also the interaction of the genotype with the environment in which it is expressed (Smith and Smith, 1992).

Kumar and Nadarajan (1994) studied the genetic divergence of 64 genotypes of soybean for 11 traits led to their grouping into 11 clusters. Grouping of genotypes in different clusters was not related to their geographic origin and genotypes from different geographic locations were grouped into one cluster while genotypes of the same geographic origin showed genetic diversity. The diversity among the genotypes measured by inter cluster distance was adequate for improvement by hybridization and selection. Based on mean performance, genetic distance and clustering pattern, hybridization involving genotypes SDP (L), KB 83, KB 85, IC 16990 and AMSS 52 are likely to give desirable segregants.

The genetic diversity was evaluated for genotypes of soybean based on the yield related traits (Rajanna *et al.*, 2000; Malik *et al.*, 2006, 2007; Ngon *et al.*, 2006). It has been reported that differences among genotypes for all the characters were highly significant and the grain yield was positively and significantly correlated with number of pods per plant. The selection for the character had positive direct effect on yield. However, some traits had negative direct effects on yield, such as the leaf area, days to 50% flowering, days to maturity, plant height, oil content and protein content.

By using morphological data for cluster analysis, Dayaman *et al.* (2009) used 45 soybean accessions and grouped them into six different clusters based on morphology. Griffin and Palmer (1995) grouped 68 genotypes of soybean into seven clusters based on morphology. Ojo *et al.*, (2012) also reported that phenotypic diversity among 40soybean genotypes using cluster analysis generated seven clusters.

Iqball *et al.* (2010) conducted an experiment to determine the variability and association among 9 traits in 139 soybean genotypes. Results of analysis of variance showed significant differences among genotypes in terms of traits under study, which indicate the existence of genetic variation. Correlation coefficient indicated that the grain yield was positively and significantly correlated with all studied traits except plant height, which showed non-significant association during both years. Oil content showed significant and positive correlation with grain yield, 100 seed weight and harvest index

while significantly negative correlation were observed with days to maturity, plant height and number of branches per plant.

According to Shadakshari *et al.* (2011) among the 12 morphological characters used to analyse the genetic diversity of 50 soybean germplasm, number of seeds per plant accounted for 40.24 % in assessing the diversity; followed by seed yield per plant contributing 20.12 %. Dayaman *et al.* (2009) also reported that among the 22 morphological traits used to investigate diversity of selected Indian soybean accessions, seed yield recorded the highest coefficient of variation of 40.06 followed by number of branches per plant.

Mebatsion *et al.* (2012) evaluated grain shape variability using principal component analysis (PCA) and 99 % of the variation in the shape of grains was captured by the first two principal components. Similarly, Bhartiya *et al.* (2011) used PCA to determine the variability of both indigenous and exotic black soybean from different eco-geographic regions of the world for which the first four principal components together accounted for 70.28 % of the total variation.

Athoni and Basavaraja (2012) studied the genetic variability, association analysis and genetic diversity for productivity on 84 soybean genotypes. The analysis of variance revealed the prevalence of significant difference among the genotypes for all the 11 characters studied. Plant height was the only character which showed high phenotypic and genotypic coefficient of variation while days to maturity, number of pods per plant and oil content recorded a low phenotypic and genotypic coefficient of variation and rest of characters recorded moderate phenotypic and genotypic coefficient of variation. There was not much amount of diversity was obtained in the material representing diverse eco-geographical regions of the country revealed no relationship between geographic diversity and genetic diversity.

Salimi *et al.* (2014) studied the relationships between morphological characters of soybean plant an experiment was conducted in randomized complete blocks design in two replications under drought stress condition at Agricultural College of Guilan University in 2008. Result of analysis of variance showed that there was significant difference among the studied soybean genotypes in the majority of traits. A similarity factor was constructed using nearest neighbor method for morphological characters

and varieties were classified into 7 groups. Classifying the results of the cluster analysis identified TNH56 and BP genotypes suitable for drought stress condition and further these genotypes could be used as source of germplasm for breeding for drought tolerance.

Adsul and Monpara (2014) studied the hundred genotypes of soybean for 15 characters in randomized block design with three replications and grouped them into fifteen clusters. The cluster I was the largest with 55 genotypes followed by cluster III containing 17 genotypes and cluster IV containing 16 genotypes. The remaining clusters were solitary with single genotype each. Genotypes falling in these clusters may serve as potential parents for a hybridization programme. The presence of clear phenotypic and genotypic differences in the characters under consideration between or among clusters gives us an opportunity to bring about improvement through hybridization of genotypes between these clusters and subsequent selection in the segregating generations.

2.2.2 Genetic diversity in soybean germplasm based on use of molecular markers for breeding

Traditionally, genetic diversity of cultivars of *Glycine max* is determined by a combination of morphological or agronomic traits and biochemical tests/assays (Chowdhury *et al.*, 2001: Dayaman *et al.*, 2009). Most commercial and released soybean cultivars arose from hybridization between members of an elite group of genotypes; hence the amount of genetic variability among those cultivars is small (Chowdhury *et al.*, 2001). Such cultivars are often indistinguishable based on agro morphological traits or biochemical tests which are often subjected to environmental influence interplaying with a number of genes and thus may not represent genetic divergence in the entire genome (Diwan and Cregan, 1997; Brown *et al.*, 2000). A large number of polymorphic markers are required to measure genetic relationships and genetic diversity; as a result, it is now widely accepted that information generated from DNA-based analyses using Restricted Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPDs), Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) alone or with morphological analyses provide the best estimate of genetic diversity (Chowdhury *et al.*, 2001).

2.2.3 Use of Microsatellites as molecular markers

Microsatellites also known as simple sequence repeats (SSR) consist of tandemly repeated, short DNA sequence motifs (Maughan *et al.*, 1995). The popularity of microsatellites from a unique combination of several important advantages; they are codominant markers, high genomic abundance in a population, and random distribution throughout the genome (Morgante *et al.*, 2002). They exhibit allelic diversity. Their reproducibility is much higher than RAPDs (Demeke *et al.*, 1997; Karp *et al.*, 1997). The flanking sequences of microsatellites are usually highly conserved, making it possible to design universal primers for their study across genomes (Akkaya *et al.*, 1992; Diwan and Cregan, 1997).

Although microsatellites are very useful in general, they also have certain disadvantages; including relatively high cost of marker development, occasional occurrence of artefacts such as stutter bands (Walsh *et al.*, 1996).

In general, microsatellites show a high level of polymorphism, so they are very informative markers. They can be used for population genetic studies and gene mapping, ranging from the individual level (e.g. clone and strain identification) to that of closely related species (Jarne and Lagoda, 1996).

2.2.3.1 Assessing genetic diversity in soybean germplasm using SSR markers

Molecular markers are frequently used in the analysis of soybean germplasm. Simple sequence repeats markers have been shown to be highly polymorphic in soybean (Akkaya *et al.*, 1992; Diwan and Cregan, 1997). The analysis of the polymorphism in DNA sequences allow for a more accurate genetic characterization.

Abe *et al.* (2003) used 20 SSR loci in 131 accessions introduced from 14 Asian countries to detect genetic diversity among them. Morgante and Olivieri (1994) detected similar levels of polymorphism in seven SSR loci in a group of soybean genotypes. Akkaya *et al.* (1992) used several types of SSRs to analyze the diversity of 43 soybean genotypes including ancestral and domestic cultivars representing the northern and southern U.S germplasm. Doldi *et al.* (1997) found two to six alleles per locus in a group of 18 soybean cultivars using 12 microsatellite loci. Diwan and Cregan (1997)

observed an average of 10.1 alleles per locus in a total of 20 loci studied in soybean genotypes that represented 95% of all alleles of the germplasm cultivated in the north of the United States.

In a study on 186 Brazilian soybean cultivars, Priolli *et al.* (2002) found four to eight alleles per loci using 12 SSR loci studied. They determined that SSR with AT and ATT repeat motifs were highly polymorphic in soybean and identified up to eight alleles at each locus.

Rongwen *et al.* (1995) identified 11 to 26 alleles at each of seven SSR loci in a diverse sample of soybean genotypes that included U.S. cultivars, *G. max*, *G. Soja*, plant introductions and Chinese landraces. Maughan *et al.* (1995) detected 79 alleles across five SSR loci in a sample of 94 soybean accessions of *G. max* and *G. soja* genotypes.

Tantasawat *et al.* (2011) used 11 SSR primers to analyse genetic relationships among 25 soybean genotypes. They reported that genetic similarity between genotypes and those 25 genotypes formed four major clusters. Singh *et al.* (2010) also reported a cluster analysis based on coefficient of similarity classified 44 soybean genotypes into four major clusters derived from 120 SSR makers. Dayamann *et al.* (2009) used 11SSR markers to analyse genetic diversity of 45 soybean genotypes and these accessions were grouped into 14 different clusters.

Anthropologists adapted Karl Pearson coefficient of racial likeness (CRL) (Morant, 1923) for the purpose of discriminating any two populations having unknown origin. Mahalanobis (1936) after identifying that CRL was a test of divergence between two samples rather than a measure of actual magnitude of divergence between them developed the D^2 statistic, which actually provides a measure of magnitude of divergence between two groups under consideration.

Mahalanobis (1936) first used this technique in the form of generalized distance, which considers the variation produced by any character and their co-joint effect that it bears on other characters. Mahalonobis also pointed out that D^2 would remain constant when samples were drawn from two different populations irrespective of size of the representative samples which indicated that D^2 supplied measure of actual magnitude of

divergence between two groups under comparison. Its application to the field of botany was started with the work of Nair and Mukharji (1960), who applied this method in classifying the natural plantation teak tree types. Its application was also extended to taxonomic studies.

In soybean, the Mahalanobis D^2 analysis was applied to discriminate one group of genotypes with another and studies carried out on this aspect have been presented in Table 1.

2.3 Studies on inheritance pattern of soybean rust

Rust is the most wide spread disease of soybean, as it occurs in all the parts of world, wherever soybean is cultivated. Usually in the beginning, it appears on the lower surface of the leaves as small, yellow lesions, which later develop into light brown to dark pustules. As the disease progresses it causes yellowing, premature drying and defoliation. Heavily infected plants have severe defoliation before maturity with fewer pods and reduced seed size (Hartman *et al.* 1991; Yang *et al.* 1991). The yield losses have been reported from 30-100 per cent in India (Sarbhay and Pal, 1997).

The soybean rust fungus belongs to genus *Phakopsora* (family: Phakopsoraceae, order: Uredinales) and is caused by two described species, *P. pachyrhizi* Sydow, which is predominant in Australia and Asia and *P. meibomiae* which is found in North America, Caribbean area and South America down to Argentina. The causal agent of rust in Africa has not been described taxonomically (Hartman *et al.* 1999). *P. pachyrhizi* is more aggressive than *P. meibomiae*. It is principally spread by windborne spores. The rust caused by *P. pachyrhizi* is more severe under conditions of moderate temperatures (18°C to 26°C) and extended leaf wetness. Long periods of temperatures above 28°C are unfavourable for rust development (Bromfield, 1984). Studies on effect of weather variables on soybean rust severity indicated that minimum temperature was the best predictor for soybean rust severity in Meghalaya (Baiswar *et al.* 2012). The severity of the disease is favoured by continuous rainfall/ high humidity combined with moderate temperatures and extended leaf wetness (Bromfield 1984).

Table 1. Summary of review of literature on genetic diversity using D^2 analysis in Soybean

Material Distance (genotypes)	Number of clusters	Maximum cluster Distance		References
		Intra	Inter	
40	10	-	842.3	Bains and Sood (1984)
58	7	87.04	771.72	Ghatge and Kodu (1993)
81	12	93.91	715.28	Kalaimagal (1991)
103	24	74.66	2020.64	Maharaddi (1996)
50	10	101.67	600.77	Ganeshmoorthy and Sheshadri (2002)
50	10	02.134	07.310	Gawande *et al.* (2002)
62	15	4.43	8.49	Sharma (2005)
50	19	8.77	26.76	Gaikwad *et al.* (2007)
81	5	32.69	246.81	Parameshwar (2006)
80	12	45.13	183.49	Aravind (2006)
36	6	60.68	379.08	Patil *et al* (2011)

Although the disease was first reported in Japan during 1902, however it did not reach epidemic proportions until the late 1940's. It has been reported in various countries including Australia, China, Korea, India, Japan, Nepal, Taiwan, Thailand, Philippines, Mozambique, Nigeria, Uganda, Zimbabwe, South Africa, Brazil, Argentina, Paraguay and USA. In 2004, soybean rust reached the United States (Schneider *et al.* 2005), and movement of the pathogen from South America to North America was believed to be facilitated by Hurricane Ivan (Isard *et al.* 2005). The possible long distance transmissibility of the uredinio spores (Isard *et al.* 2005) has enabled the fungi to inflict large damage to soybean related industries in North and South American countries.

In India, the rust pathogen was first collected at Pune in 1906 by Sydow and Butler but rust was observed for the first time on soybean at Pantnagar during September 1970 crop season (Thapliyal 1971; Yang 1977). Further the disease was observed at Kalyani in West Bengal and low in the hills of Uttarakhand. It subsequently became severe in 1971 and 1974 but was mild during 1972 and 1973 (Singh and Thapliyal 1977). The disease appeared in epiphytotic form in parts of Karnataka, Maharashtra, Madhya Pradesh, Andhra Pradesh and Rajasthan during *Kharif* 1994 and 1995 (Patil *et al.*1997). Khot *et al.* (2010) reported that the disease was widely prevalent in Krishna valley of Maharashtra and Karnataka during the rainy season from 2002 to 2005. Early infection was noticed in area adjoining to Krishna river belt from Kolhapur district from where disease spread to adjoining area.

A few genetic studies have been conducted with the goal of understanding the genetics of soybean rust resistance. Some studies have shown that rust resistance is qualitatively inherited and controlled by single dominant gene. For instance, Bromfield and Hartwig (1980) determined the inheritance of soybean rust resistance in two F_2 populations with PI 230970 and PI 230971 as the resistant parents. Their analysis of F_2's showed that their rust resistance was dominant and qualitatively (simply) inherited. Other studies have reported partial to complete dominance action in the inheritance of soybean rust resistance (Garcia *et al.*, 2008; Ray *et al.*, 2009).

Quantitative inheritance has also been reported to control inheritance to soybean rust resistance. Ribeiro *et al.* (2007) used a 6x6 full diallel mating design and reported

that soybean rust resistance was quantitatively inherited, which was predominantly controlled by additive gene action. These findings were supported by Maphosa *et al.* (2012) who found that soybean rust resistance was predominantly controlled by additive gene action.

Studies were conducted in containment facilities at Frederick, MD to determine the genetic basis of resistance to *Phakopsora pachyrhizi* Syd. carried by the soybean [*Glycine max* (L.) Merrill] line PI 459025. Previous studies showed that soybean genotypes PI 200492, PI 230970, and PI 462312 each carried a single dominant gene conferring resistance to a specific soybean rust isolate. Line PI 459025 was identified as resistant to Taiwan 80-2 as well as Taiwan 72-1 and India 73-1. Line PI 459025 was crossed with each of the three previously identified sources of resistance. The F_1 plants, F_2 populations and selected F_3 lines were inoculated with each of the three rust isolates to determine their reaction. For each plant evaluated, a leaflet of a single trifoliolate leaf was inoculated with a different rust isolate. The results showed that PI 459025 carried a single dominant gene for resistance to all three rust isolates and that this gene was at a different loci from the three previously identified genes conferring resistance to specific rust isolates. The genotype assigned for rust resistance of PI 459025 is *Rpp1 Rpp1, Rpp2 Rpp2, Rpp3Rpp3, and Rpp4 Rpp4*. (Hartwig, 1986).

Four independent dominant genes for specific resistance have been identified. The gene symbols assigned to each of specific resistant genes are Rpp1 in PI 200492, Rpp2 in PI 230970, Rpp3 in PI 462312 (Hartwig and Bromfield, 1983) and Rpp4 in PI 459025 (Hartwig, 1986).

Information on the host differential response and genetics of resistance has lead to identification of six different rust resistant genes (*Rpp*: Resistance to *P. pachyrhizi*), named *Rpp1* to *Rpp6*, against specific isolates of *P. pachyrhizi* (Hartman *et al.* 2005; Bonde *et al.* 2006; Miles *et al.* 2011). *Rpp1* confers an immune response for which there are no visible symptoms in the plant (Miles *et al.* 2006). Resistance responses mediated by the *Rpp*2 to *Rpp*5 loci results in the formation of visible reddish brown lesions which limit fungal growth and sporulation, there by suggesting of a hypersensitive like response (Bonde *et al.* 2006; Garcia *et al.*, 2008). The susceptible interaction with rust

results in Tan colored lesions and fully sporulating uredenia (Bromfield and Hartwig 1980; Bromfield, 1984; Miles *et al.* 2006).

Six dominant genes have been implicated in the rust resistance in perennial *Glycine* but the inter relationship among them and the genes for resistance in *G. max* have not been studied. The soybean lines Tainung-4, PI459024 and one *G. soja* line PI239871B are reported to have additional specific genes for resistance (Bromfield and Melching 1982; McLean and Byth 1980). Three independent dominant genes conferring resistance to specific races of soybean rust in different lines has been reported. The line PI462312 (Ankur) was assigned the genotype *Rpp*3*Rpp*3, PI200492 *Rpp*1*Rpp*1 and PI230970 *Rpp*2*Rpp*2 (Hartwig and Bromfield 1983). Additional research indicated that PI239871A, TK-5, and Tainung-4 might have single dominant gene for resistance (Tschanz *et al.* 1986). Resistance in PI459025 was controlled by a dominant gene while in cultivars AGS 129 and AGS 181 it was controlled by multiple genes (Tan *et al.* 1991).

Evaluation of wild perennial *Glycine* species for resistance to *P. pachyrhizi* revealed that accessions of *G. tabacina* and *G. tomentella* were resistant to soybean rust. *G. tomentella,* the resistance in aneuploids (2n = 78) was controlled by single dominant gene and in tetraploid (2n=80) by two or three gene loci**.** Specific resistance has been also reported in wild *Glycine* spp and some of these have been used as differential hosts for the identification of rust patho type (Burdon and Speer, 1984). In *G. canescens,* one of the seven host lines studied had two independently inherited genes of resistance, while rest six lines had single resistance gene.

Rahangdale and Raut (2004) they studied the inheritance of rust resistance in soybean with nine crosses involving two susceptible and five resistant genotypes. They analyzed the F_2 segregants and they concluded that rust resistance is governed by single dominant gene and in some crosses they found no segregation for rust resistance in turn which revealed that presence of the same gene for resistance in both the parental lines.

The soybean rust resistance was found to be controlled by the single dominant gene *Rpp*2 in the genotype PI230970 (Bromfield and Hartwig 1980). Later on *Rpp*2 gene was also reported from other donor sources *viz.,* L86-1752, PI 197182, PI 230971, PI 417125 (Kyushu31). Single dominant gene (*Rpp*2) for resistance to soybean rust has

been reported by studying 3:1 ratio in two F_2 populations of PI 230970 and PI 230971 under green house conditions using four different races *viz.*, Australia-72-1, India-73-1, Taiwan-72-1 and Phillippines-77-1 (Bromfield and Hartwig 1980). Like *Rpp*1, the *Rpp*2 gene also showed susceptible reaction to Tw 80-1 isolate. The parents of resistant x susceptible cross gave hypersensitive response to rust and develop necrotic spots restricting the pathogen. Soybean rust resistant genes derived from PI 197182, PI 230971 and PI 417125 did not segregate in crosses with PI 230970, which indicates that these genotypes have a single resistance gene in the *Rpp*2 locus (Laperuta *et al.* 2008).

Cheng and Chan, (1968) reported single dominant gene identified in the Indian accession PI 462312 (Ankur). The name *Rpp*3 gene was assigned in the genotype PI 462312 (Ankur) (Bromfield and Melching, 1982, Hartwig and Bromfield 1983). The *Rpp*3 gene was found resistant to In 73-1 and susceptible to Tw 72-1 and Tw 80-1 isolates. Hyten *et al.* (2009) mapped *Rpp*3 to chromosome 6 (LG C2) which was between markers Satt_460 and Sat_263.

Silva *et al.* (2008) used a $F_{2:3}$ mapping population derived from PI 459025B (*Rpp*4 resistant) and BRS184 (susceptible) to map the *Rpp*4 locus to soybean chromosome18 (linkage group [LG] G). *Rpp*4 mapped within 1.9 cM of simple sequence repeat (SSR) marker Satt288. The mapping populations were screened with SSR markers, using the bulk segregant analysis (BSA) to identify linked markers. Resistance genes showed an expected segregation ratio for a dominant trait and allowed mapping of *Rpp*4 loci on the linkage groups. Their *Rpp*4 locus position was consistent with that of Garcia *et al.* (2008), who mapped *Rpp*4 within 2.8 cM of Satt288. The associated markers will be of great value on marker assisted selection for this trait.

Three recessive genes conferring resistance to *P. pachyrhizi* have also been reported (Calvo *et al.* 2008; Pierozzi *et al.* 2008). Two independent single recessive resistance genes were reported in resistant parents (PI 200456 and PI 224270) by crossing each of them with susceptible cultivar (Calvo *et al.* 2008). They further suggested that use of recessive genes governed soybean rust resistance may represent a different type of resistance for breeding programs aimed at more durable resistance (Pierozzi *et al.*, 2008) reported that in the genotype, BR01-18437 resistance to soybean rust was controlled by a single recessive major gene.

Soybean rust resistance is sometimes controlled by single dominant genes (Tan *et al.*, 1991; Kiryowa *et al.*, 2005). Six single independently dominant genes *Rpp1*, *Rpp2*, *Rpp3 Rpp4*, *Rpp5*, and *Rpp6 for* specific resistance to *P. pachyrhizi* have been identified in different soybean genotypes (Hartman *et al.*, 2005; Hyten *et al.*, 2007; Garcia *et al.*, 2008). These genes are located at different loci and provide resistance to specific races of *P. pachyrhizi* (Hartman *et al.*, 2004; Bonde *et al.*, 2006). The single dominant genes may not be durable in commercial varieties and they can be ineffective with diverse *P. pachyrhizi* isolates (Yorinori, 2004; Hartman *et al.*, 2005). Several major genes that confer resistance to soybean rust have also been identified in new plant introductions (Monteros *et al.*, 2007; Garcia *et al.*, 2008; Pierozzi *et al.*, 2008; Chakrobarty *et al.*, 2009).

Specific gene resistance to *P. pachyrhizi* is unlikely to provide lasting protection due to resistance break down (Miles *et al.*, 2006). This is associated with increased selection pressure in *P. pachyrhizi* populations (Bonde *et al.*, 2006) and high genetic variability of *P. pachyrhizi* races (Hartman *et al.*, 2005). Few moderately resistant soybean varieties (Maksoy 1N, Maksoy 2N, Maksoy 3N and Namsoy 4M) are available in Uganda, but these develop rust under severe rust pressure which results into yield losses. These varieties contain specific resistance genes to soybean rust (Tukamuhabwa and Maphosa, 2011) that are likely to break down as new *P. pachyrhizi* races proliferate in the region (Kiryowa *et al.*, 2005). This was initially observed with soybean varieties Komata and Ankur which were initially resistant to soybean rust but soon become susceptible due to resistance breakdown (Bromfield, 1984). Therefore, there is need to broaden the soybean germplasm base using genetic material from many possible sources for use in developing soybean rust resistant varieties.

Calvo *et al.* (2008) investigated the genetic basis of the resistance in PI 200456 and PI 224270 by crossing them with a susceptible cultivar (CD 208). Phenotypic segregation ratios for F_2 plants and $F_{2:3}$ lines showed that the resistance in each resistant parent was controlled by a single recessive gene. A test for allelism demonstrated that these genes were non-allelic. This is the first report of recessive genes (rpp5 and rpp2) controlling soybean rust resistance in soybean. The recessive rust resistant gene rpp5 was identified from PI 200456 and rpp2 from PI 224270 respectively.

Ribeiro *et al*, (2008) investigated the quantitative genetic control of *P. pachyrhizi* and estimated parameters associated to soybean yield in the absence and presence of this phyto pathogen. Six cultivars and their 15 Diallel derived F_2 and F_3 generations were assessed in experiments carried out in the absence and presence of *P. pachyrhizi*. The results indicated that soybean yield in the presence and absence of *P. pachyrhizi* is controlled by polygenes expressing predominantly additive effects that can be selected to develop new cultivars resistant or tolerant to *P. pachyrhizi*.

Kiryowa *et al*, (2008) estimated the magnitude of genetic parameters controlling soybean rust resistance and estimated narrow sense heritability of the resistance. Crosses were made and progenies analysed according to the North Carolina II mating design with three resistant parents acting as males and three susceptible parents acting as females, F_1s and F_2s were planted in the field during two rainy seasons (2004 - 2005). Resistance gene for rust expressed complete dominance with 4VD/ 2VA = 1.1. Broad sense heritability (*hb2*) was 0.5. The ratio of Additive Variation to Phenotypic Variation (*VA/VP*) was 0.3.

Kim *et al.* (2012) suggested presence of two nucleotide binding site-leucine-rich repeat (NBS-LRR) genes in the 94.4 kb region between SSR50 and SSR1859 on the *G. max* genome of PI 56135. The 21 SNP markers mapping within the *Rpp1* region produced four distinct SNP haplotypes among the five *Rpp1* sources. However there were no SNP markers or haplotypes that could distinguish between the five soybean rust-resistant accessions and the 33 susceptible ancestral accessions. Results of Kim *et al.* (2012) further suggested that SSR66 and SSR1859 could be useful in predicting whether soybean rust resistant accessions with unknown resistance genes have the same resistance allele in the *Rpp1* region as the five known sources used in the current study.

Iwo *et al*, (2012) screened twenty eight soybean genotypes in Nigeria during 2007 and 2008 cropping seasons. Seven soybean genotypes were identified to be resistant to rust and Genetic analysis of the parental materials after hybridization for the mode of inheritance indicated that rust resistance in soybean was monogenically controlled by dominant genes. The results revealed that dominant alleles at three loci conditioned resistance to soybean rust races found in Nigeria and the tentative symbols formulated for the three loci controlling resistance to rust in soybean were Rsbr1, Rsbr2 and Rsbr3.

Santos Martins and Juliatti (2014) studied the genetic control of rust resistance using the Caiaponia x IAC-100 and Luziania x Potenza crosses. The F_2 and F_3 generations were evaluated. Rust severity was quantified through visual assessment of the middle third leaf of three leaflets per plant and performed by three different evaluators. The average score was calculated for each individual plant. From this study they estimated the mean and variance of the genetic components by employing the weighted least squares method. The estimates of number of genes controlling the trait broad and narrow sense heritabilities were also obtained. It was concluded that rust resistance is controlled by 2 to 23 genes that are predominantly dominant.

2.4 Nature and extent of variability created in segregating populations with respect to yield and its component traits

Though soybean has witnessed increasing trends in the production and productivity over the years, there is need to develop high yielding varieties with proper plant architecture, coupled with resistant to biotic and abiotic stresses. Yield being a complex trait, several attempts have been made in different crops to understand the mechanism of yield formation through growth and yield analysis. Crop species differ from each other in their morphological and physiological make up, due to which they differ in their response to environment. For these reasons, analysis of cause and effect relationship in grain yield is extremely complex (Yoshida, 1972). Simple correlation analysis has been used to know the nature and extent of association between yield and its determinants. The extent of association between quantitative and qualitative traits is also estimated.

Plant breeders are mainly interested in increasing the overall level of production. In a short period of time, within the available genetic resources this can be attempted by estimating the magnitude of genetic variability available in crop species. Thus, this is useful in selecting the variable parents for further cultivar development.

Selection of potential genotypes from the existing germplasm, utilizing them in hybridization programme and isolation of superior segregants in the segregating population is the usual breeding strategy in highly self pollinated crops like soybean. The success of selection depends on the magnitude of variation existing in the

population. This apparent variability in the crops is divided into variability due to genotype and variability due to environment, and their interaction. The genetic variability and environmental components of variation were discussed by Johansen (1909) who attributed the variation in the segregating populations to both heritable factors and non heritable factors. The phenotypic variability is a measure of variability due to genotype, environment and their interaction. The genetic variability is the real measure of variability concealed in a population, since it is result of additive and non-additive gene effects. The extent of genetic variability existing in a crop is of great importance because greater the genetic variability, wider the scope for selection.

The review of literature pertaining to these aspects are presented in the following headings.

2.4.1 Variability

2.4.2 Heritability and genetic advance

2.4.3 Association analysis

2.4.1 Variability

The extent of genetic variability existing in a crop is of great importance because greater the genetic variability present, wider the chance for selection. The studies conducted in soybean with respect to genetic variability for different quantitative traits are summarized in Table 2.

2.4.2 Heritability and genetic advance

Heritability refers to the extent to which the variability for a quantitative character is transmitted to the progeny. It is defined as the ratio of additive variance to the total variance in narrow sense (Lush, 1949) and the ratio of genotypic variance to the total phenotypic variance in broad sense (Hanson *et al.*, 1956).

However, the gains from the selection for a particular character is the function of its heritability, selection pressure and the variation existing in the base population (Burton and Devane, 1953).

Table 2. Summary of review on variability for quantitative characters in soybean

Sl. No.	Character	Variability		References
		PCV	GCV	
1.	Plant height	High	High	Jain and Ramgiry (2000), Singh *et al.* (2000), Basavaraja (2002), Bangar *et al.* (2003), Mukesh Kumar and Singh (2009) and Patil *et al.* (2011)
		High	–	Kalaimagal (1991), Nirmalakumari and Balasubramanian (1993)
		–	High	Jagtap and Mehetre (1994), Bhandarkar (1999), Ramana *et al.* (2000) and Yadav (2006).
		Moderate	Moderate	Perraju *et al.* (1982), Mehetre *et al.* (1997) and HinaKausar (2005)
2.	Number of branches per plant	High	High	Rashid and Islam (1982), Amaranath (1986), Basavaraja (2002), Bangar *et al.* (2003), HinaKausar (2005), Mukesh Kumar and Singh (2009).
		Moderate	Moderate	Shivakumar *et al.* (2011).
3.	Days to flowering	High	High	Mahajan *et al.* (1994), Maharaddi (1996), Thorat *et al.* (1999), Ramana *etal.* (2000), Agarwal *et al.* (2001), Patil *et al.*(2011)
		Moderate	Moderate	Parameshwar (2006).
		Low	High	Harer and Deshmukh (1992).
		Low	Low	Amaranath *et al.* (1991), Kalaimagal (1991) and Bangar *et al.* (2003).
4.	Pod length	High	High	HinaKausar (2005), Yadav (2006) and Shivakumar *et al.*(2011)
		Low	Low	Upadhyaya (1985), Basavaraja (2002), Patil *et al.* (2011).
5.	Pod weight per plant	High	High	Veenakumari (1994), Basavaraja (2002) and HinaKausar (2005).
6.	Number of pods per plant	High	High	Dayarani (1985), Singh and Yadava (2000) and Basavaraja (2002), Mukesh Kumar and Singh (2009), Aditya *et al.*(2011).
		High	_	Mehetre *et al.* (1997)
		_	High	Ramana *et al.* (2000) and Yadav (2006).
		Moderate	Moderate	Bangar *et al.* (2003).
7.	Hundred seed weight	High	High	Bangar *et al.* (2003).
		Moderate	Moderate	Bangar *et al.* (2003) and Hina Kausar (2005) and Shivakumar *et al.* (2011).
		Low	Low	Amaranath (1986) and Basavaraja (2002).
8.	Harvest index	High	High	Dixit *et al.* (2002) and HinaKausar (2005).
9.	Biomass per plant	-	High	Aditya *et al* (2011) and Shivakumar *et al.* (2011).
		Moderate	Moderate	Basavaraja (2002).
10	Seed yield per plant	High	High	Ghatge and Kadu (1993), Basavaraja (2002), Yadav (2006), MukeshKumar and Singh (2009), Aditya *et al.*(2011), Patil *et al.*(2011), Shivakumar *et al.* (2011).
		High	_	Ramana *et al.* (2000).
		Moderate	Moderate	Ramana *et al.* (2000).

The review of literature on heritability and genetic advance for quantitative traits in soybean is summarized in Table 3.

2.4.3 Association Analysis

Association analysis measures the mutual relationship among various plant characters and determines the components on which selection can be based for improvement. The association of characters may be due to either genetic linkage or pleiotrophy (Harland, 1939).

2.4.3.1 Association studies

The knowledge of correlation that is existing among the important characters helps the plant breeders to formulate their selection procedures. The extent of observed relationship between two characters is known as phenotypic correlation, whereas genotypic correlation on the other hand is an inherent association between two characters.

The association among the various characters in soybean has been studied by various workers is summarized in Table 4.

2.5 Validation of molecular markers linked to rust resistance

2.5.1 Molecular basis of resistance against Asian soybean rust

The genetics of resistance of six dominant genes to specific soybean rust isolates has been described as *Rpp1, Rpp2, Rpp3,Rpp4 Rpp5* and *Rpp6* (Bromfield and Hartwig 1980, Mclean and Byth 1980, Hartwig and Bromfield 1983, Hartwig,1986, Garcia *et al.*, 2008 and Li *et al.*, 2012) respectively.

In order to identify new sources of resistance in soybean, Miles *et al.*, (2006) evaluated the entire germplasm collection (16,000 accessions) of the United States Department of Agriculture (USDA) against a mixture of five *P. pachyrhizi* Syd. isolates. After two rounds of evaluation, only 850 accessions were identified with partial tolerance or resistance reactions to *P. pachyrhizi* Syd. which correlated to less than 5 per cent of USDA germplasm collection.

Table 3. Summary of review on heritability (h^2) and genetic advance (GA) for different characters in soybean

Sl. No.	Character	h^2	GA	Reference
1.	Plant height	High	High	Ramana et al. (2000), Agarwal *et al.* (2001), Basavaraja (2002), Bangar *et al.* (2003), HinaKausar (2005) Sultana *et al.* (2005) and Yadav (2006), Mukesh Kumar and Singh(2009), Patil *et al.*(2011), Shivakumar *et al.* (2011).
		High	-	Singh and Singh (1999), Kumar and Jha (2004) and Sujatha Bhat *et al.* (2011).
		-	High	Shrivastava and Shukla (1998)
2.	Number of the branches per plant	High	High	Maharaddi (1996), Basavaraja (2002), Bangar *et al.* (2003), Hina Kausar (2005), Sultana *et al.* (2005), Mukesh Kumar and Singh (2009) and Shivakumar *et al.*(2011).
		High	_	Agarwal *et al.* (2001),Ramana *et al.* (2000), Sujatha Bhat *et al.* (2011).
		Moderate	Moderate	Parameshwar (2006).
3.	Days to flowering	High	High	Ramana *et al.* (2000), Agarwal *et al.* (2001) Bangar *et al.* (2003), Rajkumar Ramteke (2010), Aditya *et al.* (2011). Shivakumar *et al.* (2011) and Sujatha Bhat *et al.* (2011).
		Moderate	Moderate	Parameshwar (2006).
		Low	High	NirmalKumari and Balasubramanian (1993).
4.	Pod length	High	High	Mishra *et al.* (1994).
		Low	Low	Basavaraja (2002).
5.	Number of pods per plant	High	High	Thorat *et al.* (1999), Ramana *et al.* (2000), Agarwal *et al.* (2001), Basavaraja (2002), Sultana *et al.* (2005), Yadav (2006), Mukesh Kumarand Singh (2009), Aditya *et al.* (2011), Patil *et al.* (2011), Shivakumar *et al.* (2011).
		High	–	Amaranath *et al.* (1991) Srivastav and Jain (1994).
6.	Pod weight per plant	High	High	Basavaraja (2002) and HinaKausar (2005), Shivakumar *et al.* (2011).
7.	100 seed weight	High	_	Amaranath *et al.* (1991) and Aditya *et al.* (2011)
		High	High	Sultana *et al.* (2005) and Sujatha Bhat *et al.* (2011).
		Moderate	Moderate	Shivakumar *et al.* (2011).
		High	Moderate	Basavaraja (2002) and HinaKausar (2005).

Contd..

Sl. No.	Character	h^2	GA	Reference
8.	Biomass per plant	High	_	Srivastava and Jain (1994), Aditya *et al.* (2011).
		High	High	Shrivastava and Shukla (1998) and Basavaraja (2002).
9.	Harvest index	High	High	Basavaraja (2002) and Shivakumar *et al.*(2011).
		High	_	Shivakumar *et al.* (2011) and Sujatha Bhat *et al.* (2011).
10.	Seed yield per plant	High	High	Agarwal *et al.* (2001), Bangar *et al.*(2003), Basavaraja (2002), Hina Kausar (2005), Sultana *et al.* (2005) and Yadav (2006), Mukesh Kumarand Singh(2009) and Patil *et al.*(2011).
		High	_	Upadyaya and Singh (1979).

Table 4. Summary of review of literature on correlation of component traits with seed yield in soybean

Sl. No.	Component character	Correlation	References
1.	Plant height	Positive	Archana *et al.* (1999), Sharma and Phul (1999), Ramana *et al.* (2000), Basavaraja (2002), Bangar *et al.* (2003), Luis Fernando *et al.* (2004), Sultana *et al.* (2005), Bhairav *et al.* (2006) and Yadav (2006).
		Negative	Kalaimagal (1991) and Jain and Ramagisry (2000).
2.	Number of branches per plant	Positive	Singh and Singh (1999), Basavaraja (2002), Bangar *et al.* (2003), Hina Kausar (2005), Sultana *et al.* (2005), Kumar *et al.* (2006), Bhairav *et al.* (2006), Mukesh Kumarand Singh (2009),Rajkumar Ramteke *et al.* (2010), Showkat and Tyagi (2010) and Shivakumar *et al.* (2011).
		Negative	Shinde *et al.* (1996) and Sunilkumar *et al.* (1997).
3.	Days to flowering	Positive	Mahajan *et al.* (1993), Archana *et al.* (1999), Ramana *et al.* (2000), Bangar *et al.* (2003), Rajkumar Ramteke *et al.* (2010).
		Negative	Kalaimagal (1991)
4.	Number of pods per plants	Positive	Bangar *et al.*(2003), HinaKausar (2005), Bhairav *et al.* (2006), Yadav(2006), Gaikwad *et al.* (2007), Mukesh Kumar andSingh (2009),Showkatand Tyagi(2010), Aditya *et al.* (2011) and Shivakumar *et al.* (2011).
		Negative	Kalaimagal (1991).
5.	Pod length	Positive	Jadhav *et al.* (1995) and Basavaraja (2002).
		Negative	Dixit and Patil (1982).
6.	Pod weight per plant	Positive	Basavaraja (2002), HinaKausar (2005), Parameshwar (2006) and Shivakumar *et al.* (2011).
7	Hundred seed weight	Positive	Singh and Singh (1999), Rajanna *et al.* (2000), Bangar *et al.* (2003), Bhairav *et al*, (2006) Showkat and Tyagi. (2010)
		Negative	Shinde *et al.* (1996) and Archana *et al.* (1999).
8	Biomass per plant	Positive	Singh and Yadava (2000) Weilenmann and Luguez (2000), Rezaizad *et al.* (2001), Basavaraja (2002), Bhairav *et al.* (2006), and Aditya *et al.* (2011)
9	Harvest index	Positive	Weilenmann and Luquez (2000), Bhairav *et al.* (2006), Kumar *et al.* (2006), Gaikwad *et al.* (2007), Mukesh KumarandSingh (2009), Showkat and Tyagi(2010), Aditya *et al.*(2011) and Shivakumar *et al.* (2011).
10.	Seed yield per plant	Positive	Mukesh Kumar andSingh (2009), Aditya *et al.* (2011) and Shivakumar *et al.* (2011)

Resistance alleles different from those already described in the literature were also identified in several other genotypes (Laperuta *et al.*, 2008, Pierozzi *et al.*, 2008).When the disease was first detected in Brazil, all the described resistance genes were effective against the fungus. However, in 2003, a new race of *P. pachyrhizi* broke the resistance conferred by genes *Rpp1* and *Rpp3* while *Rpp2*, *Rpp4* and *Rpp5* remained resistant.

Along with single gene resistance, partial resistance to soybean rust has been described (Hartman *et al.*, 2005). This kind of resistance may be controlled by minor genes and may be expressed as reduced uredinial number and size, a longer latent period and other components related to fungal reproduction. Recently, the average number of uredinia per lesion and the average uredinial diameter were reported to be components of partial resistance in soybean rust and were a reflection of fungal growth in the host tissue (Bonde *et al.*, 2006).

All described *Rpp* genes have already been mapped on soybean chromosomes (Chr), *Rpp1* was mapped on chromosome 18, *Rpp2* on Chr 16, *Rpp3* on Chr 6, *Rpp4* on Chr 18, *Rpp5* on Chr 3 and *Rpp6* on Chr 18, (Hyten *et al.*, 2007, Garcia *et al.*, 2008, Silva *et al.*, 2008, Hyten *et al.*, 2009, Li *et al.*, 2012). Additionally, some alleles were mapped on the same chromosome, for example, *Rpp1b* was also mapped on Chr 18, while *Rpp Hyuuga* was also mapped on Chr 6 (Monteros *et al.*, 2007, Chakraborty *et al.*, 2009)

Despite the physical location of the *Rpp* genes and the recent release of the soybean genome (Schmutz*et al.*, 2010), none of them was cloned yet. However, significant progress has been made towards cloning *Rpp4*, which remained the most stable gene when challenged against isolates of fungus from different parts of the world (Yamaoka *et al.*, 2002, Bonde *et al.*, 2006)

Six resistance loci (*Rpp*: resistance to *P. pachyrhizi*, *Rpp1–6*) have been mapped with molecular markers for soybean rust (Hyten *et al.*, 2007, Silva *et al.*, 2008, Garcia *et al.*, 2008, Chakraborty *et al.*, 2009, Ray *et al.*, 2009, Monteros *et al.*, 2010, Li *et al.*, 2012). Thus, it can be tagged and pyramided using molecular markers (Yamanaka *et al.*, 2008, Lemos *et al.*, 2011).

Four dominant major soybean genes controlling resistance to soybean rust have been identified (*Rpp1, Rpp2, Rpp3* and *Rpp4*). These genes being located at different loci and provide resistance to different races of *P. pachyrhizi* Syd. *Rpp1* was identified in soybean genotype PI 200492 (McLean and Byth, 1980), *Rpp2* in PI 230970 (Bromfield and Hartwig, 1980), *Rpp3* in PI 462312 (Hartwig and Bromfield, 1983) and *Rpp4* in PI 459025 (Hartwig, 1986).

In some cases, the resistance is also associated with an immune response (no visible symptoms), as is the case of *Rpp1* in the presence of certain isolates (Miles *et al.*, 2006). To date, all known soybean rust resistance loci evaluated have been overcome by at least one isolate throughout the world (Miles *et al.*, 2006; Yamaoka *et al.*, 2002).

However, *Rpp2* and *Rpp4* loci remain effective against the Brazilian isolates, whereas *Rpp1* and *Rpp3* were defeated in 2003, just 2 years after soybean rust detection in Brazil. *Rpp2* and *Rpp4* loci were identified on the lines PI 230970 and PI459025, respectively and behave as a single dominant allele (Bromfield and Hartwig, 1980; Hartwig, 1986).

Resistance genes have also been described in cultivated soybean in Taiwan, Phillipines, Zimbabwe *etc*. Presently, four different loci carrying dominant alleles have been reported: *Rpp1* identified in PI 200492 (McLean and Byth 1980), *Rpp2* from PI 230970 (Bromfield and Hartwig 1980), *Rpp3* (PI 462312) (Bromfield and Melching 1982) and *Rpp4* (PI 459025) (Hartwig 1986). An immune response has also been described for PI 200492 when it is inoculated with a particular type of *P. pachyrhizi* Syd. isolate (Bonde *et al.*, 2006).

Recently, a new locus *Rpp5*, was reported by Garcia *et al.* (2008). *Rpp1* and *Rpp4* have been mapped to two different loci on chromosome 18 (formerly linkagegroup (LG) G; Hyten *et al.*, 2007; Silva *et al.*, 2008), *Rpp2* was mapped to chromosome 16 (LG J) by Silva *et al.* (2008), *Rpp3* was mapped to chromosome 6 (LG C2) by Hyten *et al.* (2009) and *Rpp5* was mapped to chromosome 3 (LG N) by Garcia *et al.* (2008).

All soybean rust resistance genes gave a RB (reddish brown) type resistance response except for *Rpp1*, which confers an immune response to some isolates of

P. pachyrhizi Syd. (Miles *et al.,* 2006). A field evaluation of germplasm in the USA showed that *Rpp1* provided the greatest overall resistance and that resistance reactions varied from environment to environment (Walker *et al.,* 2011).

There are examples of isolates of *P. pachyrhizi* overcoming resistance genes. For example, both *Rpp1* and *Rpp3* were reported ineffective at conferring resistance in Brazil, leaving only *Rpp2*, *Rpp4* and *Rpp5* resistant in some regions of that country (Morales *et al.,* 2012).

All six *Rpp* loci have been genetically mapped: *Rpp1*, *Rpp4* and *Rpp6* were mapped to three different regions of chromosome 18 (Hyten *et al.,* 2007; Silva *et al.,* 2008; Li *et al.,* 2012), *Rpp2* on chromosome 16 (Silva *et al.,* 2008), *Rpp3* on chromosome 6 (Hyten *et al.,* 2009) and *Rpp5* on chromosome 3 (Garcia *et al.,* 2008).

A new allele of *Rpp1*, designated as *Rpp1-b*, was mapped in PI 594583A and is likely to be present in PI 587880A, PI 587886 and PI 561356 (Chakraborty *et al.,* 2009; Kim *et al.,* 2012; Ray *et al.,* 2009). The recessive allele *rpp2* was also mapped from PI 224270 at the same region as *Rpp2* (Garcia *et al.,* 2008).

At *Rpp5*, three different alleles have been reported including a dominant allele from PI 200526 and PI 200487, an incompletely dominant allele from PI 471904 and a recessive allele from PI 200456 (Garcia *et al.,* 2008).

Although, all the soybean-rust-resistant germplasm carrying *Rpp* genes triggers a hypersensitive (HR) response, the intensity of the reaction and the behavior through the developmental stage of the plant vary considerably among different genotypes (Bonde *et al.,* 2006) and there were no developed cultivars that have an acceptable level of resistance to all strains of *P. pachyrhizi* Syd. (Monteros *et al.,* 2007). Soybean genotype used as a source of resistance to *Phakopsora pachyrhizi* Syd. and the molecular information of the mapped genes in soybean genome are presented in Table 5.

Pyramiding resistance genes in a single cultivar can provide more durable resistance against some plant diseases (Liu *et al.,* 2000; Singh *et al.,* 2001; Castro *et al.,* 2003). Because of the high diversity of *P. pachyrhizi* Syd. fungi in the field, this strategy could be very important for improving soybean rust resistant varieties.

Table 5. Soybean genotypes used as a source of resistance to *Phakopsora pachyrhizi* Syd. and the molecular information of the mapped genes in soybean genome

Variety	Gene	Chromosome (linkage group)	Molecular marker	Reference
PI 200492	Rpp1	18(G)	Satt 191- Satt 064	McLean & Byth, 1980; Hyten *et al.*, 2007
P1587866	Rpp1	18(G)	Satt 191-Satt 064	Ray *et al.*, 2009.
PI587880A	Rpp1	18(G)	Satt 191-Sat372	Ray *et al.*, 2009.
PI561356	Rpp1	18(G)	SSR50- SSR1859	Kim *et al.*, 2012.
PI587905	Rpp1	18(G)	Satt 064- SSR66/Satt 191-Sat372	Hyten, 2009
PI594760B	Rpp1	18(G)	Satt 117-Sct 187	Garcia *et al.*, 2008.
PI594767A	Rpp1	18(G)	Satt 064 – Satt 191	Hossain *et al.*, 2014.
PI594538A	Rpp1-b	18(G)	Satt 064- Satt 372	Chakraborty *et al.*, 2009.
PI230970	Rpp2	16(J)	Satt 255 – Satt 620	Hartwig & Bromfield, 1983; Silva *et al.*, 2008
PI224270	rpp2	16(J)	Satt 215- Satt 361	Garcia *et al.*, 2008
PI462312	Rpp3	6(C2)	Satt 460- Satt 263	Hartwig & Bromfield, 1983; Hyten *et al.*, 2009
PI416764	Rpp3	6(C2)	Satt 263- Satt 307	Hossain *et al.*, 2014.
PI567099	rpp3	6(C2)	Satt 460- Staga001	Ray *et al.*, 2011.
PI506764	Rpp3/rpp5	6/3(C2/N)	Satt 460- Satt 263/Satt 275/Satt 275-Satt 280	Monteros *et al.*, 2007
PI200487	Rpp3/Rpp5	6/3(C2/N)	Satt 460-Satt 263/Satt 275-Satt 280	Garcia *et al.*, 2008; Kendrick *et al.*, 2011.
PI471904	Rpp3/Rpp5	6/3(C2/N)	Sat460-Satt 263/Satt 275- Satt 280	Kendrick *et al.*, 2011
PI459025	Rpp4	18(G)	Satt 288-AF162283	Hartwig, 1986; Silva *et al.*, 2008
PI459025B	Rpp4	18(G)	Satt 288 –AF162283	Silva *et al.*, 2008
PI200456	rpp5	3(N)	Satt 275- Satt 280	Garcia *et al.*, 2008
PI200526	Rpp5	3(N)	Satt 275 – Satt 280	Garcia *et al.*, 2008
PI567102B	Rpp6	18(G)	Satt 324- Satt 394	Li *et al.*, 2012

2.5.2 Marker assisted selection for disease resistance

Since the beginning of agriculture, plant breeding has been considered as the most popular method for crop improvement. Traditionally, breeding techniques like pure line selection, mass selection, recurrent selection, backcross selection and mutation breeding have been followed to breed the crops for stress resistance (Werner *et al.*, 2005; Zhang *et al.*, 2006)

Breeding work utilizing both phenotypic and genotypic markers are more reliable and fast. Conventional breeding methods may create resistant varieties which is time consuming and intensive task. Marker assisted selection (MAS) has been proven as a highly efficient breeding method in improvement of cultivars or lines for various biotic stresses in for fast crop breeding programmes because of its efficacy in selecting plants with appropriate gene combinations in segregating population (Collard and Mackill, 2008). Recent advancements made in the field of genomics have provided a varied number of molecular markers in many crop species. This made MAS practical for application in breeding programmes due to its time saving, consistency, biosafety and accuracy in selection of complex traits (Jena and Mackill, 2008).

By employing MAS technique, public and private sectors have released new cultivars resistant to biotic stresses (Xu and Crouch, 2008). The efficiency of MAS depends on the tight linkage between the target gene and the marker (Gouda *et al.*, 2012).

The marker-assisted selection results are highly reliable because of their selective effects, which are independent of gene effects and environmental factors.

2.5.3 Molecular mapping and marker trait association in soybean

Exact demarcation of resistance is not possible under field conditions when several genes are combined because of the presence of epistatic effects, numerous virulence races along with environmental interactions (Fuentes *et al.*, 2008).

DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding through Marker Assisted Selection (MAS). Genetic markers represent genetic differences between individual organisms or species. They do

not represent the target genes themselves but act as ‘signs’ or ‘flags’. Genetic markers that are located in close proximity to genes (*i.e.* tightly linked) may be referred to as gene ‘tags’. Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or ‘linked’ to genes controlling the trait. All genetic markers occupy specific genomic positions within chromosomes (like genes) called ‘loci’ (singular ‘locus’) (Collard *et al.*, 2005).

DNA markers are the most widely used as the marker predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Peterson *et al.*, 1996). These markers are selectively neutral because they are usually located in non-coding regions of DNA. DNA markers are practically unlimited in number and are not affected by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995). Target genotypes can be more effectively selected, which may enable certain traits to be ‘fast-tracked’, resulting in quicker line development and variety release. Markers can be used as a replacement for phenotyping, which allows selection in off-season nurseries making it more cost effective to grow more generations per year (Ribaut and Hoisington, 1998).

The use of molecular markers is an effective tool for gene identification and transfer (Tanskley, 1983; Tanskley and McCouch, 1997) and can speed up the development of soybean cultivars carrying single or multiple resistance genes. Soybean has a reasonably dense molecular-marker linkage map (Song *et al.*, 2004) and the association of markers to known genes has been pursued by many groups. Molecular mapping of soybean rust-resistance genes in soybean has previously been reported.

Brogin *et al.* (2004) identified Simple Sequence Repeat (SSR) markers linked to rust resistance present on the cultivar FT-2 in the linkage group (LG) -C2 of the previous soybean consensus map reported by Cregan *et al.* (1999). However, the locus could not be identified in the study. An soybean rust resistance gene from the cultivar Hyuuga was mapped at 3-cM interval on LG-C2 between Satt 134 and Satt 460 (Monteros *et al.*, 2007). The *Rpp1* locus has been mapped to 1-cM interval on LG-G between Sct_187 and Satt 064LG-G (Hyten *et al.*, 2007).

The first soybean (*Glycine max* L. Merr.) genetic linkage map of molecular markers was reported by Keim *et al.* (1990). This map consisted of 26 genetic linkage groups containing a total of 150 restriction fragment length polymorphism (RFLP) loci and was based on a F_2 population derived from an interspecific cross of *G. max* (A81-356022) and *G. soja* (PI468916).

Lark *et al.* (1993) subsequently used 132 RFLP, isozyme and morphological markers to construct a soybean genetic map comprised of 31 linkage groups. Shoemaker and Specht (1995) mapped 110 RFLP, eight random amplified polymorphic DNA (RAPD), seven pigmentation, six morphological and seven isozyme markers in an F_2 population derived from a mating of isolines of the important soybean cultivars 'Clark' and 'Harosoy'. These early genetic maps were primarily based on RFLP markers. Due to the lack of polymorphism of RFLP loci in soybean and/or the complexity of multiple DNA banding patterns detected with most RFLP probes, simple sequence repeat (SSR) or microsatellite markers were proposed for map development (Akkaya *et al.*, 1992).

Most SSRs are single-locus markers and many SSR loci are multi-allelic. These characteristics make SSRs an ideal marker system not only for creating genetic maps, but also as an unambiguous means of defining linkage group homology across mapping populations. Cregan *et al.* (1999) reported the development of 606 SSR loci which, together with 689 RFLP, 79 RAPD, 11AFLP, ten isozyme and 26 classical loci, were mapped to one or more of three populations: the USDA/Iowa State *G. max* & *G. soja* F_2, the University of Utah 'Minsoy', 'Noir 1' recombinant inbred lines and the University of Nebraska 'Clark', 'Harosoy' F_2 population.

These three separate maps provided useful information relative to the consistency of marker order and genetic distance among the different populations. The Cregan *et al.* (1999) established, for the first time, 20 consensus linkage groups, which were assumed to be the genetic correlates of the 20 soybean chromosomes. In that report, a total of 412 SSR loci were positioned in the 'Minsoy', 'Noir 1'mapping population of 240 recombinant inbred lines. The resulting map was approximately 2,400 cM in length, but contained 36 intervals of at least 20 cM and 79 intervals of at least 10 cM, in which no microsatellite loci were positioned. Inversely, there were 67 distinct intervals with less than 0.01 cM of distance between two or more adjacent SSR

markers. In some of the 67 intervals, there was no recombination between adjacent SSR loci. Cregan *et al.* (1999) successfully developed new SSR markers targeted to two regions of the soybean genome near soybean cyst nematode-resistance loci on linkage groups G and A2. Genetic mapping confirmed that the new SSRs mapped to the correct sites in the genome. Molecular tagging of soybean rust resistance can help in the process of resistance breeding. In this study, an F_2 population of cross (susceptible cultivar 'NRC 7' × resistant exotic genotype EC 241780) was used for bulked segregant analysis (BSA) with 25 SSR (simple sequence repeat) primers linked with six *Rpp* genes (Deshmukh *et al.*, 2015).

Among them, five polymorphic SSR markers, *viz.*, Sct 187, SSR 1859, Satt 191 (*Rpp1b* like loci) and Satt 215, Satt 361 (*Rpp2* loci) distinguished the soybean rust resistant and susceptible bulks and individuals. In combined marker analysis, the markers Satt 191 (*Rpp1b* like loci) and Satt 215 (*Rpp2* loci) were linked with soybean rust severity score and were also confirmed in individual 110 F_2 segregants. Hence, these markers could be utilized in the marker assisted rust resistance breeding of *Rpp1b* like and *Rpp2* genes. Among the different molecular markers, SSRs are of interest for genetic mapping because each marker corresponds to a single position in the genome, but has several alleles yielding a high degree of polymorphism (Cregan *et al.*, 1999). Further, they are easy to use, yield consistent results and are accessible to all biotechnology labs.

3. MATERIAL AND METHODS

3.1 Experimental site

The present investigation was carried out at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. The campus is located at a latitude of 15° 26'N, longitude of 75° 07'E and altitude of 678 m above mean sea level.

3.2 Climate and weather conditions

Main Agricultural Research Station (MARS) of University of Agricultural Sciences, Dharwad it receives an average rainfall (over 50 years) of 798 mm with two peaks one in July and another in October. During 2015, about 716 mm rainfall was received in 43 rainy days at MARS, Dharwad. However, the rainfall received during the crop growth period was 99.4 mm in 14 rainy days (July to September). The mean maximum temperature varied from 20.3°C to 35.1°C during the month of April, whereas the mean minimum temperature varied from 13.3°C to 28.6°C during the month of January. The mean relative humidity was the highest (80.0 %) during the month of June and is the lowest (40.0 %) during the month of February.

During 2016, about 568.2 mm rainfall was received in 55 rainy days at MARS, Dharwad. However, the rainfall received during the crop growth period was 380.6 mm in 37 rainy days (July to October). The mean maximum temperature varied from 21.6°C to 38.0°C during the month of April, whereas the mean minimum temperature varied from 21.0°C to 26.3°C during the month of July. The mean relative humidity was the highest (86.0 %) during the month of July and is the lowest (41.0 %) during the month of March.

During 2017, about 582.8 mm rainfall was received in 51 rainy days at MARS, Dharwad. However, the rainfall received during the crop growth period was 347.8 mm in 28 rainy days (July to September). The mean maximum temperature varied from 21.2°C to 37.7°C during the month of April, whereas the mean minimum temperature varied from 21.0°C to 27.6°C during the month of July. The mean relative humidity was the highest (90.9 %) during the month of September and is the lowest (35.1 %) during the month of February.

The data on weather conditions prevailed during 2015 to 2017 (three seasons) is furnished in Appendix 1, 2 & 3.

3.3 Material used in the study

A total of five experiments were formulated comprising 144 exotic germplasm lines including resistant and susceptible checks with three genotypes *viz*., DSb 21, JS 335 and EC 241780. The seed material for experimentation was collected from AICRP on Soybean, Main Agricultural Research Station, Dharwad.

About 144 exotic germplasm lines including highly susceptible check JS 335 and resistant checks *viz.*, DSb 21, EC 241780 and EC 241778 were evaluated during *kharif* 2015 at Dharwad for identification of new sources for resistance to rust and genetic diversity. Based on the resistance reaction, 22 lines which exhibited resistant/moderately resistant reaction were selected. These lines were further evaluated to confirm their resistance reaction under natural epiphytotic condition at two hotspots for rust *viz.*, Ugarkhurd and Dharwad during *kharif* 2016.

Three genotypes *viz.*, DSb 21, JS 335 and EC 241780 obtained from AICRP on Soybean, UAS, Dharwad were utilized in crossing programme/hybridization during *summer* 2015 to study the inheritance pattern for rust resistance and variability. Subsequently, F_2 and F_3 populations were raised during *kharif* 2016 & 2017 respectively. In addition to this, validation of molecular markers linked to rust resistance in F_2 cross JS 335 x EC 241780 was carried out using 25 SSR markers.

The details of material used and techniques adopted in the present study for recording of observations, statistical analysis are briefly presented under the respective experiments separately. The experiments planned as detailed below;

Experiment 3.1. Evaluation of exotic germplasm lines for identification of new sources for resistance to rust.

Experiment 3.2. Studies on genetic diversity in exotic germplasm lines.

Experiment 3.3. Studies on inheritance pattern of rust.

Experiment 3.4. Study on the nature and extent of variability generated in the segregating populations with respect to yield and its component traits.

Experiment 3.5. Validation of molecular markers linked to rust resistance.

Experiment 3.1: Evaluation of exotic germplasm lines for identification of new sources for resistance to rust

3.1.1 Experimental material

The experimental material composed 144 exotic germplasm lines including highly resistant checks *viz.*, DSb 21, EC 241780 and EC 241778 and highly susceptible check JS 335. The experiment was conducted under rust prone condition (unprotected condition) without any fungicidal spray. List of soybean germplasm lines and checks used in the disease screening are presented in Table 6.

3.1.2 Experimental layout

About 144 exotic germplasm lines including resistant checks (DSb 21, EC 241780 and EC 241778) and susceptible check (JS 335) were evaluated in augmented block design. Each line was raised in one row of 5 m length with a spacing of 45 x 10 cm during *kharif* 2015 at The Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. Scoring of the disease was done between 65 to 90 days after sowing based on per cent leaf area infected by using 0-9 scale (Mayee and Datar, 1986) and yield components *viz.*, days to 50 per cent flowering, number of branches per plant, days to maturity, number of pods per plant and seed yield per plant were also recorded on five randomly tagged plants in each line.

3.1.3 Preparation of inoculum

The leaves from rust infected fields were collected and soaked overnight. In the morning uredospores were oozed out and the uredospore suspension was sprayed on all the entries at 45 and 55 days after sowing.

Table 6. List of soybean germplasm lines and checks used in the disease screening during *kharif* 2015

Sl. No.	Genotypes	Sl. No.	Genotypes	Sl. No.	Genotypes	Sl. No.	Genotypes
1	EC 1028	42	EC 250578	83	EC 333920	124	EC 457419
2	EC 10027	43	EC 250588	84	EC 333934	125	EC 49393
3	EC 100031	44	EC 250607	85	EC 338597	126	EC 65772
4	EC 100772	45	EC 250608	86	EC 34057	127	EC 685246
5	EC 104817	46	EC 250619	87	EC 34078	128	EC 685250
6	EC 107416	47	EC 251329	88	EC 34079	129	EC 685251
7	EC 114520	48	EC 251334	89	EC 34092	130	EC 685252
8	EC 114573	49	EC 251341	90	EC 34500	131	EC 685255
9	EC 116343	50	EC 251358	91	EC 340924	132	EC 685256
10	EC 118420	51	EC 251401	92	EC 36816	133	EC 685258
11	EC 118443	52	EC 251409	93	EC 37937	134	EC 7048
12	EC 12570	53	EC 251411	94	EC 376065	135	EC 85705
13	EC 14426	54	EC 251 456	95	EC 377552(A)	136	EC 917258
14	EC 242091	55	EC 251501	96	EC 380322	137	EC 93413
15	EC 14476	56	EC 251516	97	EC 383165	138	EC 94625
16	EC 14573	57	EC 251762	98	EC 385243	139	EC 95291
17	EC 149988	58	EC 274755	99	EC 389148	140	EC 95815
18	EC 15966	59	EC 287754	100	EC 389151	141	EC 241778 (RC)
19	EC 16119	60	EC 30832	101	EC 389178	142	EC 241780 (RC)
20	EC 16738	61	EC 308334	102	EC 389400	143	DSb 21 (RC)
21	EC 172607	62	EC 309512	103	EC 39219	144	JS 335 (SC)
22	EC 175529	63	EC 309538	104	EC 39362		
23	EC 177744	64	EC 309545	105	EC 39491		
24	EC 187456	65	EC 315213	106	EC 39516		
25	EC 184337	66	EC 3251	107	EC 39536		
26	EC 19923	67	EC 325092	108	EC 390981		
27	EC 225114	68	EC 325099	109	EC 391158		
28	EC 221329	69	EC 325101	110	EC 391336		
29	EC 2388	70	EC 325102	111	EC 391346		
30	EC 232019	71	EC 329158	112	EC 392532		
31	EC 241309	72	EC 33875	113	EC 392580		
32	EC 241761	73	EC 33917	114	EC 394839		
33	EC 241766	74	EC 33922	115	EC 396052		
34	EC 242018	75	EC 33940	116	EC 396053		
35	EC 242038	76	EC 333868	117	EC 397158		
36	EC 242104	77	EC 333875	118	EC 4435		
37	EC 242104(A)	78	EC 333881	119	EC 42081		
38	EC 245984	79	EC 333886	120	EC 457161		
39	EC 245989	80	EC 333891	121	EC 457175		
40	EC 2581	81	EC 333904	122	EC 457286		
41	EC 25269	82	EC 333909	123	EC 457406		

*RC-Resistant check, SC- Susceptible check

3.1.4 Observations recorded

Five plants in each line were tagged and observations were recorded on individual plant basis. The observations on yield and its attributes were recorded on each of the tagged plants. Observations on days to 50% flowering, number of branches per plant, days to maturity, number of pods per plant and seed yield per plant were recorded.

3.1.5 Reaction for rust

The severity of rust was scored between 65-90 days after sowing based on per cent leaf area infected by using 0-9 scale given by Mayee and Datar (1986) (Plate 1).

0: Absolute Resistant (<1%)

1: Highly resistant (1-10%)

3: Moderately resistant (11-25%)

5: Moderately susceptible (26-50%)

7: Susceptible (50-75%)

9: Highly susceptible (>75%)

3.1.5.1 Rate of development of disease (r)

The rate of development of disease (r) at different intervals was also calculated by following formula given by Van der plank (1963).

$$r = \frac{2.3}{t_2 - t_1} \left[\log \frac{X_2}{1-X_2} - \log \frac{X_1}{1-X_1} \right]$$

Where,

r = Apparent rate of infection or spread

X_1 = Per cent disease index at time t_1

X_2 = Per cent disease index at time t_2

$t_2 - t_1$ = Time interval in days between the two consecutive observations

HR - Highly resistant
S - Susceptible

MR - Moderately resistant
HS - Highly susceptible

MS - Moderately susceptible

Plate 1: Disease scoring : 0-9 scale

3.1.5.2. Area under disease progress curve (AUDPC)

Area under disease progress curve is an important feature associated with disease resistance. It is the area of graph under the line that depicts the progress of epidemics and is calculated using the formulae given by Wilcoxson (1975) as below.

$$AUDPC = \sum_{i-1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Y_i and Y_{i+1} are the disease scores done at t_i and t_{i+1} time intervals.

3.1.6 Type of the lesions

Type of lesions may be either reddish brown or tan colour. Reddish Brown lesions may produce few urediospores, whereas Tan lesions may produce numerous urediospores based on colour of lesions and these were scored either resistant or susceptible (Bromified, 1984; Pham *et al.* 2009, Sharadha and Jahagirdar 2015) respectively. The count of the number of lesions was taken per cm^2 of infected leaves from mid-vein and both sides of mid-vein. The lesion colour on the infected leaves was recorded in the form of Reddish Brown (resistant) and TAN (susceptible). The number of lesions per cm^2 square of infected leaves were recorded using a magnifying glass.

3.1.7 Evaluation of promising lines for confirmation to rust resistance

Among 144 exotic germplasm lines including resistant (DSb 21, EC 241780 and EC 241778) and susceptible (JS 335) checks were screened during *kharif* 2015, twenty two lines were selected and further evaluated to confirm their resistance along with checks *viz*., JS 335 (susceptible), DSb 21, EC 241778 and EC 241780 (resistant) under natural epiphytotic condition at two hotspots for rust *viz.*, Dharwad and Ugarkhurd. Each line was raised in one row of 5 m length with a spacing of 45 x 10 cm in two replications during *kharif* 2016. Scoring of the disease was done between 65 to 90 days after sowing based on per cent leaf area infected by using 0-9 scale (Mayee and Datar, 1986) and yield components *viz.*, days to 50% flowering, number of branches per plant, days to maturity, number of pods per plant, 100 seed weight and seed yield per plant were recorded (Plate 2, 3, 4).

Experiment 3.2: Studies on genetic diversity in exotic germplasm lines

3.2.1 Experimental material

The experimental material composed 144 exotic germplasm lines including highly resistant checks *viz.*, DSb 21, EC 241780 and EC 241778 and highly susceptible check JS 335. Origen/ source of these exotic germplasm lines are presented in Table 7.

3.2.2 Experimental layout

The lines were evaluated in augmented block design along with highly resistant checks *viz.*, DSb 21, EC 241780 and EC 241778 and highly susceptible check JS 335. Each line was raised in one row of 5 m length with a spacing of 45 x 10 cm during *kharif* 2015 at The Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. Yield components *viz.*, days to 50% flowering, number of branches per plant, days to maturity, number of pods per plant and seed yield per plant were recorded.

3.2.3 Observations recorded

In all the entries, five random plants were tagged in each line for recording the various observations. Mean of five plant observations was used for the statistical analysis. Observations were recorded on plant basis as mentioned in 3.3.4.

3.2.4 Statistical analysis for k-*means*

In the present study, Non-hierarchical Euclidean cluster analysis based on k-*means* method was used for assessing the genetic divergence for yield related traits in exotic germplasm lines. The Non-hierarchical Euclidean cluster analysis was performed employing SPSS software. k-*means* was used for describing an algorithm that assigned each item to the cluster having nearest means (Queen, 1967).

Plate 2: Evaluation of exotic germplasm lines during *kharif* 2015- Dharwad

Plate 3: Evaluation of exotic germplasm lines for confirmation during *kharif* 2016 - Dharwad

Plate 4: Evaluation of exotic germplasm lines for confirmation during *kharif* 2016 - Ugarkhurd

Table 7. Source / origin of exotic germplasm lines utilized in genetic diversity studies

Source/Origin	Name of the Germplasm
USA	EC 10031, EC 100772, EC 107416, EC 114520, EC 114573, EC 242091, EC 24139, EC 241761, EC 241766, EC 242038, EC 242104, EC 242105, EC 251501, EC 308334, EC 329158, EC 333868, EC 333875, EC 333881, EC 333886, EC 333891, EC 333904, EC 333909, EC 333920, EC 333934, EC 39491, EC 65772
China	EC 16119, EC 281762
Australia	EC 14426
Brazil	EC 399512, EC 309538, EC 309545
Argentina	EC 251329, EC 251334, EC 251341, EC 251358, EC 251401, EC 251401, EC 251409, EC 251411, EC 251456, EC 251516, EC 377552
Philippines	EC 274755, EC 287754, EC 241780, EC 241778
Hungary	EC 325092, EC 325099, EC 325101, EC 325102, EC 34057, EC 34078, EC 34079, EC 34092
Russia	EC 95815
Taiwan	EC 245984, EC 245989, EC 250588, EC 250607, EC 250608, EC 250619
Indonesia	EC 4435
Canada	EC 36816

3.2.4.1 Methods involved in k-*means* are as below

Step 1. Partitioned the items into k clusters. The value of k can be obtained by using the formulae,

$$\text{Min } E = \Sigma d^2_{i.c(i)}$$

Minimum E is over the number of k clusters.

$\Sigma d^2_{i.c(i)}$ is the squared distance of case i from the centroid (mean) of the assigned cluster.

Step 2. Compute the Euclidean distance of each item from the group centroids and reassign each item to the nearest group

$$\text{Euclidian distance } D_{ij} = \sqrt{\sum_{ki} (Xki - Xkj)^2}$$

Where,

D_{ij} distance between the object i and j, X_{kj} is the value of variable k for the object j and X_{ki} is the value of variable k for the object i.

Step 3. Step 2 repeated until no more reassignments take place. If an item was moved from the initial configuration, the cluster means were updated before proceeding. The i^{th} coordinate, $i = 1, 2, ..., p$, of the centroid was easily updated using the formulas.

$$X_{i,new} = \frac{nX_i + X_{ij}}{n + 1}$$ If the j^{th} item was added to the group

$$X_{j,new} = \frac{nX_i + X_{ij}}{n + 1}$$ If the i^{th} item was added to the group

Here, n is the number of items in the old group with centroid $x = (x_1, x_2, ……..., x_p)$

Experiment 3.3: Studies on inheritance pattern of soybean rust resistance

3.3.1 Experimental material

The experimental material for this study comprised of three genotypes *viz*., DSb 21, JS 335 and EC 241780 were used for crossing programme obtained from AICRP on Soybean. UAS, Dharwad. DSb 21 and EC 241780 are resistant genotypes and JS 335 is susceptible genotype. These three genotypes were crossed in possible combinations like susceptible x resistant (JS 335 x EC 241780), resistant x susceptible (EC 241780 x JS 335) and resistant x resistant (DSb 21 x EC 241780) during *summer* 2015. The salient features of three parents are given in Table 8.

3.3.2 Crossing programme/ hybridization

Among the three genotypes, DSb 21, EC 241780 are resistant genotypes and JS 335 is highly susceptible genotype. These were utilized in crossing programme/ hybridization during *summer* 2015. These three genotypes were crossed in combinations like susceptible x resistant (JS 335 x EC 241780), resistant x susceptible (EC 241780 x JS 335) and resistant x resistant (DSb 21 x EC 241780). Emasculation was done by removing all the anthers using forceps at the time of flowering (bud initiation stage) during evening hours (5.00 pm to 6.30 pm). The pollination was carried out during morning hours (7.30 am to 9.00 am) on next day. The crossed pods were harvested, dried and threshed separately.

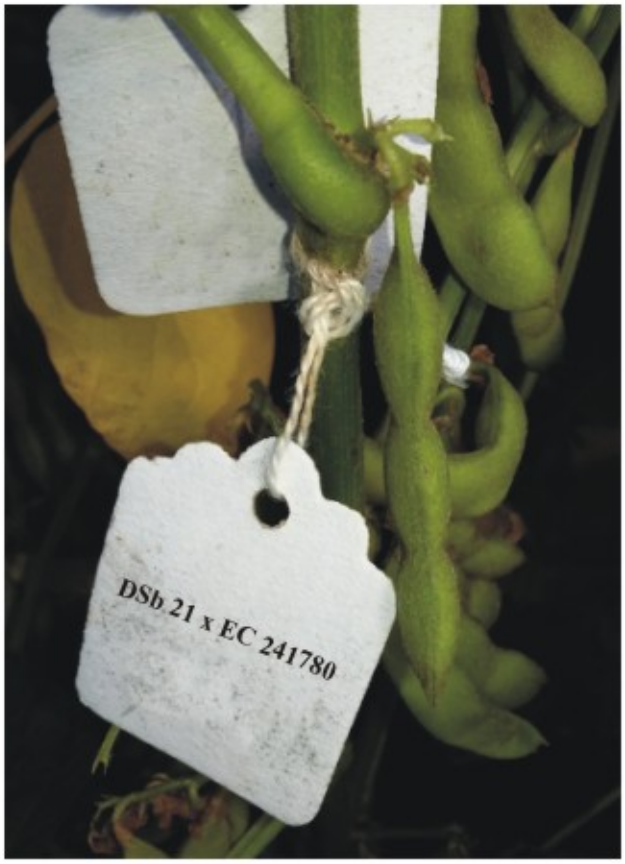
3.3.3 Experimental layout

3.3.3.1 Generation of F_1's and Identification of true F_1's

The F_1's along with parents from three crosses were raised with spacing of 45 cm between rows and 15 cm between the plants during *Kharif* 2015 and the recommended package of practices were followed for raising a good crop. True F_1's were identified based on parental characteristics (morphological characteristics) used in the crossing programme. The true F_1 plants from each cross were harvested, dried and threshed separately (Plate 5).

a) JS 335 x EC 241780

b) EC 241780 x JS 335



c) DSb 21 x EC 241780

Plate 5: F_1s of different crosses

Table 8. Salient features of the parents used in the study

Sl. No.	Variety	Pedigree	Duration (days)	Yield potential (q/ha)	Salient features
1.	JS 335	JS 78-77 x JS 71-05	85-90	25-30	Purple flowers; pubescence sparse or almost absent on stem ,leaves and pods; yellow seed coat, semi -determinate growth habit; tolerant to pod shattering up to 8-10 days after maturity and highly susceptible to rust.
2.	DSb 21	JS 335 x EC 241778	90-95	30-32	Purple flowers; pubescence - almost absent on stem, leaves and pods; yellow seed coat; semi-determinate growth habit; brown hilum; tolerant to pod shattering up to 8-10 days after maturity; highly resistant to rust.
3.	EC 241780	An exotic Germplasm line	110-120	Low Yielding	Purple flowers, pubescence-tawny on stem, leaves and pods, semi-determinate growth habit; yellow seed coat, brown hilum and highly resistant to rust.

3.3.3.2 Evaluation of F_2's

F_2 population of three crosses i.e. susceptible x resistant (JS 335 x EC 241780), resistant x susceptible (EC 241780 x JS 335) and resistant x resistant (DSb 21 x EC 241780) with 350, 456 and 432 seeds respectively were sown in field along with parents with spacing of 45 x 10 cm during *Kharif* 2016 to study the inheritance of resistance and evaluated for morphological and yield traits (Plate 6).

3.3.3.3 Evaluation of F_3's

The seeds from the F_2 generation were used for raising F_3 families and about hundred progenies were selected from each cross randomly. The plant to progeny rows were sown in field with spacing of 45 x 10 cm during *kharif* 2017 to study the inheritance of rust disease in F_2:F_3 populations and evaluated for morphological and yield traits (Plate 7).

The crop was raised under rainfed condition. The crop stand and the crop growth were satisfactory. All the recommended practices were followed for raising a good crop.

3.3.4 Observations recorded

All plants in a cross were tagged and observations were recorded on individual plant basis. The observations on yield and its attributes were recorded on each of the tagged plants in F_2 population of all the three crosses. In F_3 families of three crosses, five random plants were tagged in each progeny row for recording the observations. Morphological and yield traits *viz*., days to 50 % flowering, plant height (cm), number of branches per plant, days to maturity, number of pods per plant, pod length (cm), pod weight per plant (g), number of seed per pod, 100 seed weight (g), harvest index (%) and seed yield per plant (g) were recorded and the procedure followed in recording of these observations are described below;

3.3.4.1 Days to 50 % flowering (DFF)

Number of days taken from the date of sowing to the day on which 50 per cent of plants flowered on each individual line was recorded as days to 50 per cent flowering and in F_2 population on the basis of individual plant basis, in F_3 families 50 per cent of plants flowered in each progeny rows.

a) JS 335 x EC 241780

b) EC 241780 x JS 335

c) DSb 21 x EC 241780

Plate 6: Evaluation of F_2 population during *kharif* 2016 at Dharwad

a) JS 335 x EC 241780

b) EC 241780 x JS 335

c) DSb 21 x EC 241780

Plate 7: Evaluation of F_3 population during *kharif* 2017 at Dharwad

3.3.4.2 Plant height at harvest (cm) (PH)

Height of the main stem from the ground level to the top of the stem was measured in centimeters at the time of harvest.

3.3.4.3 Number of branches per plant (NB)

This was recorded by counting the total number of branches present on main stem of each plant at the time of harvest.

3.3.4.4 Days to maturity (DM)

Number of days taken from date of sowing to physiological maturity of the plant was recorded as days to maturity.

3.3.4.5 Number of pods per plant (NPP)

Total number of pods produced in each plant.

3.3.4.6 Pod length (cm) (PL)

The length of ten randomly selected pods were measured in centimeters.

3.3.4.7 Pod weight per plant (g) (PWP)

The weight of all the pods present on a plant were weighed in grams.

3.3.4.8 Number of seeds per pod (NSP)

Seeds present in ten randomly selected pods were counted and recorded as seeds per pod.

3.3.4.9 100 Seed weight (g) (100SW)

Randomly selected hundred seeds were weighed in grams and recorded as test weight.

3.3.4.10 Harvest index (%) (HI)

It is the ratio of economic yield (seed) to the total biological yield expressed in percentage.

$$\text{Harvest Index (\%)} = \frac{\text{Seed yield per plant}}{\text{Total biological yield per plant}} \times 100$$

3.3.4.11 Seed yield per plant (g) (SYP)

Seeds obtained from each individual plant were weighed in grams.

3.3.5 Reaction to rust

The severity of rust was scored between 65-90 days after sowing based on per cent leaf area infected by using 0-9 scale given by Mayee and Datar (1986).

3.3.6 Statistical analysis

3.3.6.1 Chi- square test

The segregation pattern was studied in F_2 and F_3 population. The disease resistance was classified into two groups. First group consisting of highly resistant, resistant and moderately resistant and second group consisting of highly susceptible and susceptible. The reactions for disease were recorded as resistant and susceptible in all the individual F_2.The recorded observations were subjected to chi-square test based on expected ratios.

$$\chi^2 = \frac{\Sigma (O-E)^2}{E}$$

Where, O = Observed frequency, E = Expected frequency

The goodness of fit between observed and expected segregation ratio was tested by comparing the calculated chi-square value with table value at 5 per cent level of significance at appropriate degrees of freedom (n-1), (n = number of classes of trait consideration)

Experiment 3.4: Study on the nature and extent of variability generated in the segregating populations with respect to yield and its component traits

3.4.1 Experimental material

The experimental material for this study comprised of F_2 population of three crosses *viz.*, JS 335 x EC 241780 (Susceptible x Resistant) consisting of 350 plants, EC 241780 x JS 335 (Resistant x Susceptible) with 456 plants and DSb 21 × EC 241780 (Resistant x Resistant) consisting of 432 plants and F_3 families of above three same F_2 crosses, each cross consisting of 100 progeny rows.

3.4.2 Experimental layout

The F_2 population of three crosses i.e. JS 335 X EC 241780 (Susceptible x Resistant), EC 241780 x JS 335 (Resistant x Susceptible) and DSb 21 × EC 241780 (Resistant x Resistant) were sown in field with spacing of 45 x 10 cm during *kharif* 2016 and F_3 families of above three same F_2 crosses, each cross consisting of 100 progeny rows were sown in field with spacing of 45 x 10 cm during *kharif* 2017 for evaluation of morphological and yield traits.

3.4.3 Statistical analysis

3.4.3.1 Mean, range and variance

The mean and variance were analyzed based on the formula given by Singh and Chaudhary (1977).

3.4.3.2 Mean: The parent and population means of crosses derived for different characters were computed as given below:

$$\bar{y} = \frac{1}{n} \sum_{i=1}^{n} (y_i)$$

Where,

$\bar{y}$ = Population mean

yi = Individual value

n = Total number of observations

3.4.3.3 Range: The minimum and maximum value on the basis of individual plant observations were used to indicate the limits of range for a given character.

3.4.3.4 Variance: In all the populations and parents, variance was computed by using formula.

$$\text{Variance} = \frac{1}{n-1} \left[\sum_{i=1}^{n} (y_i - \bar{y})^2\right]$$

Where,

yi = Individual value

y = Population mean

n = Number of observations

Standard deviation (SD) = √ Variance

3.4.3.5 Estimation of variance components

Genotypic and phenotypic variances and coefficients of variance were computed based on mean and variance calculated using the data of unreplicated treatments. Phenotypic and genotypic variances were estimated using the following formula:

(i) Phenotypic variance

The individual observation made for each trait on F_2 population is used for calculating the phenotypic variance.

$$\text{Phenotypic variance } (\sigma^2 p) = \text{Var } F_2$$

Where,

$\text{Var } F_2$ = variance of F_2 population

(ii) Environmental variance

The average variance of parents and their corresponding F_1 is used as environmental variance for single crosses.

$$\text{Environmental variance } (\sigma^2 e) = \frac{(\sigma^2 p_1) + (\sigma^2 p_2) + (\sigma^2 F_1)}{3}$$

Where, $\sigma^2 p_1$ = Variance of parent P_1

$\sigma^2 p_2$ = Variance of parent P_2

$\sigma^2 F_1$ = Variance of cross F_1

(iii) Genotypic variance

Genotypic variance $(\sigma^2 g) = \sigma^2 p - \sigma^2 e$

$\sigma^2 p$ = Phenotypic variance

$\sigma^2 e$ = Environmental variance

3.4.3.6 Coefficient of variation

Both genotypic and phenotypic coefficients of variation were computed as per the method suggested by Burton and Devane (1953).

0-10 per cent : Low

10-20 per cent : Moderate

20 per cent and above : High

(i) Genotypic coefficient of variation (GCV)

$$GCV = \frac{\sqrt{\sigma^2 g}}{\overline{x}} \times 100$$

(ii) Phenotypic coefficient of variation (PCV)

$$PCV = \frac{\sqrt{\sigma^2 p}}{\overline{x}} \times 100$$

Where,

$\sigma^2 g$ = genotypic variance

$\sigma^2 p$ = phenotypic variance

$\bar{x}$ = General mean of the characters

3.4.3.7 Heritability (h^2)

Heritability in broad sense was computed as the ratio of genetic variance to the total phenotypic variance as suggested by Hanson *et al.* (1956) and expressed as percentage.

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where,

$\sigma^2 g$ = genotypic variance

$\sigma^2 p$ = phenotypic variance

Heritability estimates were classified into low, moderate and high as given by Hanson *et al.* (1956).

0-30 per cent : Low

30-60 per cent : Moderate

60 per cent and above : High

3.4.3.8 Genetic advance (GA)

Genetic advance as per cent mean was categorized as low, moderate and high as per Johnson *et al.* (1955).

$$GA = h^2 K \sigma p$$

Where,

h^2 = Heritability in broad sense

K = Selection differential which is equal to 2.06 at 5 per cent intensity of selection (Lush, 1949).

σp = Phenotypic standard deviation

The GA as per cent of mean was categorized as low, moderate and high as given by Robinson *et al.* (1949) as follows:

0 - 10 per cent	: Low
10 – 20 per cent	: Moderate
20 and above	: High

3.4.3.9 Genetic advance as per cent of mean (GAM)

$$GAM = \frac{GA}{\overline{x}} \times 100$$

Where,

GA = Genetic advance

$\overline{x}$ = General mean of the character

GAM was categorized as per Johnson *et al.* (1955).

0-10 per cent	: Low
10-20 per cent	: Moderate
20 per cent and above	: High

3.4.3.10 Association analysis

The correlation coefficients were calculated to determine the degree of association of characters with yield and also among the yield components themselves in each environment.

Phenotypic correlations were computed by using the formula given by Webber and Moorty (1952).

$$\text{Phenotypic correlation} = r_{xy}(g) = \frac{COVxy(p)}{\sqrt{Vx(p) \times Vy(p)}}$$

Where,

Cov_{xy} (p) = Phenotypic covariance between x and y

V_x (p) = Phenotypic variance of characters x

V_y (p) = Phenotypic variance of characters y

Experiment 3.5: Validation of molecular markers linked to rust resistance

3.5.1 Genomic DNA extraction from soybean leaves

Genomic DNA was extracted by following the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980) with few modifications. DNA was extracted from individual plants from all susceptible, resistant and F_2 (350) plants. Leaves of three to four weeks old seedlings were taken from the field and frozen in liquid nitrogen. DNA was extracted as follows:

1. Frozen tissue sample (g) was ground into fine powder in liquid nitrogen in 2 ml microcentrifuge tube using autoclaved micropestle.

2. Extraction buffer was added to this microcentrifuge tube. The contents were mixed well and incubated at 65°C for one hour with occasional mixing by gentle swirling.

3. After incubation, the contents were spin for 5 minute at 8,000 rpm. About 750 µl of supernatant was transferred to fresh 1.5 ml microcentrifuge tube and the remaining was discarded.

4. About 750 µl of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added to this supernatant. The contents were mixed thoroughly and centrifuged for 10 minute at 13,000 rpm.

5. The aqueous phase was extracted and transferred to fresh 1.5 ml microcentrifuge tube and equal volume of Chloroform: Isoamyl alcohol (24:1) was added and contents were centrifuged for 10 minute at 13,000 rpm.

6. The aqueous phase was extracted and transferred to fresh 1.5 ml microcentrifuge tube and equal volume of Isopropanol was added and mixed by gentle inversion and incubated at -20 ^{0}C for overnight.

7. After overnight incubation, the tubes were centrifuged at 10,000 rpm for 10 minutes and supernatant was gently decanted.

8. Pellet (DNA) was washed with 50 µl of 70 per cent Ethanol and tubes were inverted till the pellet was air dried completely.

9. Pellet was dissolved in $T_{10}E_1$ buffer (40-50 µl) and stored at -20 ^{0}C.

3.5.2 Purification of extracted genomic DNA

The DNA was purified as follows:

1. The DNA samples were treated with 2 µl RNase A solution (1mg/ml) per 40 µl of $T_{10}E_1$ and tubes were incubated at 37 ^{0}C in waterbath for one hr.

2. After incubation temperature was increased to 65 ^{0}C for 10-15 minutes to denature the RNase A.

3. Equal volume (~50 µl) of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added and the content was mixed thoroughly and centrifuged at 11,000 rpm for 5 mins.

4. Aqueous phase was extracted and transferred to a fresh sterile 1.5 ml micro-centrifuge tube. Equal volume of Chloroform: Isoamyl alcohol (24:1) was added and tubes were centrifuged at 10,000 rpm for 5 mins.

5. Supernatant was extracted and transferred to a fresh sterile 1.5 ml micro-centrifuge tube and equal volume of isopropanol was added and content was mixed by gentle inversion and tubes were kept at -20 ^{0}C for two hrs.

6. The tubes were centrifuged at 10,000 rpm for 10 minutes and supernatant was gently decanted.

7. Pellet (DNA) was washed with 50 µl of 70 per cent Ethanol and pellet was air dried completely.

8. Pellet was dissolved in $T_{10}E_1$ buffer and stored at -20 ^{0}C.

3.5.3 Quantification of DNA

The amount of DNA in each sample was quantified by taking absorbance reading at 260 nm and 280 nm in Nano Drop spectrophotometer (ND-1000 V3.5.2, Nano Drop Technologies Inc., USA).

1. Initialization of the instrument was done with autoclaved distilled water.

2. The instrument was set blank with 2 µl $T_{10}E_1$ buffer.

3. The quantity of DNA was measured by loading 1 µl DNA sample on Nano Drop spectrophotometer pedestal.

4. The DNA quantity in ng/µl and OD value for each sample was noted.

The ratio between the readings at 260 and 280 nm (OD 260/OD 280) was used as an estimate of the purity of the DNA samples. Pure preparations of DNA have 260 nm/280 nm OD ratio between 1.7 and 1.8 (Sambrook and Russel, 2001). Computed OD values were used to dilute the DNA samples to the working concentrations of 100 ng/µl. Amount of stock DNA solution to be taken for dilution was calculated using following formula, where M_1 is stock DNA concentration (for example, 1,000 ng/µl), V_1 is volume of stock to be diluted, M_2 is concentration of working solution (100 ng/µl) and V_2 is volume of working solution to be prepared.

$$M_1V_1 = M_2V_2$$

$$(1000 \text{ ng/µl})\ V_1 = (100 \text{ ng /µl})\ (100 \text{ µl})$$

$$V_1 = (100 \text{ ng/µl})\ (100 \text{ µl})/\ (1000 \text{ ng/µl})$$

$$V_1 = 10 \text{ µl}$$

Thus, the amount of stock DNA solution to be taken for dilution was calculated. The appropriate volume from the stock was transferred to 1.5 ml microcentrifuge tube and the volume was made to 100 µl using $T_{10}E_1$ buffer. The DNA working solutions were stored at - 20 °C till further use.

3.5.4 DNA quality check by agarose gel electrophoresis

The gel casting tray was cleaned with distilled water and open ends were sealed with a tape. The comb was then positioned parallel to open edges about 2 mm above the surface of tray. Agarose (1.2 g) was added to 150 ml 1X TAE buffer and dissolved by melting. The solution was then allowed to cool. After cooling 7.5 µl of ethidium bromide was added as a staining agent. Then the solution was poured into the gel casting tray and allowed to solidify. After setting, the gel was placed in the electrophoresis unit with wells towards the cathode and tank was filled with 1X TAE buffer just enough to cover the surface of the gel. The DNA sample was pipetted onto a para film and mixed well with 2 µl of 6X loading dye. DNA samples were loaded in individual wells. The electrodes were connected to power supply and electrophoresis was carried out at 60 volts for 1-1.5 hours till the dye migrates to the end of the gel. The DNA was visualized and documented using a gel documentation system (Syngene Pvt. Ltd. USA).

3.5.5 Parental polymorphism study using SSR markers

Parental polymorphism is a pre requisite to begin marker assisted selection of superior genotypes. A clear polymorphism between resistant and susceptible parent is used to identify the difference among them. Polymorphism between the resistant (EC 241780) and susceptible (JS 335) parents was evaluated by using already reported 25 SSR markers and among them, some of SSR markers showed polymorphism between susceptible and resistant parents Table 9. These SSR markers were used for screening of F_2 population derived from the cross JS 335 x EC 241780. For gel electrophoresis the concentration of agarose 2.5 to 3.5 per cent with ethidium bromide staining procedure was followed. The annealing temperature of different markers used in this study are given in Table 10.

Table 9. List of SSR markers used in the present study

Sl. No.	Primer	Sequence	Location	Gene
1.	Sct 187	F: CATGCTCCCATTCTCT R: AACATTGGCTTTTTACTTAG	Chr.18	*Rpp1/Rpp1b*
2.	Sct 064	F: CCACAATTCCCAAAATAC R: ATAAAAATGGCTGAATAATAGAC		
3.	SSR 50	F: AGCACTAACAACTTTCTTTG R: GTTCTTAAATCTTACCCTCAC		
4.	SSR 60	F: AGATTGGGTGAGAACATAAG R: GGAGAGCGTAAAAGAAATTC		
5.	SSR 1859	F: CTCAATCGCATCCTTGCATA R: GCCTTCCAACTCATGTTTCAA		
6.	Satt 191	F: CGCGATCATGTCTCTG R: GGGAGTTGGTGTTTTCTTGTG		
7.	Satt 064	F: TAGCTTTATAATGAGTGTGATAGAT R: GTATGCAAGGGATTAATTAAG		
8.	Satt 372	F: GCGTCTCGAGGTAATTATCTATTTATCTTTT R: GCGAGTTTGGTAACATCGAGTATTGAT		
9.	Satt 255	F: GCGTCTCGAGGTAATTATCTATTTATCTTTT R: GCGAGTTTGGTAACATCGAGTATTGAT	Chr16	*Rpp2*
10.	Satt 215	F: GCGCCTTCTTCTGCTAAATCA R: CCCATTCAATTGAGATCCAAAATTAC		
11.	Satt 361	F: GCGTTAGATTTCCTTAGAATACATTGCTTCC R: GCGTTGACACTCATGATGTTATCTTACACC		
12.	Satt 366	F: GCGGCACAAGAACAGAGGAAACTATT R: GCGGACATGGTACATCTATATTACGAGTATT		
13.	Satt 460	F: GCGCGATGGGCTGTTGGTTTTTAT R: GCGCATACGATTTGGCATTTTTCTATTG	Chr.6	*Rpp3/Hyugaa*
14.	Satt 263	F: CACCCAATCATGATAGCATTTTAT R: CTCATGGAATTGTCTTTCAGTTTC		
15.	SSR 1788	F: TGAAATTGGAAACGATCGCAACG R: TGCTTCTTTCTTTCTTTATCCGCTCC		
16.	SSR 079	F: AGTCGAAGATACACAATTAGAT R: CTTTTAGACACAAATTTATCACT	Chr 18	*Rpp*4
17.	Satt 288	F: AGTCGAAGATACACAATTAGAT R: CTTTTAGACACAAATTTATCACT		
18.	Satt 143	F: GTGCCACAAATTTAAAATTACTCA R: TCCCTCCCTTTTGATTTACAC		
19.	Satt 612	F: GTGCCACAAATTTAAAATTACTCA R: TCCCTCCCTTTTGATTTACAC		
20.	Rpp4 TM	F: GTTTGCTTCAAGGGGTCCACA R: AACATCCCGCACAATGTCATGC		
21.	Satt 280	F:GGCGGTGGATATGAAACTTCAATAACTACAA R: GGCGGGCTTCAAATAATTACTATAAAACTACGG	Chr.3	*Rpp5*
22.	SSR 0469	F: GGTACACCATCACATTTCCAAGGCA R: TGGAAGTTTTTGGATGTGGTGCG		
23.	Satt 275	F: GCGGGATAATTGGTTTTACGAAAATGC R: GCGCCTAATCACCTAAAAAAACGTTTA		
24.	Satt 394	F: GCGTTTTTTCAATTTAAAGAGAATTGAC R: GCGTAACTTGCATGTGTATATCGAGATG	Chr. 18	*Rpp6*
25.	Satt 324	F: GTTCCCAGGTCCCACCATCTATG R: GCGTTTCTTTTATACCTTCAAG		

Chr. – Chromosome

Table 10. The annealing temperature for different markers used in this study

Sl. No.	Primer	Annealing temperature (°C)
1.	SSR 0469	71.5
2.	Satt 275	69.55
3.	Satt 394	66.95
4.	Satt 324	63.95
5.	SSR 1788	70.1
6.	Satt 361	69.65
7.	Satt 366	68.3
8.	Satt 460	72.75
9.	Satt 263	62.55
10.	SSR 079	52.85
11.	Satt 288	67.55
12.	Satt 143	61.2
13.	Satt 612	63.85
14.	Rpp4 TM	69.65
15.	Satt 280	70.7
16.	SSR 50	53.65
17.	SSR 66	56.35
18.	SSR 1859	64.35
19.	Satt 191	60.2
20.	Satt 064	54.7
21.	Satt 372	66.25
22.	Satt 255	71.5
23.	Satt 215	65.65
24.	Sct 187	53.35
25.	Sct 064	55.1

Note: The annealing temperature was calculated by taking the average of temperatures of forward and reverse primers for each of the markers used in this study.

3.5.6 Dilution of SSR primers

1. Primers were diluted by giving a brief spin to collect the lyophilized primer stock at bottom of tubes supplied by the company.
2. Nano pure water was added to prepare stock solution of 100 pM.
3. The tubes were incubated at 37 ^{0}C for 30 minutes. Then the working solution of 10 pM concentration was prepared.
4. Primers stock and working solutions of all the primers were stored in -20 ^{0}C for further use.

3.5.7 Polymerase chain reaction

Amplification of genomic DNA was done using forward and reverse primers pair through Polymerase chain reaction as (PCR) explained below.

3.5.7.1 PCR amplification

PCR amplification of SSR markers was done using forward and reverse primer pairs. PCR reaction mixture was prepared as master mix for all the templates in single microcentrifuge tube. Then it was distributed to all 0.2 ml PCR tubes and 1 µl of respective DNA template was added. Short spin was given to mix template with all reaction components and then tubes were loaded in a thermal cycler. The reaction in thermal cycler (Master cycler gradient 5331-Eppendorf version 2.30.31-09, Germany) was programmed as follows for respective microsatellite.

Contents of PCR reaction mixture

Reagents	Volume (µl)
Taq assay buffer (10X)	2
dNTPs (2.5 mM)	1.5
Forward primer (10 picoMole)	1
Reverse primer (10 picoMole)	1
Taq DNA Polymerase (3U/µl)	0.3
Template (DNA 100 ng/µl)	1
Sterile distilled water	13.2
Total	**20**

Touch-down PCR profile used for polymorphic marker

Reaction step	Temperature (^{0}C)	Time
Initial denaturation	95.0	5 min
Denaturation	94.0	1 min
Annealing*	65.0*	1 min
Primer extension	72.0	1 min
Repeat 10 cycles		
Denaturation	94.0	1 min
Annealing*	60.0	1 min
Primer extension	72.0	1 min
Final extension	72.0	10 min
Repeat 25 cycles		
Hold	4.0	

*Annealing temperature varied with primer

3.5.8 Gel electrophoresis

In order to visualize the DNA bands or amplification of PCR products, 3 per cent of Agarose: Metaphor at the rate of 1:1 gel was prepared with 1X TAE buffer. Ethidium bromide was added at a concentration of 0.5 µg ml^{-1} of gel for autoradiography. The gel was allowed to set fully before removing the comb and loading the sample. 5 µl of tracking dye (Bromophenol blue) was added to 15 µl of PCR products and mixed well before loading into the wells. A voltage of 60 Volts was given for period of three hours for separation of PCR fragments and then autoradiography was done under UV light using an *AlphaImager* Gel Documentation System (Alpha Innotech, USA). Photographs were taken from the gels using the gel documentation system and saved for later use during marker scoring.

3.5.9 Analysis of polymorphism

Clearly resolved unambiguous polymorphic bands were scored visually for base pair difference between the resistant and susceptible parent in comparison with 100 bp ladder.

3.5.10 Validation of molecular markers for rust resistance in F_2 population *via,* scoring of gel images

Polymorphic SSR markers identified between the susceptible (JS 335) and resistant (EC 241780) parents were considered for genotyping of 350 individual F_2 plants derived from the cross JS 335 × EC 241780. The genotypic scoring was done as, susceptible parent type scored as 'A', resistant parent type scored as 'B', heterozygote F_1 type scored as 'H'.

3.5.11 Single marker analysis (SMA)

Methods of QTL mapping are based on three broad classes namely regression, maximum likelihood and Bayesian models. The single marker analysis identifies QTL's based on the difference between the mean phenotypic values of different marker groups, but cannot separate recombination fraction and QTL effects.

Rust disease resistance scores of F_2 individual plants were subjected to associate with corresponding marker score for its significance by single marker analysis (SMA) using software WinQTLCart 2.5 version. SMA was performed to tag and confirm potential SSR markers linked to the trait based on phenotypic and genotypic data pertaining to the F_2 population of the cross JS 335 × EC 241780 which is based on simple linear regression coefficient.

4. EXPERIMENTAL RESULTS

Soybean [*Glycine max* (L.) Merrill] being a potentially high yielding crop can play an important role in boosting oil seed production in the country. It is referred as "miracle crop of 20th century" as it contains 40 per cent high quality protein and 20 per cent oil. It is also rich in Lysine and Vitamin A, B and D. Quality of soybean protein is next to animal protein and better than cereals and pulses. The edible oil in soybean is approximately 85 per cent unsaturated and contains essential fatty acids. The present investigation was carried out during 2015-17 at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad.

About 144 exotic germplasm lines including highly susceptible check JS 335 and resistant checks *viz.*, DSb 21, EC 241780 and EC 241778 were evaluated during *kharif* 2015 at Dharwad for identification of new sources for resistance to rust and genetic diversity. Based on the resistance reaction, 22 lines which exhibited resistant/moderately resistant reaction were selected. These lines were further evaluated to confirm their resistance reaction under natural epiphytotic condition at two hotspots for rust *viz.*, Ugarkhurd and Dharwad during *kharif* 2016.

Two varieties *viz.*, DSb 21, JS 335 and one genotype EC 241780 obtained from AICRP on Soybean, UAS, Dharwad were utilized in crossing programme/hybridization during *summer* 2015 to study the inheritance pattern for rust resistance and variability. Subsequently, F_2 and F_3 populations were raised during *kharif* 2016 & 2017 respectively.

In addition to this, validation of molecular markers linked to rust resistance in F_2 population of cross JS 335 x EC 241780 was carried out using 25 SSR markers.

The results of the above studies are categorised and presented as below:

4.1 Evaluation of exotic germplasm lines for identification of new sources for resistance to rust

4.2 Studies on genetic diversity in exotic germplasm lines

4.3 Studies on inheritance pattern of soybean rust

4.4 Study on the nature and extent of variability generated in the segregating populations with respect to yield and its component traits

4.5 Validation of molecular markers linked to rust resistance

4.1 Evaluation of exotic germplasm lines for identification of new sources for resistance to rust

The experiment on screening of 144 exotic germplasm lines including resistant and susceptible checks for rust caused by *Phakopsora pachyrhizi* Syd. under field condition was conducted at MARS, Dharwad during *kharif* 2015. Based on the resistance reaction, 22 lines which exhibited resistant/moderately resistant reaction were selected. These lines were further evaluated to confirm their resistance reaction along with susceptible check (JS 335) and resistant checks (DSb 21, EC 241780 and EC 241778) under natural epiphytotic condition at two hotspots for rust *viz.*, Ugarkhurd and Dharwad during *kharif* 2016. Scoring of the disease was done between 65 to 85 days after sowing based on per cent leaf area infected by using 0-9 scale (Mayee and Datar, 1986) and yield components *viz.*, days to 50 per cent flowering, number of branches per plant, days to maturity, number of pods per plant, 100 seed weight and seed yield per plant were recorded on five randomly selected plants in each line.

4.1.1 Rust severity at 10 days interval

The severity of soybean rust disease index was recorded at 10 days interval. Per cent disease index was calculated using formula given by Wheeler (1969) and the results are presented in Table 11.

Among 144 exotic germplasm lines including resistant and susceptible checks screened under field condition, four lines recorded highly resistant reaction (DSb 21, EC 241780, EC 241778 and EC 242104), nine lines recorded moderately resistant reaction, five lines registered moderately susceptible reaction, 46 lines were found to be susceptible and 80 lines exhibited highly susceptible reaction.

In post flowering stage (65 DAS), the range of PDI in 142 exotic germplasm lines and checks varied from 4.44 per cent (EC 241778) to 91.11 per cent (JS 335). The highest PDI was recorded in JS 335 (91.11%) followed by EC 685252 (82.22 %),

Table 11. Per cent disease index for rust at 10 days interval in soybean exotic germplasm lines during *kharif* 2015 at Dharwad

Sl. No.	Germplasm lines	PDI / DAS			Grade (0-9 Scale)	Reaction	No. of lesions/cm^2	Type of lesion
		65	75	85				
1	EC 1028	15.56	55.56	73.33	7	S	18.80	TAN
2	EC 10027	37.78	60.00	64.44	7	S	14.23	TAN
3	EC 100031	20.00	24.44	24.44	3	MR	9.23	RB
4	EC 100772	46.67	55.56	68.89	7	S	18.40	TAN
5	EC 104817	55.56	68.89	91.11	9	HS	21.13	TAN
6	EC 107416	51.11	68.89	82.22	9	HS	22.33	TAN
7	EC 114520	51.11	68.89	91.11	9	HS	21.27	TAN
8	EC 114573	37.78	60.00	91.11	9	HS	23.14	TAN
9	EC 116343	46.67	64.44	86.67	9	HS	27.40	TAN
10	EC 118420	28.89	42.22	46.67	5	MS	20.47	TAN
11	EC 118443	64.44	73.33	95.56	9	HS	21.27	TAN
12	EC 12570	51.11	64.44	82.22	9	HS	21.93	TAN
13	EC 14426	24.44	24.44	28.89	5	MS	19.27	TAN
14	EC 242091	46.67	60.00	73.33	7	S	23.80	TAN
15	EC 14476	15.56	24.44	24.44	3	MR	10.14	RB
16	EC 14573	37.78	68.89	77.78	9	HS	19.20	TAN
17	EC 149988	46.67	51.11	60.00	7	S	22.07	TAN
18	EC 15966	8.89	15.56	20.00	3	MR	10.25	RB
19	EC 16119	46.67	60.00	64.44	7	S	19.00	TAN
20	EC 16738	28.89	46.67	73.33	7	S	15.73	TAN
21	EC 172607	28.89	51.11	68.89	7	S	20.53	TAN
22	EC 175529	37.78	42.22	51.11	7	S	12.20	TAN
23	EC 177744	24.44	46.67	64.44	7	S	20.47	TAN
24	EC 187456	33.33	46.67	60.00	7	S	18.87	TAN
25	EC 184337	33.33	64.44	73.33	7	S	20.60	TAN
26	EC 19923	42.22	60.00	68.89	7	S	18.40	TAN
27	EC 225114	33.33	60.00	73.33	7	S	23.00	TAN

Contd……

Sl. No.	Germplasm lines	PDI / DAS			Grade (0-9 Scale)	Reaction	No. of lesions/cm^2	Type of lesion
		65	75	85				
28	EC 221329	28.89	42.22	46.67	5	MS	19.33	TAN
29	EC 2388	24.44	46.67	64.44	7	S	21.73	TAN
30	EC 232019	42.22	60.00	68.89	7	S	21.47	TAN
31	EC 241309	37.78	60.00	73.33	7	S	21.07	TAN
32	EC 241761	42.22	60.00	64.44	7	S	21.73	TAN
33	EC 241766	42.22	64.44	68.89	7	S	22.27	TAN
34	EC 242018	55.56	68.89	82.22	9	HS	23.53	TAN
35	EC 242038	46.67	60.00	68.89	7	S	20.40	TAN
36	EC 242104	6.67	8.89	8.89	1	HR	5.00	RB
37	EC 242105	77.78	82.22	91.11	9	HS	26.60	TAN
38	EC 245984	64.44	68.89	73.33	7	S	21.13	TAN
39	EC 245989	64.44	82.22	91.11	9	HS	24.47	TAN
40	EC 2581	68.89	77.78	86.67	9	HS	21.73	TAN
41	EC 25269	46.67	60.00	73.33	7	S	23.47	TAN
42	EC 250578	13.33	15.56	20.00	3	MR	9.82	RB
43	EC 250588	64.44	77.78	82.22	9	HS	20.53	TAN
44	EC 250607	60.00	68.89	73.33	7	S	25.13	TAN
45	EC 250608	46.67	64.44	73.33	7	S	21.27	TAN
46	EC 250619	46.67	64.44	73.33	7	S	26.07	TAN
47	EC 251329	46.67	55.56	64.44	7	S	23.73	TAN
48	EC 251334	42.22	55.56	68.89	7	S	22.33	TAN
49	EC 251341	46.67	60.00	68.89	7	S	20.60	TAN
50	EC 251358	24.44	55.56	60.00	7	S	22.07	TAN
51	EC 251401	28.89	46.67	51.11	7	S	20.87	TAN
52	EC 251409	73.33	77.78	86.67	9	HS	22.60	TAN
53	EC 251411	68.89	77.78	82.22	9	HS	25.40	TAN
54	EC 251 456	60.00	68.89	73.33	7	S	21.53	TAN
55	EC 251501	64.44	68.89	77.78	9	HS	21.67	TAN
56	EC 251516	64.44	73.33	82.22	9	HS	20.67	TAN

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Sl. No.	Germplasm lines	PDI / DAS			Grade (0-9 Scale)	Reaction	No. of lesions/cm^2	Type of lesion
		65	75	85				
57	EC 251762	37.78	46.67	73.33	7	S	18.93	TAN
58	EC 274755	64.44	73.33	86.67	9	HS	17.47	TAN
59	EC 287754	13.33	24.44	24.44	3	MR	8.65	RB
60	EC 30832	51.11	64.44	86.67	9	HS	21.87	TAN
61	EC 308334	8.89	20.00	24.44	3	MR	9.23	RB
62	EC 309512	73.33	77.78	86.67	9	HS	21.40	TAN
63	EC 309538	68.89	73.33	86.67	9	HS	23.56	TAN
64	EC 309545	64.44	73.33	82.22	9	HS	27.67	TAN
65	EC 315213	68.89	68.89	77.78	9	HS	23.20	TAN
66	EC 3251	42.22	42.22	46.67	5	MS	20.32	TAN
67	EC 325092	68.89	82.22	82.22	9	HS	21.40	TAN
68	EC 325099	64.44	68.89	82.22	9	HS	17.73	TAN
69	EC 325101	42.22	46.67	51.11	7	S	22.07	TAN
70	EC 325102	42.22	46.67	51.11	7	S	26.20	TAN
71	EC 329158	46.67	51.11	77.78	9	HS	20.13	TAN
72	EC 33875	64.44	73.33	82.22	9	HS	24.47	TAN
73	EC 33917	42.22	64.44	95.56	9	HS	31.00	TAN
74	EC 33922	24.44	42.22	46.67	5	MS	23.40	TAN
75	EC 33940	64.44	68.89	82.22	9	HS	24.20	TAN
76	EC 333868	55.56	68.89	73.33	7	S	19.53	TAN
77	EC 333875	68.89	73.33	82.22	9	HS	22.00	TAN
78	EC 333881	64.44	73.33	86.67	9	HS	21.60	TAN
79	EC 333886	51.11	64.44	77.78	9	HS	32.80	TAN
80	EC 333891	64.44	77.78	86.67	9	HS	28.53	TAN
81	EC 333904	37.78	60.00	68.89	7	S	26.13	TAN
82	EC 333909	42.22	55.56	64.44	7	S	21.13	TAN
83	EC 333920	46.67	60.00	77.78	9	HS	21.93	TAN
84	EC 333934	20.00	24.44	24.44	3	MR	9.45	RB
85	EC 338597	42.22	55.56	82.22	9	HS	23.87	TAN

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Sl. No.	Germplasm lines	PDI / DAS			Grade (0-9 Scale)	Reaction	No. of lesions/cm^2	Type of lesion
		65	75	85				
86	EC 34057	64.44	73.33	82.22	9	HS	24.80	TAN
87	EC 34078	60.00	73.33	86.67	9	HS	21.40	TAN
88	EC 34079	60.00	68.89	86.67	9	HS	27.13	TAN
89	EC 34092	68.89	73.33	82.22	9	HS	32.25	TAN
90	EC 34500	73.33	82.22	86.67	9	HS	28.53	TAN
91	EC 340924	68.89	82.22	91.11	9	HS	25.80	TAN
92	EC 36816	68.89	77.78	86.67	9	HS	30.73	TAN
93	EC 37937	51.11	73.33	95.56	9	HS	26.33	TAN
94	EC 376065	68.89	73.33	77.78	9	HS	25.20	TAN
95	EC 377552	60.00	73.33	91.11	9	HS	26.80	TAN
96	EC 380322	73.33	77.78	91.11	9	HS	25.47	TAN
97	EC 383165	60.00	77.78	91.11	9	HS	35.69	TAN
98	EC 385243	13.33	20.00	24.44	3	MR	9.89	RB
99	EC 389148	60.00	82.22	91.11	9	HS	32.07	TAN
100	EC 389151	73.33	82.22	86.67	9	HS	33.27	TAN
101	EC 389178	55.56	68.89	73.33	7	S	41.53	TAN
102	EC 389400	77.78	82.22	86.67	9	HS	37.27	TAN
103	EC 39219	77.78	82.22	91.11	9	HS	32.67	TAN
104	EC 39362	73.33	82.22	95.56	9	HS	32.33	TAN
105	EC 39491	73.33	82.22	86.67	9	HS	36.53	TAN
106	EC 39516	51.11	68.89	77.78	9	HS	29.93	TAN
107	EC 39536	64.44	73.33	77.78	9	HS	28.73	TAN
108	EC 390981	60.00	82.22	91.11	9	HS	23.40	TAN
109	EC 391158	42.22	46.67	51.11	7	S	22.67	TAN
110	EC 391336	8.89	15.56	20.00	3	MR	9.21	RB
111	EC 391346	68.89	68.89	73.33	7	S	22.20	TAN
112	EC 392532	60.00	73.33	77.78	9	HS	27.80	TAN
113	EC 392580	55.56	68.89	73.33	7	S	27.47	TAN
114	EC 394839	73.33	77.78	91.11	9	HS	24.13	TAN

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Sl. No.	Germplasm lines	PDI / DAS			Grade (0-9 Scale)	Reaction	No. of lesions/cm^2	Type of lesion
		65	75	85				
115	EC 396052	60.00	73.33	73.33	7	S	23.27	TAN
116	EC 396053	55.56	68.89	73.33	7	S	32.87	TAN
117	EC 397158	55.56	73.33	91.11	9	HS	27.20	TAN
118	EC 4435	68.89	82.22	82.22	9	HS	24.93	TAN
119	EC 42081	64.44	68.89	73.33	7	S	28.87	TAN
120	EC 457161	64.44	73.33	82.22	9	HS	31.53	TAN
121	EC 457175	42.22	55.56	60.00	7	S	20.93	TAN
122	EC 457286	60.00	73.33	73.33	7	S	33.60	TAN
123	EC 457406	73.33	82.22	86.67	9	HS	37.85	TAN
124	EC 457419	73.33	77.78	86.67	9	HS	27.13	TAN
125	EC 49393	77.78	82.22	91.11	9	HS	26.93	TAN
126	EC 65772	64.44	73.33	86.67	9	HS	35.27	TAN
127	EC 685246	68.89	82.22	86.67	9	HS	25.87	TAN
128	EC 685250	77.78	86.67	91.11	9	HS	30.07	TAN
129	EC 685251	73.33	82.22	91.11	9	HS	36.20	TAN
130	EC 685252	82.22	82.22	86.67	9	HS	34.60	TAN
131	EC 685255	73.33	82.22	95.56	9	HS	23.87	TAN
132	EC 685256	73.33	82.22	91.11	9	HS	32.00	TAN
133	EC 685258	64.44	77.78	86.67	9	HS	24.93	TAN
134	EC 7048	73.33	82.22	82.22	9	HS	23.93	TAN
135	EC 85705	68.89	82.22	91.11	9	HS	31.80	TAN
136	EC 917258	73.33	77.78	91.11	9	HS	38.20	TAN
137	EC 93413	55.56	82.22	91.11	9	HS	24.13	TAN
138	EC 94625	68.89	86.67	91.11	9	HS	32.53	TAN
139	EC 95291	64.44	82.22	95.56	9	HS	29.60	TAN
140	EC 95815	68.89	82.22	95.56	9	HS	36.33	TAN
141	EC 241778 (RC)	4.44	6.67	8.89	1	HR	8.73	RB
142	EC 241780 (RC)	6.67	8.89	8.89	1	HR	7.56	RB
143	DSb 21 (RC)	6.67	8.89	8.89	1	HR	6.87	RB
144	JS 335 (SC)	91.11	95.56	95.56	9	HS	42.80	TAN

*RC-Resistant check, SC- Susceptible check

EC 685250 (77.78 %) and EC 49393 (77.78 %). The lowest PDI was recorded by EC 241778 (4.44 %) followed by DSb 21(6.67 %), EC 242104 (6.67 %) and EC 241780 (6.67 %).

At 75 days after sowing, the range of PDI varied from 6.67 per cent (EC 241778) to 95.56 per cent (JS 335). The highest PDI was recorded in JS 335 (95.56 %) followed by EC 94625 (86.67 %), EC 685250 (86.67 %) and EC 95815 (82.22 %). The lowest PDI was recorded EC 241778 (6.67 %) by followed by DSb 21, EC 242104 and EC 241780 (8.89 %).

At maturity stage (85 DAS), the range of PDI varied from 8.89 per cent (DSb 21, EC 242104, EC 241778, EC 241780) to 95.56 per cent (JS 335). The highest PDI was observed in JS 335 (95.56%) followed by EC 95815 (95.56%), EC 95291 (95.56%) and EC 685255 (95.56%).Whereas lowest PDI was recorded by DSb 21, EC 242104, EC 241778, EC 241780 (8.89 %) followed by EC 391336, EC 250578 and EC 15966 (20.0 %).

In general the lines with a low initial per cent disease index invariably resulted with a low terminal disease index. PDI status at different interval observed in lines as EC 242104 recorded 6.67 % at 65 DAS and 8.89 % at 85 DAS, DSb 21 recorded 6.67 % at 65 DAS and 8.89 % at 85 DAS, EC 241780 recorded 6.67 % at 65 DAS and 8.89 % at 85 DAS, EC 241778 recorded 4.44 % at 65 DAS and 8.89 % at 85 DAS, EC 391336 recorded 8.89 % at 65 DAS and 20.0 % at 85 DAS and EC 15966 recorded 8.89 % at 65 DAS and 20.0 % at 85 DAS.

4.1.2 Count for the number of lesions

Count for the number of rust lesions on both the sides of mid-vein of infected leaves were recorded after 65 days of sowing. The data for the lesions count per cm^2 on mid-vein and both the sides of mid-vein of infected leaves is presented in Table 11. The minimum number of lesions per leaf was recorded in EC 242104 (5.0) followed by DSb 21 (6.87), EC 241780 (7.56), EC 241778 (8.73), EC 287754 (8.65) and EC 391336 (9.21). The maximum number of lesions were recorded in JS 335 (42.80) followed by EC 389178 (41.53), EC 917258 (38.20), EC 457406 (37.85) and EC 389400 (37.27) for mid-vein and on both the sides of mid-vein of infected leaves.

4.1.3 Observations for lesion colour (reddish brown/ tan colour)

Phenotypic observations for the lesion colour of the genotypes under study were recorded after 65 days of sowing and are presented in Table 11. The rust pathogen produces mainly two types of lesion colour after infestation, reddish brown and tan. The reddish brown lesions symbolize the resistance reaction while the tan coloured lesions symbolize susceptible reaction to the rust pathogen. Thirteen lines exhibited reddish brown colour while 131 lines showed tan coloured lesions.

4.1.4 Area under disease progress curve (AUDPC) for rust disease at 10 days interval

The per cent disease index obtained at 10 days interval for each line was used for estimation of AUDPC and are presented in Table 12. The Area Under Disease Progress Curve (AUDPC) value for each genotype was worked out using the formulae given by Wilcoxson *et al.* (1975).

The range of AUDPC at 65-75 DAS was from 55.56 (EC 241778) to 933.33 (JS 335). The highest AUDPC value was observed in JS 335 (933.33) followed by EC 685252 (822.22), EC 685250 (822.22) and EC 49393 (800). The least AUDPC value was recorded in EC 241778 (55.56) followed by DSb 21, EC 242104 and EC 241780 (77.78).

The range of AUDPC at 75-85 DAS was from 77.78 (EC 241778) to 955.56 (JS 335). The highest AUDPC value was observed in JS 335 (955.56) followed by EC 95815 (888.89), EC 95291 (888.89) and EC 94625 (888.89). The lowest AUDPC value was recorded in EC 241778 (77.78) followed by DSb 21, EC 242104 and EC 241780 (88.89).

The AUDPC in lines revealed a wide variation among the different lines at different intervals. Among, the lines tested, the highest average AUDPC value was observed in the lines JS 335 (944.44) followed by EC 685250 (855.55), EC 94625, EC 685255, EC 685252 (833.33). While, the least average AUDPC value was recorded in lines EC 241778 (66.67) followed by DSb 21, EC 242104, EC 241780 (83.33) and EC 15966, EC 391336 (150.00).

Table 12. Area under disease progress curve (AUDPC) for rust disease at 10 days interval in soybean exotic germplasm lines

Sl. No.	Germplasm lines	Grade (0-9 Scale)	AUDPC / DAS	
			65-75	75-85
1	EC 1028	7	355.56	644.44
2	EC 10027	7	488.89	622.22
3	EC 100031	3	222.22	244.44
4	EC 100772	7	511.11	622.22
5	EC 104817	9	622.22	800.00
6	EC 107416	9	600.00	755.56
7	EC 114520	9	600.00	800.00
8	EC 114573	9	488.89	755.56
9	EC 116343	9	555.56	755.56
10	EC 118420	5	355.56	444.44
11	EC 118443	9	688.89	844.44
12	EC 12570	9	577.78	733.33
13	EC 14426	5	244.44	266.67
14	EC 242091	7	533.33	666.67
15	EC 14476	3	200.00	244.44
16	EC 14573	9	533.33	733.33
17	EC 149988	7	488.89	555.56
18	EC 15966	3	122.22	177.78
19	EC 16119	7	533.33	622.22
20	EC 16738	7	377.78	600.00
21	EC 172607	7	400.00	600.00
22	EC 175529	7	400.00	466.67
23	EC 177744	7	355.56	555.56
24	EC 187456	7	400.00	533.33
25	EC 184337	7	488.89	688.89
26	EC 19923	7	511.11	644.44
27	EC 225114	7	466.67	666.67

Contd……

Sl. No.	Germplasm lines	Grade (0-9 Scale)	AUDPC / DAS	
			65-75	75-85
28	EC 221329	5	355.56	444.44
29	EC 2388	7	355.56	555.56
30	EC 232019	7	511.11	644.44
31	EC 241309	7	488.89	666.67
32	EC 241761	7	511.11	622.22
33	EC 241766	7	533.33	666.67
34	EC 242018	9	622.22	755.56
35	EC 242038	7	533.33	644.44
36	EC 242104	1	77.78	88.89
37	EC 242105	9	800.00	866.67
38	EC 245984	7	666.67	711.11
39	EC 245989	9	733.33	866.67
40	EC 2581	9	733.33	822.22
41	EC 25269	7	533.33	666.67
42	EC 250578	3	144.44	177.78
43	EC 250588	9	711.11	800.00
44	EC 250607	7	644.44	711.11
45	EC 250608	7	555.56	688.89
46	EC 250619	7	555.56	688.89
47	EC 251329	7	511.11	600.00
48	EC 251334	7	488.89	622.22
49	EC 251341	7	533.33	644.44
50	EC 251358	7	400.00	577.78
51	EC 251401	7	377.78	488.89
52	EC 251409	9	755.56	822.22
53	EC 251411	9	733.33	800.00
54	EC 251 456	7	644.44	711.11
55	EC 251501	9	666.67	733.33
56	EC 251516	9	688.89	777.78

Contd……..

Sl. No.	Germplasm lines	Grade (0-9 Scale)	AUDPC / DAS	
			65-75	75-85
57	EC 251762	7	422.22	600.00
58	EC 274755	9	688.89	800.00
59	EC 287754	3	188.89	244.44
60	EC 30832	9	577.78	755.56
61	EC 308334	3	144.44	222.22
62	EC 309512	9	755.56	822.22
63	EC 309538	9	711.11	800.00
64	EC 309545	9	688.89	777.78
65	EC 315213	9	688.89	733.33
66	EC 3251	5	422.22	444.44
67	EC 325092	9	755.56	822.22
68	EC 325099	9	666.67	755.56
69	EC 325101	7	444.44	488.89
70	EC 325102	7	444.44	488.89
71	EC 329158	9	488.89	644.44
72	EC 33875	9	688.89	777.78
73	EC 33917	9	533.33	800.00
74	EC 33922	5	333.33	444.44
75	EC 33940	9	666.67	755.56
76	EC 333868	7	622.22	711.11
77	EC 333875	9	711.11	777.78
78	EC 333881	9	688.89	800.00
79	EC 333886	9	577.78	711.11
80	EC 333891	9	711.11	822.22
81	EC 333904	7	488.89	644.44
82	EC 333909	7	488.89	600.00
83	EC 333920	9	533.33	688.89
84	EC 333934	3	222.22	244.44
85	EC 338597	9	488.89	688.89

Contd……..

Sl. No.	Germplasm lines	Grade (0-9 Scale)	AUDPC / DAS	
			65-75	75-85
86	EC 34057	9	688.89	777.78
87	EC 34078	9	666.67	800.00
88	EC 34079	9	644.44	777.78
89	EC 34092	9	711.11	777.78
90	EC 34500	9	777.78	844.44
91	EC 340924	9	755.56	866.67
92	EC 36816	9	733.33	822.22
93	EC 37937	9	622.22	844.44
94	EC 376065	9	711.11	755.56
95	EC 377552	9	666.67	822.22
96	EC 380322	9	755.56	844.44
97	EC 383165	9	688.89	844.44
98	EC 385243	3	166.67	222.22
99	EC 389148	9	711.11	866.67
100	EC 389151	9	777.78	844.44
101	EC 389178	7	622.22	711.11
102	EC 389400	9	800.00	844.44
103	EC 39219	9	800.00	866.67
104	EC 39362	9	777.78	888.89
105	EC 39491	9	777.78	844.44
106	EC 39516	9	600.00	733.33
107	EC 39536	9	688.89	755.56
108	EC 390981	9	711.11	866.67
109	EC 391158	7	444.44	488.89
110	EC 391336	3	122.22	177.78
111	EC 391346	7	688.89	711.11
112	EC 392532	9	666.67	755.56
113	EC 392580	7	622.22	711.11
114	EC 394839	9	755.56	844.44

Contd……..

Sl. No.	Germplasm lines	Grade (0-9 Scale)	AUDPC / DAS	
			65-75	75-85
115	EC 396052	7	666.67	733.33
116	EC 396053	7	622.22	711.11
117	EC 397158	9	644.44	822.22
118	EC 4435	9	755.56	822.22
119	EC 42081	7	666.67	711.11
120	EC 457161	9	688.89	777.78
121	EC 457175	7	488.89	577.78
122	EC 457286	7	666.67	733.33
123	EC 457406	9	777.78	844.44
124	EC 457419	9	755.56	822.22
125	EC 49393	9	800.00	866.67
126	EC 65772	9	688.89	800.00
127	EC 685246	9	755.56	844.44
128	EC 685250	9	822.22	888.89
129	EC 685251	9	777.78	866.67
130	EC 685252	9	822.22	844.44
131	EC 685255	9	777.78	888.89
132	EC 685256	9	777.78	866.67
133	EC 685258	9	711.11	822.22
134	EC 7048	9	777.78	822.22
135	EC 85705	9	755.56	866.67
136	EC 917258	9	755.56	844.44
137	EC 93413	9	688.89	866.67
138	EC 94625	9	777.78	888.89
139	EC 95291	9	733.33	888.89
140	EC 95815	9	755.56	888.89
141	EC 241778 (RC)	1	55.56	77.78
142	EC 241780 (RC)	1	77.78	88.89
143	DSb 21 (RC)	1	77.78	88.89
144	JS 335 (SC)	9	933.33	955.56

*RC-Resistant check, SC- Susceptible check

4.1.5 Apparent rate of infection (r) for rust disease at different stages of crop growth

The apparent rate of soybean rust infection per unit per day (r) was calculated from PDI by using the formula given by Van der Plank (1963) and are presented in Table 13.

The range of 'r' at 65-75 DAS was from 0.00 (EC 14426, EC 315213, EC 3251, EC 391346 and EC 685252) to 0.191 (EC 1028). The highest 'r' value was observed in the line EC 1028 (0.191) and EC 251358 (0.135). The least average 'r' value was recorded in five lines *viz.*, EC 14426, EC 315213, EC 3251, EC 391346 and EC 685252 (0.00) followed by EC 149988 (0.018) and EC 250578 (0.018).

The range of 'r' at 75-85 DAS was from 0.018 (EC 118420, EC 221329, EC 251358, EC 251401, EC 325101, EC 391158 and EC 3251) to 0.191 (EC 1028). The highest 'r' value was observed in the line EC 1028 (0.191) and EC 251358 (0.135). However, least average 'r' value was recorded in lines *viz.*, EC 118420, EC 221329, EC 251358, EC 251401, EC 325101, EC 391158 and EC 3251 (0.018) followed by EC 10027, EC 241761 (0.019) and EC 241766 (0.020).

The rate of apparent infection in exotic lines revealed a wide variation among the different exotic lines at different intervals. Among, the exotic lines tested, the highest average 'r' value was observed in the lines EC 33917 (0.169) followed by EC 37937 (0.151), EC 114573 (0.141) and EC 95291 (0.124). While, the lowest average 'r' value was recorded by EC 3251 (0.009) followed by EC 391346, EC 14426 (0.011) and EC 685252 (0.017).

4.1.6 Reaction of germplasm lines during *kharif* 2016 at Dharwad and Ugarkhurd

Among 22 exotic germplasm lines including resistant and susceptible checks screened under natural epiphytotic condition at two hotspots for rust *viz.*, Ugarkhurd and Dharwad during *kharif* 2016. Only one line (EC 242104), resistant checks *viz.*, DSb 21, EC 241780, EC 241778 recorded disease grade of 1 and found to be highly resistant reaction, 9 lines recorded disease grade of 3 and found to be moderately resistant reaction,

Table 13. Apparent rate of infection (r) for rust disease at different stages of crop growth in soybean exotic germplasm lines

Sl. No.	Germplasm lines	Rate of spread 'r' / DAS		Average 'r'
		65-75	75-85	
1	EC 1028	0.191	0.079	0.135
2	EC 10027	0.090	0.019	0.055
3	EC 100031	0.026	0.000	0.013
4	EC 100772	0.036	0.057	0.046
5	EC 104817	0.057	0.153	0.105
6	EC 107416	0.075	0.074	0.074
7	EC 114520	0.075	0.153	0.114
8	EC 114573	0.090	0.192	0.141
9	EC 116343	0.073	0.128	0.100
10	EC 118420	0.059	0.018	0.038
11	EC 118443	0.042	0.205	0.124
12	EC 12570	0.055	0.094	0.074
13	EC 14426	0.000	0.023	0.011
14	EC 242091	0.054	0.061	0.057
15	EC 14476	0.056	0.000	0.028
16	EC 14573	0.129	0.046	0.087
17	EC 149988	0.018	0.036	0.027
18	EC 15966	0.063	0.031	0.047
19	EC 16119	0.054	0.019	0.036
20	EC 16738	0.077	0.114	0.096
21	EC 172607	0.094	0.075	0.085
22	EC 175529	0.019	0.036	0.027
23	EC 177744	0.099	0.073	0.086
24	EC 187456	0.056	0.054	0.055
25	EC 184337	0.129	0.042	0.085
26	EC 19923	0.072	0.039	0.055
27	EC 225114	0.110	0.061	0.085

Contd……

Sl. No.	Germplasm lines	Rate of spread 'r' / DAS		Average 'r'
		65-75	75-85	
28	EC 221329	0.059	0.018	0.038
29	EC 2388	0.099	0.073	0.086
30	EC 232019	0.072	0.039	0.055
31	EC 241309	0.090	0.061	0.075
32	EC 241761	0.072	0.019	0.045
33	EC 241766	0.091	0.020	0.055
34	EC 242018	0.057	0.074	0.065
35	EC 242038	0.054	0.039	0.046
36	EC 242104	0.031	0.000	0.015
37	EC 242105	0.028	0.079	0.054
38	EC 245984	0.020	0.022	0.021
39	EC 245989	0.094	0.079	0.087
40	EC 2581	0.046	0.062	0.054
41	EC 25269	0.054	0.061	0.057
42	EC 250578	0.018	0.031	0.024
43	EC 250588	0.066	0.028	0.047
44	EC 250607	0.039	0.022	0.030
45	EC 250608	0.073	0.042	0.057
46	EC 250619	0.073	0.042	0.057
47	EC 251329	0.036	0.037	0.036
48	EC 251334	0.054	0.057	0.055
49	EC 251341	0.054	0.039	0.046
50	EC 251358	0.135	0.018	0.077
51	EC 251401	0.077	0.018	0.047
52	EC 251409	0.024	0.062	0.043
53	EC 251411	0.046	0.028	0.037
54	EC 251 456	0.039	0.022	0.030
55	EC 251501	0.020	0.046	0.033
56	EC 251516	0.042	0.052	0.047

Contd……

Sl. No.	Germplasm lines	Rate of spread 'r' / DAS		Average 'r'
		65-75	75-85	
57	EC 251762	0.037	0.114	0.075
58	EC 274755	0.042	0.086	0.064
59	EC 287754	0.074	0.000	0.037
60	EC 30832	0.055	0.128	0.091
61	EC 308334	0.094	0.026	0.060
62	EC 309512	0.024	0.062	0.043
63	EC 309538	0.022	0.086	0.054
64	EC 309545	0.042	0.052	0.047
65	EC 315213	0.000	0.046	0.023
66	EC 3251	0.000	0.018	0.009
67	EC 325092	0.074	0.000	0.037
68	EC 325099	0.020	0.074	0.047
69	EC 325101	0.018	0.018	0.018
70	EC 325102	0.018	0.018	0.018
71	EC 329158	0.018	0.121	0.069
72	EC 33875	0.042	0.052	0.047
73	EC 33917	0.091	0.247	0.169
74	EC 33922	0.081	0.018	0.050
75	EC 33940	0.020	0.074	0.047
76	EC 333868	0.057	0.022	0.039
77	EC 333875	0.022	0.052	0.037
78	EC 333881	0.042	0.086	0.064
79	EC 333886	0.055	0.066	0.060
80	EC 333891	0.066	0.062	0.064
81	EC 333904	0.090	0.039	0.065
82	EC 333909	0.054	0.037	0.045
83	EC 333920	0.054	0.085	0.069
84	EC 333934	0.026	0.000	0.013
85	EC 338597	0.054	0.131	0.092

Contd……

Sl. No.	Germplasm lines	Rate of spread 'r' / DAS		Average 'r'
		65-75	75-85	
86	EC 34057	0.042	0.052	0.047
87	EC 34078	0.061	0.086	0.073
88	EC 34079	0.039	0.108	0.073
89	EC 34092	0.022	0.052	0.037
90	EC 34500	0.052	0.034	0.043
91	EC 340924	0.074	0.079	0.077
92	EC 36816	0.046	0.062	0.054
93	EC 37937	0.097	0.205	0.151
94	EC 376065	0.022	0.024	0.023
95	EC 377552	0.061	0.131	0.096
96	EC 380322	0.024	0.107	0.066
97	EC 383165	0.085	0.107	0.096
98	EC 385243	0.048	0.026	0.037
99	EC 389148	0.112	0.079	0.096
100	EC 389151	0.052	0.034	0.043
101	EC 389178	0.057	0.022	0.039
102	EC 389400	0.028	0.034	0.031
103	EC 39219	0.028	0.079	0.054
104	EC 39362	0.052	0.153	0.103
105	EC 39491	0.052	0.034	0.043
106	EC 39516	0.075	0.046	0.060
107	EC 39536	0.042	0.024	0.033
108	EC 390981	0.112	0.079	0.096
109	EC 391158	0.018	0.018	0.018
110	EC 391336	0.063	0.031	0.047
111	EC 391346	0.000	0.022	0.011
112	EC 392532	0.061	0.024	0.042
113	EC 392580	0.057	0.022	0.039
114	EC 394839	0.024	0.107	0.066

Contd……

Sl. No.	Germplasm lines	Rate of spread 'r' / DAS		Average 'r'
		65-75	75-85	
115	EC 396052	0.061	0.000	0.030
116	EC 396053	0.057	0.022	0.039
117	EC 397158	0.079	0.131	0.105
118	EC 4435	0.074	0.000	0.037
119	EC 42081	0.020	0.022	0.021
120	EC 457161	0.042	0.052	0.047
121	EC 457175	0.054	0.018	0.036
122	EC 457286	0.061	0.000	0.030
123	EC 457406	0.052	0.034	0.043
124	EC 457419	0.024	0.062	0.043
125	EC 49393	0.028	0.079	0.054
126	EC 65772	0.042	0.086	0.064
127	EC 685246	0.074	0.034	0.054
128	EC 685250	0.062	0.045	0.054
129	EC 685251	0.052	0.079	0.066
130	EC 685252	0.000	0.034	0.017
131	EC 685255	0.052	0.153	0.103
132	EC 685256	0.052	0.079	0.066
133	EC 685258	0.066	0.062	0.064
134	EC 7048	0.052	0.000	0.026
135	EC 85705	0.074	0.079	0.077
136	EC 917258	0.024	0.107	0.066
137	EC 93413	0.131	0.079	0.105
138	EC 94625	0.108	0.045	0.077
139	EC 95291	0.094	0.153	0.124
140	EC 95815	0.074	0.153	0.114
141	EC 241778 (RC)	0.043	0.031	0.037
142	EC 241780 (RC)	0.031	0.000	0.015
143	DSb 21 (RC)	0.031	0.000	0.015
144	JS 335 (SC)	0.074	0.000	0.037

*RC-Resistant check, SC- Susceptible check

9 lines registered moderately susceptible reaction, 3 lines were found to be susceptible and susceptible check JS 335 exhibited highly susceptible reaction. Reactions of these exotic germplasm lines for rust during *kharif* 2016 are presented in Table 14. Sever incidence of rust at Dharwad are presented in Plate 8.

4.2 Studies on genetic diversity in exotic germplasm lines

In the present investigation the algorithm k-*means* cluster analysis was employed to group the 144 genotypes into eight clusters, based on the mean values of yield related traits. The number of genotypes, name of genotypes and the cluster to which they belong are presented in the Table 15. Maximum number of genotypes were grouped in the cluster V (39 genotypes) followed by cluster I (35 genotypes), cluster VII and cluster VIII (20 genotypes each). *Per se* performance of 140 exotic germplasm lines and four checks for yield related traits in soybean during *kharif* 2015 are presented in Appendix 4

4.2.1 Inter-cluster distances

Estimates of average inter-cluster distances for yield related traits in soybean are presented in Table 16. The maximum inter-cluster distance was 55.64 observed between cluster II and VI followed by 53.07 between cluster VII and VIII. The lowest inter-cluster distance was observed between cluster I and III (7.07) followed by cluster II and VIII (9.41). Cluster I is farthest to cluster VI (33.69) and nearest to cluster III (7.07). similarly, cluster II is nearest to cluster VIII (9.41) and farthest to cluster VI (55.64), cluster III is nearest to cluster VII (12.08) and farthest to cluster VI (32.56), cluster IV is nearest to cluster VI (20.07) and farthest to cluster VIII (42.29), cluster V is nearest to cluster VIII (9.71) and farthest to cluster VI (44.26), while cluster VI is nearest to cluster VII (20.82) and farthest to cluster VIII (53.07). With respect to cluster VII, the cluster VIII is farthest (32.90) and cluster VIII nearest to the cluster II (9.41).

4.2.2 Cluster mean analysis and per cent contribution of traits towards the divergence

The cluster mean values and per cent contribution of yield related traits are presented in Table 17. Significant cluster mean was observed for all the traits studied.

a) F_2 population (*kharif* 2016)

b) F_3 population (*kharif* 2017)

Plate 8: Severe incidence of rust at Dharwad

Table 14. Disease severity for rust, yield and yield components in soybean exotic germplasm lines during *kharif* 2016 at Dharwad and Ugarkhurd

Sl. No.	Genotypes	Disease scoring (0-9 Scale)				Yield and Yield components					
		Dharwad		Ugarkhurd							
		Grade	Reaction	Grade	Reaction	DFF	DM	NBP	NPP	100 SW (g)	SYP (g)
1	EC 14426	3	MR	3	MR	39	90	4.0	46.0	11.79	11.48
2	EC 15966	3	MR	3	MR	39	90	3.8	52.0	12.80	10.12
3	EC 16119	7	S	7	S	41	93	3.4	39.6	9.20	8.66
4	EC 33922	5	MS	5	MS	39	88	6.0	43.2	11.60	9.68
5	EC 100031	3	MR	3	MR	43	95	3.2	44.8	12.70	8.44
6	EC 118420	5	MS	5	MS	41	90	3.4	43.2	11.85	9.42
7	EC 149988	7	S	7	S	39	89	4.4	39.2	10.80	9.18
8	EC 175529	5	MS	5	MS	41	89	8.0	53.4	12.05	10.40
9	EC 221329	5	MS	5	MS	38	88	5.0	40.6	11.89	8.38
10	EC 242104	1	HR	1	HR	41	93	4.6	52.4	13.90	11.6
11	EC 250578	3	MR	3	MR	43	95	5.0	67.2	12.57	11.32
12	EC 251358	5	MS	5	MS	39	89	4.6	39.2	11.00	9.58
13	EC 251401	5	MS	5	MS	38	89	3.8	49.4	12.40	9.74
14	EC 257754	3	MR	3	MR	36	85	3.4	38.0	12.39	10.96
15	EC 308334	3	MR	3	MR	43	93	7.2	53.8	10.48	11.08
16	EC 325101	5	MS	5	MS	39	88	3.6	52.8	11.80	9.60
17	EC 325102	5	MS	5	MS	43	95	4.8	45.8	12.00	10.46
18	EC 333909	7	S	7	S	41	93	5.4	55.4	11.90	10.84
19	EC 333934	3	MR	3	MR	40	91	4.2	41.2	12.79	9.10
20	EC 385243	3	MR	3	MR	43	90	8.6	54.6	12.80	10.52
21	EC 391158	5	MS	5	MS	44	92	7.2	33.0	12.00	8.24
22	EC 391336	3	MR	3	MR	40	91	4.4	39.6	12.80	10.28
23	EC241778 (RC)	1	HR	1	HR	49	102	5.4	48.4	13.90	10.60
24	EC241780 (RC)	1	HR	1	HR	50	101	4.4	44.2	14.10	9.80
25	DSb 21 (RC)	1	HR	1	HR	42	92	4.4	54.8	14.00	15.72
26	JS 335 (SC)	9	HS	9	HS	37	84	3.4	43.4	8.10	9.30

*RC-Resistant check, SC- Susceptible check
DFF- Days to 50 % flowering; DM- Days to maturity; NB- Number of branches per plant;
NPP- Number of pods per plant; 100 SW- 100 Seed weight (g); SYP- Seed yield per plant (g).

Table 15. Sources and clustering of 140 exotic germplasm lines and four checks in eight clusters

Cluster	Number of germplasm lines	Name of the Exotic germplasm
I	35	EC 100031, EC 118420, EC 149988, EC 187456, EC 242038, EC 242104, EC 245984,EC 25269, EC 250607, EC 251341, EC 251358, EC 251411, EC 251501, EC 251516, EC 274755, EC 30832, EC 309512, EC 315213, EC 33922, EC 33940, EC 333868, EC 333875, EC 333920, EC 333934, EC 34092, EC 389178, EC 39219, EC 39516, EC 390981, EC 394839,EC 42081, EC 457161, EC 457286, EC 457419, EC 65772.
II	16	EC 10027, EC 14573, EC 250588, EC 251401, EC 325099, EC 325101, EC 325102, EC 329158, EC 33917, EC 333886, EC 333909, EC 34079, EC 392532, EC 392580, EC 685250, EC 685258.
III	10	EC 1028,EC 116343, EC 250619, EC 251334, EC 251762, EC 34057, EC 377552, EC 39536, EC 685252, EC 95291.
IV	2	EC 241780 (C), EC 241778 (C).
V	39	EC 100772, EC 104817, EC 107416, EC 114573, EC 12570, EC 15966, EC 16119, EC 177744, EC 184337, EC 225114, EC 221329, EC 2388, EC 232019, EC 241309, EC 242018, EC 242105, EC 245989, EC 250578, EC 250608, EC 251329, EC 251 456,EC 287754, EC 308334, EC 309538, EC 333891, EC 338597, EC 340924, EC 36816, EC 389151, EC 39491, EC 391336, EC 396052, EC 457175, EC 457406, EC 49393, EC 685246, EC 685255, EC 685256, JS 335(C).
VI	2	EC 385243, DSb 21 (C).
VII	20	EC 114520, EC 118443, EC 14426, EC 242091, EC 14476, EC 16738, EC 19923, EC 2581, EC 33875, EC 380322, EC 383165, EC 389400, EC 396053, EC 4435, EC 7048, EC 85705, EC 9172587, EC 93413, EC 94625, EC 95815.
VIII	20	EC 172607, EC 175529, EC 241761, EC 241766, EC 251409, EC 309545, EC 3251, EC 325092, EC 333881, EC 333904, EC 34078, EC 34500, EC 37937, EC 376065, EC 389148, EC 39362, EC 391158, EC 391346, EC 397158, EC 685251.

Table 16. Estimates of average inter-cluster distances for yield related traits in soybean

Cluster	I	II	III	IV	V	VI	VII	VIII
I		22.607	7.076	26.235	10.687	33.69	13.404	19.636
II			23.591	II-47.806	12.422	I-55.641	34.907	9.417
III				29.361	13.868	32.569	12.084	23.378
IV					35.766	20.071	20.144	42.29
V						44.265	23.701	9.71
VI							20.82	53.07
VII								32.908
VIII								

Table 17. Cluster mean values and percent contribution for yield related traits in soybean

Characters	Cluster								F	Percent contribution
	I	II	III	IV	V	VI	VII	VIII		
Days 50 % Flowering	36.54	33.75	34.7	46	34.9	37.5	34.9	37.15	7.56**	13.42
Number of branches/ plant	4.72	4.28	5.06	5.8	4.63	6.2	5.39	4.35	8.38**	17.47
Days to maturity	89.83	82.75	83.4	103.5	88.79	91.5	88.35	91.35	24.06**	9.05
Number of pods per plant	56.31	35.26	58.5	76.5	45.87	89.9	69.51	37.03	243.19**	41.82
Seed yield per plant (g)	12.62	9.45	13.27	10.86	11.39	13.49	12.87	9.29	51.57**	18.24

The cluster VI recorded maximum cluster mean value of 91.5 for days to maturity, 89.9 for number of pods per plant, 37.5 for days to 50 % flowering, 13.49 for seed yield per plant and 6.2 for number of branches per plant. Whereas, cluster II exhibited minimum cluster mean value of 82.75 for days to maturity, 35.26 for number of pods per plant, 33.75 for days to 50 % flowering and 4.28 for number of branches per plant. The maximum cluster means for seed yield per plant was registered by cluster IV (13.49) followed by cluster III (13.27) while, minimum was observed in cluster VIII (9.29). Cluster II showed minimum cluster mean values of 33.75 and 82.75 for the days to 50 % flowering and days to maturity respectively. The maximum cluster mean observed for days to 50 % flowering (37.5) and days to maturity (91.5) was in cluster VI.

The traits number of pods per plant (41.82), number of branches per plant (17.47) and days to 50 % flowering (13.42) exhibited major contribution towards the diversity while, days to maturity (9.05) contributed the least towards the diversity for seed yield.

4.3 Studies on inheritance pattern of soybean rust

4.3.1 Selection of parents

The disease incidence of three F_2 population of soybean against rust disease under natural conditions and their segregation pattern is given in Table 18.

Three parents *viz.*, JS 335, EC 241780 and DSb 21 were selected to study the inheritance of rust resistance. The DSb 21 which is already identified as a highly rust resistant variety, another line EC 241780 which is also resistant to rust and highly susceptible variety JS 335 was used for inheritance studies. These three parents were used in hybridization programme in three different combinations i.e. susceptible x resistant (JS 335 x EC 241780), resistant x susceptible (EC 241780 x JS 335) and resistant x resistant (DSb 21 x EC 241780).

4.3.2 Phenotyping of the F_2 populations for reaction to rust disease

A total of 350 F_2 segregants from the cross JS 335 x EC 241780, 456 F_2 segregants from the cross EC 241780 x JS 335 and 432 F_2 segregants from the cross DSb 21 x EC 241780 were screened for rust reaction by creating artificial epiphytic

disease condition using same procedure as that for the parental screening in *Kharif* 2016. Rust reaction was recorded on a 0- 9 scale as suggested Mayee and Datar (1986).

Out of total 350 F_2 segregants of the cross JS 335 x EC 241780, 272 plants recorded resistant reaction with the score of 1 and 78 plants recorded susceptible reaction with the score of 9. The proportion of which was closer to the expected number of plants with simple monogenic gene inheritance with 3:1 ratio for resistance and susceptible reaction. The chi-square analysis of the number of resistant and susceptible plants indicating the probability value of 1.37 which is less than table value 3.84. Therefore it can be concluded that rust resistance is controlled by a single dominant gene in the cross susceptible (JS 335) x resistant (EC 241780).

In the cross EC 241780 x JS 335 (resistant x susceptible) out of 456 F_2 segregants, 352 plants were exhibited resistant reaction with the score of 1 and 104 plants were exhibited susceptible reaction with the score of 9. The chi-square analysis of the number of resistant and susceptible plants indicating the probability value of 1.16 which is less than table value 3.84. Therefore it can be concluded that rust resistance is controlled by a single dominant gene in the cross resistant (EC 241780) x susceptible (JS 335) for 3:1 segregation pattern.

The calculated value was non- significant in both the crosses susceptible x resistant (JS 335 x EC 241780) and resistant x susceptible (EC 241780 x JS 335) indicating single dominant gene is responsible for resistance to soybean rust with resistance being dominant over susceptibility.

In the cross DSb 21 x EC 241780 which represent resistant x resistant combination for rust. Out of 432 F_2 segregants, 414 plants recorded resistant reaction and 18 segregants recorded susceptible reaction to rust disease, thus fitting well for 15:1 segregation ratio. The chi- square analysis for this population recorded the probability value 3.2, which is less than table value (3.84) for 15:1 segregation pattern. The results indicated presence of the different resistance genes in these genotypes.

4.3.3 Phenotyping of the F_3 populations for reaction to rust disease

The details of the resistance and susceptible reaction observed in F_3 population is given in Table 18.

Table 18. Chi- square analysis of three F_2 and $F_{2:3}$ population for soybean rust disease

Crosses/ Generations	JS 335 x EC 241780 (Susceptible x Resistant)	EC 241780 x JS 335 (Resistant x Susceptible)	DSb 21 x EC 241780 (Resistant x Resistant)
F_1	R	R	R
F_2 plants			
Resistant	272	352	414
Susceptible	78	104	18
Total	350	456	432
x^2	1.37	1.16	3.2
d.f.	1	1	1
Expt. Ratio	3 : 1	3 : 1	15 : 1
Table value	3.84	3.84	3.84
F_3 progenies			
Resistant	25	23	46
Segregating	49	51	49
Susceptible	26	26	5
Total	100	100	100
x^2	0.06	0.22	0.385
d.f.	2	2	2
Expt. Ratio	1 : 2 : 1	1 : 2 : 1	7 : 8 : 1
Table value	5.99	5.99	5.99

df- degrees of freedom.

To confirm the mode of inheritance, 100 plants were selected from each F_2 population of three crosses *viz.,* JS 335 x EC 241780, EC 241780 x JS 335 and DSb 21 x EC 241780 based on randomisation table and were sown at MARS, Dharwad during *kharif* 2017 and screened under natural condition.

Out of the total 100 F_3 progeny rows of the cross JS 335 x EC 241780, 25 lines were exhibited resistant reaction, 49 lines were segregating and 26 lines were exhibited susceptible reaction. The chi- square analysis for this population recorded 0.06, which is less than table value (5.99) for 1:2:1 segregation pattern. In the cross EC 241780 x JS 335, out of the total 100 F_3 progeny rows, 23 lines were recorded resistant reaction, 51 lines were segregating and 26 lines were recorded susceptible reaction to rust disease thus fitting well for 1:2:1 segregation ratio. The chi- square analysis for this population recorded 0.22, which is less than table value (5.99) for 1:2:1 segregation pattern.

In the cross DSb 21 x EC 241780, out of the total 100 F_3 progeny rows, 46 lines were recorded resistant reaction, 49 lines were segregating and 5 lines were recorded susceptible reaction to rust disease thus fitting well for 7:8:1 segregation ratio. The chi- square analysis for this population recorded 0.38, which is less than table value (5.99) for 7:8:1 segregation pattern.

Each F_3 family was classified as resistant (homozygous), susceptible (homozygous), and segregating (heterozygous) based on reaction to rust. In F_3, cross with susceptible x resistant and resistant x susceptible parents exhibited goodness of fit with 1:2:1 ratio, (1 resistant: 2 segregating: 1 susceptible) and in the cross involving resistant x resistant parent the goodness of fit observed was 7:8:1 ratio, (7 resistant: 8 segregating: 1 susceptible), confirming the results observed in F_2 generation.

4.4 Nature and extent of variability created in the segregating populations with respect to yield and its component traits

4.4.1 Genetic variability studies for yield and yield attributing traits in F_2 populations of three crosses

Estimates of mean, range, phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance as per cent mean for yield and

its component traits *viz.*, days to flowering, plant height (cm), number of branches per plant, days to maturity, number of pods per plant, pod length (cm), pod weight per plant (g), number of seeds per pod, 100 seed weight (g), harvest index (%) and seed yield per plant (g) in all the crosses are presented in Table 19, 20 and 21.

4.4.1.1 Days to 50 % flowering

The overall mean number of days for flowering in cross 1 (JS 335 x EC 241780) was 40.42 days with a range of 36 to 44 days, whereas overall mean number of days for flowering in cross 2 (EC 241780 x JS 335) was 38.39 days with range of 31 to 46 days and overall mean number of days for flowering in cross 3 (DSb 21 x EC 241780) was 41.73 days with a range of 34 to 48 days. In cross 2, plant number 45 (31 days) flowered early followed by plant numbers 211, 240 and 269 with 33 days to flower. Similarly in cross 3, plant number 7 (34 days) flowered early followed by plant number 191 with 36 days to flower. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were low for all the traits studied in all the three crosses, whereas, heritability values were moderate in the cross 2, low in cross 1 and cross 3 and genetic advances as per cent mean values were low in all the three crosses.

4.4.1.2 Plant height (cm)

The overall mean of plant height in cross 1 was 46.88 cm ranged from 15.00 cm to 76.00 cm. Overall mean of plant height in cross 2 was 48.43 cm with a range of 22.00 cm to 76.00 cm. In case of cross 3 mean plant height was 52.50 cm with a range of 18.00 cm to 77.00 cm. Plant number 249 (77 cm) of cross 3 was tallest followed by plant numbers 330 and 223 of cross 2 and cross 1 (76 cm) respectively. Among the three crosses, plant numbers 217, 218 (15.00 cm) followed by plant number 211 (18.00 cm) of cross 1 and plant number 186 (18.00 cm) of cross 3 were dwarf types. Moderate estimates of GCV and PCV values were recorded in all the three crosses for this trait. High heritability associated with high GAM was exhibited in all the three crosses.

4.4.1.3 Number of branches per plant

The plants differed for this trait in all the three crosses. It exhibited a range of 2 to 6 with an overall mean of 5.03, 4.61 and 4.65 in cross 1, 2 and 3, respectively.

Table 19. Genetic variability parameters for seed yield and its components in F_2 population of cross JS 335 x EC 241780

Sl. No.	Traits	Mean	Minimum	Maximum	PCV (%)	GCV (%)	h^2 (bs)	Genetic advance	GAM (%)
1	Days to 50 % flowering	40.42	36.00	44.00	3.88	1.74	20.14	0.65	1.61
2	Plant height (cm)	46.88	15.00	76.00	20.64	18.83	83.22	16.59	35.38
3	Number of branches per plant	5.03	2.00	6.00	21.20	19.60	85.42	1.88	37.31
4	Days to maturity	90.89	80.00	96.00	2.31	1.34	33.92	1.47	1.61
5	Number of pods per plant	61.00	19.00	108.00	32.64	32.16	97.08	39.83	65.29
6	Pod length (cm)	3.80	2.88	4.74	10.21	9.10	79.49	0.64	16.72
7	Pod weight per plant (g)	25.67	7.78	38.25	25.79	25.28	96.10	13.10	51.05
8	Number of seeds per pod	2.84	2.20	3.60	6.32	4.55	51.77	0.19	6.74
9	100 Seed weight (g)	12.52	8.6	15.70	12.98	12.42	91.56	3.07	24.49
10	Harvest index (%)	44.95	30.47	57.17	11.04	9.04	67.02	6.85	15.25
11	Seed yield per plant (g)	17.22	6.55	25.50	24.51	23.70	93.48	8.13	47.20

Table 20. Genetic variability parameters for seed yield and its components in F_2 population of cross EC 241780 x JS 335

Sl. No.	Traits	Mean	Minimum	Maximum	PCV (%)	GCV (%)	h^2 (bs)	Genetic advance	GAM (%)
1	Days to 50 % flowering	38.39	31.00	46.00	6.41	4.35	46.09	2.34	6.09
2	Plant height (cm)	48.43	22.00	76.00	20.23	18.41	82.84	16.72	34.52
3	Number of branches per plant	4.61	2.00	6.00	22.11	18.79	72.22	1.52	32.90
4	Days to maturity	90.06	83.00	96.00	2.13	1.52	50.93	2.02	2.24
5	Number of pods per plant	57.43	15.00	98.00	30.75	30.16	96.21	35.00	60.94
6	Pod length (cm)	3.73	2.30	4.60	8.84	7.47	71.30	0.48	12.99
7	Pod weight per plant (g)	27.12	14.85	46.37	28.97	28.27	95.22	15.41	56.82
8	Number of seeds per pod	2.78	2.20	3.00	4.94	3.35	45.94	0.13	4.68
9	100 Seed weight (g)	11.95	9.10	15.80	12.36	11.80	91.13	2.77	23.20
10	Harvest index (%)	43.10	31.02	65.62	14.40	11.00	58.28	7.45	17.29
11	Seed yield per plant (g)	16.95	9.28	28.98	28.97	28.02	93.57	9.46	55.83

Table 21. Genetic variability parameters for seed yield and its components in F_2 population of cross DSb 21 x EC 241780

Sl. No.	Traits	Mean	Minimum	Maximum	PCV (%)	GCV (%)	h^2 (bs)	Genetic advance	GAM (%)
1	Days to 50 % flowering	41.73	34.00	48.00	5.26	2.44	21.48	0.97	2.33
2	Plant height (cm)	52.50	18.00	77.00	16.30	14.92	83.80	14.77	28.14
3	Number of branches per plant	4.65	2.00	6.00	23.30	19.75	71.84	1.60	34.49
4	Days to maturity	92.71	88.00	102.00	2.69	1.98	54.32	2.79	3.01
5	Number of pods per plant	59.99	10.00	99.00	32.43	31.67	95.38	38.23	63.72
6	Pod length (cm)	3.72	2.80	4.70	11.43	10.23	80.11	0.70	18.87
7	Pod weight per plant (g)	28.61	12.23	47.88	28.51	27.94	96.08	16.14	56.42
8	Number of seeds per pod	2.78	2.20	3.40	5.92	4.43	55.82	0.19	6.81
9	100 Seed weight (g)	12.12	10.00	15.60	10.35	9.69	87.67	2.27	18.69
10	Harvest index (%)	44.03	31.20	61.94	11.34	9.12	64.76	6.66	15.12
11	Seed yield per plant (g)	17.88	7.64	27.93	28.38	27.61	94.70	9.84	55.35

Plant numbers 1, 2,3,6,9 of cross 1, recorded highest number of branches per plant (6). In cross 2 plant numbers 8, 20, 21, 16, 31, 58 and in cross 3 plant numbers 220, 171, 166, 152 117, 44 recorded highest number of branches per plant (6). A moderate estimate of GCV was recorded in all the three crosses. Whereas, PCV values were high in all the three crosses. High heritability associated with high GAM was exhibited for this trait in all the three crosses.

4.4.1.4 Days to maturity

The overall mean number of days for maturity in cross 1 (JS 335 x EC 241780) was 90.89 days with a range of 80 to 96 days, whereas overall mean number of days for maturity in cross 2 (EC 241780 x JS 335) was 90.06 days with range of 83-96 days and overall mean number of days for maturity in cross 3 (DSb 21 x EC 241780) was 92.71 days with range of 88- 102 days. In cross 1, plant number 53 (80 days) was earliest to mature followed by plant number 36 with 85 days to mature. Plant number 443 of cross 2 was earliest to mature (83 days) followed by plant number 338 with 84 days to mature. Similarly in cross 3, plant number 132 was earliest to mature (88 days) followed by plant numbers 7, 17, 49 and 128 with 89 days to mature. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were low in all the three crosses for this trait. Heritability values were moderate in all the three crosses. Genetic advances as per cent mean values were low in all the three crosses.

4.4.1.5 Number of pods per plant

The trait exhibited a wide range from 19 to 108 pods per plant with a mean of 61 pods per plant in cross 1. In cross 2 and cross 3 range values were 15 to 98 and 10 to 99 with a mean value of 57.43 and 59.99 respectively. Among 350 plants evaluated, in cross 1 plant numbers 19 and 285 recorded highest number of pods per plant (108). In cross 3, plant numbers 102, 395, 343, and 231 recorded highest number of pods per plant (99). Similarly in cross 2, plant numbers 430, 406 and 373 recorded highest number of pods per plant (98). High estimates of PCV and GCV values were recorded in all the three crosses for this trait. High heritability associated with high GAM was noticed in all the three crosses. The high heritability value (97.08%) was recorded in cross 1 followed by cross 2 (96.21 %) and cross 3 (95.38 %), respectively.

4.4.1.6 Pod length (cm)

Variability was observed among the plants in all the three crosses for this character. The range was 2.88 to 4.74 cm with a mean value of 3.80 cm in cross 1, whereas in cross 2 and cross 3 the trait varied from 2.30 cm to 4.60 cm and 2.80 cm to 4.70 cm with a mean value of 3.73 cm and 3.72 cm, respectively. Plant numbers 2, 88, 187 and 188 of cross 1 recorded highest pod length (4.74 cm). Similarly in cross 3, the plant numbers 56, 108, 132 recorded highest pod length (4.70 cm). Moderate estimates of PCV and GCV values were recorded in cross 3 and moderate PCV and low GCV values were recorded in cross 1. Whereas, cross 2 recorded low estimates of PCV and GCV values. High heritability estimates associated with moderate GAM was noticed in all the three crosses. The heritability value was maximum (80.11%) in cross 3 followed by cross 1 (79.49 %) and cross 2 (71.30 %).

4.4.1.7 Pod weight per plant (g)

The variability was observed among the plants in all the three crosses for this trait. Mean pod weight per plant was 25.67 g ranged from 7.78 g to 38.25 g in cross 1. Similarly in cross 2 and 3, the mean value was 27.12 g and 28.61 g with a wide range of 14.85 g to 46.35 g and 12.23 g to 47.88 g, respectively. Highest pod weight (47.88 g) was recorded in plant numbers 150, 110, 1 of cross 3, whereas in cross 2 and cross 1, plant numbers 268 and 145 exhibited highest pod weight per plant (46.37 g and 38.25 g, respectively). High estimates of PCV and GCV values were recorded in all the three crosses for this trait. High heritability associated with high GAM was noticed in all the three crosses. The high heritability value (96.10 %) was recorded in cross 1 followed by cross 3 (96.08 %) and cross 2 (95.22 %), respectively.

4.4.1.8 Number of seeds per pod

The plants differed for this trait in all the three crosses. Mean number of seeds per pod was 2.84 ranged from 2.20 to 3.60 in cross 1. Similarly in cross 2 and 3, the mean value was 2.78 and 2.78 with a wide range of 2.20 to 3.00 and 2.20 to 3.40, respectively. Highest number of seeds per pod (3.60) recorded in plant number 285 followed by 120 and 9 (3.40) of cross 1, whereas in cross 3, plant number 72, 200, 19 exhibited highest number of seeds per pod (3.40). Low estimates of PCV and GCV

values were recorded in all the three crosses for this trait. The moderate heritability and low GAM values were recorded in all the three crosses.

4.4.1.9 100 seed weight (g)

The data revealed high variability observed among the plants for this trait in all three crosses. The mean value in cross 1 was 12.52 g with a wide range of 8.60 g to 15.70 g, whereas in cross 2 and 3, the mean value was 11.95 g and 12.12 g with a wide range of 9.10 g to 15.80g and 10.00 g to 15.60 g, respectively. The plant numbers 184, 122 cross 2 recorded highest 100 seed weight (15.80 g), whereas in cross 1 plant numbers 185, 257 (15.70 g) and plant number 407 (15.60 g) in cross 3 recorded highest 100 seed weight. Moderate PCV and GCV values were recorded in cross 1 and 2. In cross 3 moderate estimate of PCV and low estimates of GCV values were recorded. High heritability (91.56 % & 91.13%) associated with high GAM was recorded in cross 1 and cross 2, whereas in cross 3 high heritability (87.67 %) associated with moderate GAM was recorded.

4.4.1.10 Harvest index (%)

The overall mean value of harvest index was 44.95 % ranged from 30.47 % to 57.17 % in cross 1. Similarly in cross 2 and cross 3, the mean value was 43.10 % and 44.03 % with a range of 31.02 % to 65.62 % and 31.20 % and 61.94 %, respectively. In cross 2, plant number 183 (65.62 %) recorded highest harvest index. Similarly in cross 3, plant number 215 (61.94 %) followed by plant numbers 212 (60.58 %) and 103 (59.22 %) recorded highest harvest index. High PCV and GCV values were recorded in the cross 2, whereas moderate estimates of PCV and low estimates of GCV values were recorded in cross 1 and cross 3. High heritability associated with moderate GAM was recorded in cross 1 and 3, whereas cross 2 recorded moderate heritability associated with moderate GAM.

4.4.1.11 Seed yield per plant (g)

The mean seed yield of Cross-3 (resistant x resistant) recorded highest seed yield per plant of 17.88 g among the three crosses studied followed by the Cross-1 (susceptible x resistant (17.22 g) and the cross resistant x susceptible (16.95 g).

Similarly variability for the trait was more in cross resistant x susceptible (9.28 g to 28.98 g) followed by resistant x resistant (7.64 g to 27.93 g) and susceptible x resistant (6.55 g to 25.50 g) cross. Plant number 268 of cross 2 recorded highest seed yield per plant (28.98 g) followed by plant numbers 3, 160, 164, 192, 308 and 373 (28.81 g). In cross 3 plant numbers 92, 368, 414 and 145 recorded highest seed yield per plant (27.93 g). Similarly in cross 1, plant number 145 recorded highest seed yield per plant (25.50 g). High estimates of PCV and GCV values were recorded in all the three crosses for this trait. High heritability associated with high GAM was noticed in all the three crosses. Whereas, cross 3 recorded highest heritability (94.70 %) followed by cross 2 (93.57 %) and cross 1 (93.48 %), respectively.

4.4.2 Genetic variability studies for yield and yield attributing traits in F_3 populations of three crosses

4.4.2.1 Analysis of variance

The segregating lines of three F_3 crosses *viz.*, JS 335 x EC 241780, EC 241780 x JS 335 and DSb 21 x EC 241780 exhibited significant variability for all the traits studied. Analysis of variance for eleven characters of three crosses are presented in Table 22, 23 & 24. The Estimates of mean, range, phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance as per cent mean for yield and its component traits *viz.*, days to flowering, plant height (cm), number of branches per plant, days to maturity, number of pods per plant, pod length (cm), pod weight per plant (g), number of seeds per pod, 100 seed weight (g), harvest index (%) and seed yield per plant (g) in all the three crosses are presented in Table 25, 26 & 27. *Per se* performance of F_3 progeny lines of three crosses are summarized in Appendix-V, VI & VII.

4.4.2.2 Days to 50 per cent flowering

The overall mean number of days to flowering in Cross-1 (JS 335 x EC 241780) was 38.44 days ranged from 35.00 to 42.00 days. Overall mean number of days to flowering in Cross-2 (EC 241780 x JS 335) was 38.85 days with a range of 36.00 to 43.00 days and overall mean number of days for flowering in Cross-3 (DSb 21 x EC 241780) was 41.38 days with a range of 38 to 45 days. In cross 1, line number 30

Table 22. Analysis of variance for different characters in F_3 population of cross JS 335 x EC 241780

Source of Variation	d.f	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100 SW	HI	SYP
Replication	1	0.180	24.780	1.051	0.405	53.700	0.331	15.790	0.020	4.286	0.721	0.861
Genotypes	99	3.214**	122.530**	1.279**	3.286**	200.990**	0.134**	43.680**	0.020*	1.631**	15.480**	17.070**
Error	99	0.755	1.390	0.055	1.162	2.559	0.024	0.676	0.0139	0.214	0.862	0.762
SEm ±		0.622	0.834	0.166	0.762	1.131	0.111	0.581	0.083	0.327	0.656	0.617
C.V. (%)		2.291	2.441	5.480	1.210	3.090	4.000	3.540	4.130	3.700	2.060	5.590
C.D. at 5%		1.747	2.340	0.460	2.130	3.170	0.310	1.630	0.230	0.910	1.840	1.730

*Significant at 5% level of probability, **Significant at 1% level of probability

df- degrees of freedom; CV- Coefficient of variation; CD- Critical difference; SEm ± - Standard error of mean; DFF- Days to 50 % flowering; PH- Plant height (cm); NB- Number of branches per plant; DM- Days to maturity; NPP- Number of pods per plant; PL- Pod length (cm); PWP- Pod weight per plant (g); NSP- Number of seeds per pod; 100 SW- 100 Seed weight (g); HI- Harvest index (%); SYP- Seed yield per plant (g).

Table 23. Analysis of variance for different characters in F_3 population of cross EC 241780 x JS 335

Source of Variation	d.f	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100 SW	HI	SYP
Replication	1	20.480	1.630	0.200	24.500	16.130	0.080	14.670	0.006	0.120	41.480	17.970
Genotypes	99	3.700**	107.820**	1.100**	2.300**	104.560**	0.100**	27.010**	0.024**	2.450**	12.310**	7.850**
Error	99	0.380	10.170	0.019	0.430	0.650	0.010	0.550	0.014	0.047	0.580	0.310
SEm ±		0.440	2.250	0.097	0.468	0.570	0.094	0.527	0.086	0.153	0.541	0.398
C.V. (%)		1.600	7.230	3.300	0.740	1.740	3.680	3.340	4.320	1.730	1.760	4.010
C.D. at 5%		1.230	6.330	0.270	1.310	1.600	0.260	1.470	0.240	0.430	1.510	1.110

*Significant at 5% level of probability, **Significant at 1% level of probability

df- degrees of freedom; CV- Coefficient of variation; CD- Critical difference; SEm ± - Standard error of mean; DFF- Days to 50 % flowering; PH- Plant height (cm); NB- Number of branches per plant; DM- Days to maturity; NPP- Number of pods per plant; PL- Pod length (cm); PWP- Pod weight per plant (g); NSP- Number of seeds per pod; 100 SW- 100 Seed weight (g); HI- Harvest index (%); SYP- Seed yield per plant (g).

Table 24. Analysis of variance for different characters in F_3 population of cross DSb 21 x EC 241780

Source of Variation	d.f	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100 SW	HI	SYP
Replication	1	41.400	11.470	0.390	105.120	63.730	0.210	82.620	0.002	0.290	90.120	22.200
Genotypes	99	1.960**	151.970**	1.420**	3.800**	213.240**	0.030**	60.220**	0.030**	1.660**	16.730**	19.500**
Error	99	0.550	4.780	0.020	0.910	0.870	0.010	0.810	0.010	0.016	0.730	0.260
SEm ±		0.527	1.547	0.114	0.675	0.661	0.073	0.637	0.089	0.091	0.606	0.365
C.V. (%)		1.800	3.930	3.600	1.030	1.820	2.890	3.360	4.460	1.010	1.970	3.290
C.D. at 5%		1.480	4.340	0.320	1.890	1.850	0.200	1.780	0.250	0.250	1.700	1.020

*Significant at 5% level of probability, **Significant at 1% level of probability

df- degrees of freedom; CV- Coefficient of variation; CD- Critical difference; SEm ± - Standard error of mean; DFF- Days to 50 % flowering; PH- Plant height (cm); NB- Number of branches per plant; DM- Days to maturity; NPP- Number of pods per plant; PL- Pod length (cm); PWP- Pod weight per plant (g); NSP- Number of seeds per pod; 100 SW- 100 Seed weight (g); HI- Harvest index (%); SYP- Seed yield per plant (g).

Table 25. Genetic variability parameters for seed yield and its components in F_3 population of cross JS 335 x EC 241780

Sl. No.	Traits	Mean	Minimum	Maximum	PCV (%)	GCV (%)	h^2 (bs)	Genetic advance	GAM (%)
1	Days to 50 % flowering	38.44	35.00	42.00	3.68	2.87	61.10	1.78	4.63
2	Plant height (cm)	48.33	31.20	66.00	16.29	16.10	97.80	15.85	32.80
3	Number of branches per plant	4.27	2.40	5.20	19.08	18.27	91.70	1.54	36.05
4	Days to maturity	88.78	84.00	93.00	1.68	1.16	47.70	1.47	1.65
5	Number of pods per plant	51.63	30.20	86.35	19.53	19.29	97.50	20.26	39.23
6	Pod length (cm)	3.95	3.21	4.65	7.16	5.94	68.70	0.40	10.14
7	Pod weight per plant (g)	23.20	12.35	37.75	20.30	19.99	96.90	9.41	40.54
8	Number of seeds per pod	2.85	2.60	3.20	4.62	2.05	19.70	0.05	1.88
9	100 Seed weight (g)	12.50	10.25	14.56	7.68	6.73	76.70	1.52	12.15
10	Harvest index (%)	44.95	38.21	53.96	6.36	6.01	89.40	5.26	11.71
11	Seed yield per plant (g)	15.61	8.88	25.17	19.14	18.30	91.40	5.63	36.05

Table 26. Genetic variability parameters for seed yield and its components in F_3 population of cross EC 241780 x JS 335

Sl. No.	Traits	Mean	Minimum	Maximum	PCV (%)	GCV (%)	h^2 (bs)	Genetic advance	GAM (%)
1	Days to 50 % flowering	38.85	36.00	43.00	3.68	3.31	81.00	2.39	6.14
2	Plant height (cm)	44.12	17.52	66.52	17.41	15.84	82.70	13.09	29.67
3	Number of branches per plant	4.17	2.36	4.56	17.95	17.65	96.60	1.49	35.73
4	Days to maturity	89.17	86.00	92.00	1.31	1.08	68.00	1.64	1.84
5	Number of pods per plant	46.22	31.20	71.25	15.69	15.59	98.80	14.76	31.92
6	Pod length (cm)	3.63	3.12	4.95	6.74	5.64	70.10	0.35	9.73
7	Pod weight per plant (g)	22.27	14.09	36.21	16.67	16.33	96.00	7.34	32.95
8	Number of seeds per pod	2.82	2.60	3.30	4.96	2.42	23.80	0.07	2.44
9	100 Seed weight (g)	12.51	10.36	14.80	8.95	8.78	96.20	2.22	17.74
10	Harvest index (%)	43.41	37.36	52.36	5.85	5.58	90.90	4.75	10.95
11	Seed yield per plant (g)	14.03	9.30	22.25	14.41	13.84	92.20	3.84	27.37

Table 27. Genetic variability parameters for seed yield and its components in F_3 population of cross DSb 21 x EC 241780

Sl. No.	Traits	Mean	Minimum	Maximum	PCV (%)	GCV (%)	h^2 (bs)	Genetic advance	GAM (%)
1	Days to 50 % flowering	41.38	38.00	45.00	2.71	2.03	55.80	1.29	3.12
2	Plant height (cm)	55.63	32.25	74.80	15.91	15.42	93.90	17.12	30.78
3	Number of branches per plant	4.49	2.40	5.40	18.98	18.63	96.40	1.69	37.68
4	Days to maturity	92.26	89.00	97.00	1.66	1.30	61.30	1.94	2.10
5	Number of pods per plant	51.10	29.60	77.00	20.25	19.91	97.70	20.49	40.32
6	Pod length (cm)	3.59	3.16	4.20	4.38	3.28	56.30	0.18	5.07
7	Pod weight per plant (g)	26.77	16.25	41.25	20.64	20.36	97.30	11.08	41.38
8	Number of seeds per plant	2.83	2.60	3.60	5.88	3.82	42.30	0.15	5.12
9	100 Seed weight (g)	12.67	10.25	14.32	7.24	6.89	96.00	1.55	13.72
10	Harvest index (%)	43.53	36.45	51.20	6.79	6.49	91.60	5.57	12.80
11	Seed yield per plant (g)	15.68	10.46	23.21	20.05	19.78	97.30	6.30	40.18

(35.00 days) flowered early followed by line numbers 3, 7, 37, 48, 114, 204, 233, 269, 295, 301 and 320 with 36.50 days to flower. In cross 2, line numbers 3, 8, 10, 32 (36.00 days) flowered early followed by line numbers 25 and 60 with 37 days to flower. Similarly in cross 3, line numbers 73 and 100 (38 days) flowered early followed by line number 75, 82, 88 and 94 with 40 days to flower. The GCV and PCV were low for this trait in all the three crosses. Heritability values were high in Cross-1 and 2, whereas low in Cross-3 and genetic advances as per cent mean values were low in all the three crosses.

4.4.2.3 Plant height (cm)

The overall mean of plant height in Cross-1 was 48.33 cm ranged from 31.20 cm to 66.00 cm. Overall mean of plant height in Cross-2 was 44.12 cm with a range of 17.52 cm to 66.52 cm. In Cross-3 overall mean of plant height was 55.63 cm with a range of 32.25 cm to 74.80 cm. In cross 3, Line- 83 (74.80 cm) was tallest followed by line numbers 61 (72.91 cm), 4 (70.93 cm). In Cross-1, line- 19 (66.00 cm) was tallest followed by line number 140 (62.56 cm). Similarly in cross 2, line number 53 (66.52 cm) was tallest followed by line number 92 (60.38 cm). The line number 21 (17.52 cm) of Cross-2 followed by line number 30 (31.20 cm) of Cross-1 was dwarf types. The moderate estimates of GCV and PCV values were recorded in all the three for this trait. High heritability associated with high GAM was exhibited for the trait.

4.4.2.4 Number of branches per plant

The lines differed for this trait in all the three crosses. It exhibited a range of 2.40 to 5.20 with an overall mean of 4.27 in Cross-1. Overall mean for number of branches per plant in Cross-2 and Cross-3 was 4.17, 4.49 with a range of 2.36 to 4.56 and 2.40 to 5.40, respectively. Line numbers 50 (5.40), 29 (5.38) of Cross-3 and line numbers 62 (5.20), 24 (5.15) of Cross-1 were recorded highest number of branches per plant. Whereas, in Cross-2 line number 46 followed by 92 were recorded highest number of branches per plant (4.56). Moderate estimates of PCV and GCV values were recorded in all the three crosses for this trait. High heritability associated with high GAM was recorded in all the three crosses.

4.4.2.5 Days to maturity

The overall mean number of days for maturity in Cross-1 (JS 335 x EC 241780) was 88.78 days with a range of 84.00 to 93.00 days, whereas overall mean number of days for maturity in Cross-2 (EC 241780 x JS 335) was 89.17 days with a range of 86.00 to 92.00 days and overall mean number of days for maturity in Cross-3 (DSb 21 x EC 241780) was 92.26 days with a range of 89.00 to 97.00 days. In Cross-1, line numbers 4, 28 and 47 (84 days) was earliest to mature followed by line number 5, 32, 38, 74, 77 and 88 with 85 days to mature. In Cross-2, line numbers 3, 16 (86 days) was earliest to mature followed by line number 83, 2, 5, 14, 18 and 19 with 87 days to mature. Similarly in Cross-3, line numbers 66, 68 was earliest to mature (89 days) followed by line numbers 17, 42, 46, 48 and 73 with 90 days to mature. The GCV and PCV were low for this trait in all the three crosses. High heritability associated with low GAM values were recorded in Cross-2 and Cross-3, whereas moderate heritability associated with low GAM values were recorded in Cross-1.

4.4.2.6 Number of pods per plant

The average number of pods per plant was 51.63 in Cross-1 with a wide range of 30.20 to 86.35. Whereas, in Cross-2 and Cross-3 range values were 31.20 to 71.25 and 29.60 to 77.00 with a mean values of 46.22 and 51.10. The line number 8 (86.35) of Cross-1 followed by line numbers 21 (79.82), 13 (78.35) recorded highest number of pods per plant, whereas line numbers 42 (77.00), 46 (74.76) of Cross-3 and line numbers 97 (71.25), 15 (64.82) of Cross-2 recorded highest number of pods per plant. Moderate estimates of PCV and GCV values were recorded in Cross-1 and Cross-2, whereas high PCV and moderate GCV values recorded in Cross-3. Heritability followed by genetic advances as per cent mean values were high in all the three crosses.

4.4.2.7 Pod length (cm)

Variability was observed among the lines in all the three crosses for this character. The range was 3.21 cm to 4.65 cm with a mean value of 3.95 cm in Cross-1, whereas in Cross-2 and Cross-3 values ranged from 3.12 cm to 4.95 cm and 3.16 cm to 4.20 cm with a mean value of 3.63 cm and 3.59 cm, respectively. In Cross-2, Line-71 (4.95 cm) recorded highest pod length followed by line- 42 (4.12 cm), whereas in

Cross-1 and Cross-3, line numbers 59, 75 and 17, 19 recorded highest pod length (4.65 cm, 4.40 cm & 4.20 cm, 3.89 cm, respectively). Low estimates of PCV and GCV values were recorded in all the three crosses for the trait studied. High heritability estimates was noticed in Cross-1 and Cross-2, whereas Cross-3 recorded moderate estimates of heritability. Genetic advances as per cent mean values were moderate in Cross-1 and low in Cross-2 and 3.

4.4.2.8 Pod weight per plant (g)

The variability was observed among the lines in all the crosses for this trait. Mean pod weight per plant was 23.20 g ranging from 12.35 g to 37.75 g in Cross-1, whereas in Cross-2 and 3, the mean values were 22.27 g and 26.77 g with a wide range of 14.09 g to 36.21 g and 16.25 g to 41.25 g, respectively. In Cross-3, line number 3 (41.25 g) recorded highest pod weight per plant followed by line numbers 29 (39.12 g), 42 (38.23 g) and 46 (37.49 g). In Cross-1, line number 8 (37.75 g) recorded highest pod weight per plant followed by line numbers 13 (36.70 g) and 21 (35.63 g). Similarly, in Cross-2, line numbers 97 (36.21 g) and 15 (32.83 g) exhibited highest pod weight per plant. High estimates of PCV and GCV values were recorded in Cross-3. Moderate estimates of PCV and GCV values were recorded in Cross-2, high PCV and moderate GCV values were recorded in Cross-1. High heritability associated with high GAM was recorded in all the three crosses. The high heritability value (97.30 %) was recorded in Cross-2 followed by Cross-1 (96.90 %) and Cross-3 (96.00 %), respectively.

4.4.2.9 Number of seeds per pod

The plants differed for this trait in all the three crosses. Mean number of seeds per pod was 2.85 ranged from 2.60 to 3.20 in Cross-1. Similarly, in Cross-2 and 3, range values were 2.60 to 3.30 and 2.60 to 3.60 with mean values of 2.82 and 2.83. The line-81 (3.20) of Cross-1 followed by line numbers 67, 59, 9 (3.10) recorded highest number of seeds per pod. Line-19 (3.60) of Cross-3 and line-81 (3.30) of Cross-2 recorded highest number of seeds per pod. Low estimates of PCV and GCV values were recorded in all the three crosses. Moderate estimates of heritability were observed in Cross-3. Low estimates of heritability were recorded in Cross-1 and Cross-2. Genetic advances as per cent mean values were low in all the three crosses.

4.4.2.10 100 seed weight (g)

The data revealed high variability observed among the lines for all the three crosses for this trait. The mean value in Cross-1 was 12.50 g ranged from 10.25 g to 14.56 g. In Cross-2 and Cross-3, the mean values were 12.51 g and 12.67 g with a wide range of 10.36 g to 14.80 g and 10.25 g to 14.32 g, respectively. The line-11 of Cross-2 recorded highest 100 seed weight (14.80 g). Line numbers 98 (14.56 g), 16 (14.13 g) of Cross-1 and line numbers 3 (14.32 g), 31 (14.26 g) of Cross-3 were recorded highest 100 seed weight. Low estimates of PCV and GCV values were recorded in all the three crosses. High heritability associated with moderate GAM was recorded in all the three crosses.

4.4.2.11 Harvest index (%)

The overall mean value for harvest index was 44.95 (%) with a range of 38.21 (%) to 53.96 (%) in Cross-1. In Cross-2 and Cross-3, the mean values were 43.41 (%) and 43.53 (%) with a range of 37.36 (%) to 52.36 (%) and 36.45 (%) to 51.20 (%), respectively. The highest harvest index noticed in line number 3 (53.96 %) followed by line-21 (52.00 %) of Cross-1, whereas in Cross-2 line numbers 97 (52.36 %), 59 (48.63 %) and in Cross-3 line-29 (51.20 %) followed by line-9 (48.98 %) recorded highest harvest index. Low estimates of PCV and GCV values were recorded in all the three crosses. High heritability associated with moderate GAM was recorded in all the three crosses.

4.4.2.12 Seed yield per plant (g)

The mean seed yield of Cross-3 (resistant x resistant) recorded highest seed yield per plant of 15.68 g among the three crosses studied followed by the Cross-1 (susceptible x resistant) 15.61 g and the Cross-2 (resistant x susceptible) 14.03 g. Similarly variability for the trait was more in Cross-1 (8.88 g to 25.17 g) followed by Cross-3 (10.46 g to 23.21 g) and Cross-2 (9.30 g to 22.25 g). Line number 8 of Cross-1 recorded highest seed yield per plant (25.17 g) followed by line numbers 13 (24.39 g) and 21 (23.92 g). In Cross-2, line numbers 97 (22.25 g) and 25 (19.08 g) recorded highest seed yield per plant. Similarly in Cross-3, line numbers 29 (23.21 g), 42 (22.61 g) recorded highest seed yield per plant. Moderate estimates of PCV and

GCV values were recorded in Cross-1 and Cross-2. High PCV and moderate GCV values were recorded in Cross-3. High heritability associated with high GAM was recorded in all the three crosses.

4.4.3 Character association

The phenotypic correlation co-efficients were computed to know the nature and magnitude of relationship existing between yield and its component characters as well as the association among the component characters themselves.

Phenotypic correlations for different characters in F_3 population of three crosses.

4.4.3.1 Association studies in the cross JS 335 x EC 241780

a) Association between yield components and seed yield per plant

Seed yield per plant was found to have highly significant positive association with plant height (0.262), number of branches per plant (0.492), number of pods per plant (0.919), pod weight per plant (0.977), number of seeds per pod (0.208) and harvest index (0.930). But exhibited non-significant positive association with days to 50 % flowering (0.064), days to maturity (0.098), pod length (0.098) and 100 seed weight (0.136) (Table 28).

b) Association among yield components

1. Days to 50 % flowering

The trait exhibited highly significant positive association with plant height (0.193) and days to maturity (0.631). Non-significant negative association was observed with 100 seed weight (-0.019).

2. Plant height (cm)

The trait exhibited highly significant positive association with days to 50 % flowering (0.193), days to maturity (0.252), number of pods per plant (0.289), pod weight per plant (0.250), 100 seed weight (0.378), harvest index (0.273) and seed yield per plant (0.262). The trait recorded non-significant negative association with number of branches per plant (-0.039) and number of seeds per pod (-0.054).

Table 28. Phenotypic correlation coefficients for seed yield and its components in F_3 population of cross JS 335 x EC 241780

Traits	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100 SW	HI	SYP
DFF	**1.000**	0.193 **	0.084	0.631 **	0.041	0.077	0.062	0.105	-0.019	0.058	0.064
PH		**1.000**	-0.039	0.252 **	0.289 **	0.055	0.250 **	-0.054	0.378 **	0.273 **	0.262**
NB			**1.000**	0.009	0.470 **	0.244 **	0.484 **	0.072	0.026	0.442 **	0.492**
DM				**1.000**	0.066	0.082	0.061	0.072	0.071	0.059	0.098
NPP					**1.000**	0.074	0.937 **	0.011	0.128	0.871 **	0.919**
PL						**1.000**	0.090	0.107	0.099	0.094	0.098
PWP							**1.000**	0.212 **	0.111	0.929 **	0.977**
NSP								**1.000**	-0.047	0.217 **	0.208**
100 SW									**1.000**	0.129	0.136
HI										**1.000**	0.930**
SYP											**1.000**

*Significant at 5% level of probability, **Significant at 1% level of probability

DFF- Days to 50 % flowering; PH- Plant height (cm); NB- Number of branches per plant; DM- Days to maturity; NPP- Number of pods per plant; PL- Pod length (cm); PWP- Pod weight per plant (g); NSP- Number of seeds per pod; 100 SW- 100 Seed weight (g); HI- Harvest index (%); SYP- Seed yield per plant (g).

3. Number of branches per plant

It showed highly significant positive correlation with number of pods per plant (0.470), pod length (0.244), pod weight per plant (0.484), harvest index (0.442) and seed yield per plant (0.492). Non-significant positive association was observed with days to maturity (0.009), number of seeds per pod (0.072) and 100 seed weight (0.026).

4. Days to maturity

Highly significant positive association of the trait was observed with the traits days to 50 % flowering (0.631) and plant height (0.252) and exhibited non-significant positive association with other traits like number of pods per plant (0.066), pod weight per plant (0.061), 100 seed weight (0.071), harvest index (0.059) and seed yield per plant (0.098).

5. Number of pods per plant

Association of plant height (0.289), number branches per plant (0.470), pod weight per plant (0.937), harvest index (0.871) and seed yield per plant (0.919) was highly significant and positive. Exhibited non-significant positive association with days to 50 % flowering (0.041), days to maturity (0.066) and 100 seed weight (0.128).

6. Pod length (cm)

Association of number of branches per plant (0.244) was highly significant and positive. The trait recorded non-significant positive association with days to 50 % flowering (0.077), days to maturity (0.082), number of pods per plant (0.074), pod weight per plant (0.090), 100 seed weight (0.099), harvest index (0.094) and seed yield per plant (0.098).

7. Pod weight per plant (g)

This trait exhibited highly significant positive association with plant height (0.250), number of branches per plant (0.484), number of pods per plant (0.937), number of seeds per pod (0.212), harvest index (0.929) and seed yield per plant (0.977). While non-significant positive association was observed with days to 50 % flowering (0.062), days to maturity (0.061) and 100 seed weight (0.111).

8. Number of seeds per pod

It was found to have highly significant positive association with pod weight per plant (0.212), harvest index (0.217) and seed yield per plant (0.208). The trait recorded non-significant negative association with plant height (-0.054) and 100 seed weight (-0.147).

9. 100 seed weight (g)

The trait recorded highly significant positive association with plant height (0.0.378). Non significant negative association was observed with days to 50 % flowering (-0.019) and number of seeds per pod (-0.047).

10. Harvest index (%)

It was found to have highly significant positive association with plant height (0.273), number of branches per plant (0.442), number of pods per plant (0.871), pod weight per plant (0.929), number of seeds per pod (0.217) and seed yield per plant (0.930).

4.3.2 Association studies in cross EC 241780 x JS 335

a) Association between yield components and seed yield per plant

Seed yield per plant recorded highly significant positive association with plant height (0.175), number of branches per plant (0.505), number of pods per plant (0.911), pod weight per plant (0.973), number of seeds per pod (0.311) and harvest index (0.832). But exhibited non-significant positive association with days to 50 % flowering (0.051), days to maturity (0.028), however non-significant negative association with pod length (-0.005) and 100 seed weight (-0.106). (Table 29)

b) Association among yield components

1. Days to 50 per cent flowering

The trait exhibited highly significant positive association with plant height (0.211), number of branches per plant (0.158), days to maturity (0.701), 100 seed weight

Table 29. Phenotypic correlation coefficients for seed yield and its components in F_3 population of cross EC 241780 x JS 335

Traits	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100 SW	HI	SYP
DFF	**1.000**	0.211 **	0.158 *	0.701 **	0.104	-0.109	0.070	-0.131	0.161 *	0.141 *	0.051
PH		**1.000**	0.479 **	0.234 **	0.246 **	-0.091	0.187 **	-0.101	0.115	0.197 **	0.175*
NB			**1.000**	0.194 **	0.541 **	0.028	0.504 **	0.031	-0.004	0.522 **	0.505**
DM				**1.000**	0.029	-0.015	0.036	0.023	0.171 *	0.185 **	0.028
NPP					**1.000**	-0.031	0.925 **	0.062	-0.157 *	0.801 **	0.911**
PL						**1.000**	-0.016	0.108	-0.083	0.079	-0.005
PWP							**1.000**	0.305 **	-0.135	0.828 **	0.973**
NSP								**1.000**	-0.010	0.249 **	0.311**
100 SW									**1.000**	-0.077	-0.106
HI										**1.000**	0.832**
SYP											**1.000**

*Significant at 5% level of probability, **Significant at 1% level of probability

DFF- Days to 50 % flowering; PH- Plant height (cm); NB- Number of branches per plant; DM- Days to maturity; NPP- Number of pods per plant; PL- Pod length (cm); PWP- Pod weight per plant (g); NSP- Number of seeds per pod; 100 SW- 100 Seed weight (g); HI- Harvest index (%); SYP- Seed yield per plant (g).

(0.161) and harvest index (0.141). But exhibited non-significant negative association with pod length (-0.109) and number of seeds per pod (-0.131).

2. Plant height (cm)

It was found to have highly significant positive association with days to 50 % flowering (0.211), number of branches per plant (0.479), days to maturity (0.234), number of pods per plant (0.246), pod weight per plant (0.187), harvest index (0.197) and seed yield per plant (0.175). The trait recorded non-significant negative association with pod length (-0.091) and number of seeds per pod (-0.101).

3. Number of branches per plant

It showed highly significant positive correlation with days to 50 % flowering (0.158), plant height (0.479),days to maturity (0.194), number of pods per plant (0.541), pod weight per plant (0.504), harvest index (0.522) and seed yield per plant (0.505). Non-significant positive association was observed with pod length (0.028) and number of seeds per pod (0.031).

4. Days to maturity

Highly significant positive association of the trait was observed with the traits namely days to 50 % flowering (0.701), plant height (0.234), number of branches per plant (0.194), 100 seed weight (0.071) and harvest index (0.185) and exhibited non-significant positive association with other traits like number of pods per plant (0.029), pod weight per plant (0.036) and seed yield per plant (0.028).

5. Number of pods per plant

Association of plant height (0.246), number branches per plant (0.541), pod weight per plant (0.925), harvest index (0.801) and seed yield per plant (0.911) was highly significant and positive. The trait recorded non-significant positive association with days to 50 % flowering (0.104), days to maturity (0.029) and number of seeds per pod (0.062).

6. Pod length (cm)

The trait exhibited non-significant positive association with number of branches per plant (0.028), number of seeds per pod (0.108) and harvest index (0.079). Non-significant negative association was observed with days to 50 % flowering (-0.109), plant height (-0.091), days to maturity (-0.015), number of pod per plant (-0.031), pod weight per plant (-0.016), 100 seed weight (-0.083) and seed yield per plant (-0.005).

7. Pod weight per plant (g)

Association of plant height (0.187), number of branches per plant (0.504), number of pods per plant (0.925), number of seeds per pod (0.305), harvest index (0.828) and seed yield per plant (0.973) was noticed highly significant and positive. But exhibited non-significant negative association with pod length (-0.016) and 100 seed weight (-0.135).

8. Number of seeds per pod

The trait exhibited highly significant positive association with pod weight per plant (0.305), harvest index (0.249) and seed yield per plant (0.311). Non-significant negative association was observed with days to 50 % flowering (-0.131), plant height (-0.101) and 100 seed weight (-0.010).

9. 100 seed weight (g)

Highly significant positive association was exhibited with days to 50 % flowering (0.161) and days to maturity (0.171). Significant negative association was observed with number of pods per plant (-0.157). Non significant negative association was observed with number of branches per plant (-0.004), pod length (-0.083), pod weight per plant (-0.135), number of seeds per pod (-0.010), harvest index (-0.077) and seed yield per plant (-0.106).

10. Harvest index (%)

Highly significant positive association was exhibited with days to 50 % flowering (0.141), plant height (0.197), number of branches per plant (0.522), days to maturity (0.185), number of pod per plant (0.801), pod weight per plant (0.828), number

of seeds per pod (0.249) and seed yield per plant (0.832). The trait recorded non-significant positive association with pod length (0.079) and non-significant negative association with 100 seed weight (-0.077).

4.3.3 Association studies in cross DSb 21 x EC 241780

a) Association of yield components with seed yield per plant

Seed yield per plant recorded highly significant positive association with plant height (0.200), number of branches per plant (0.707), number of pods per plant (0.931), pod length (0.237), pod weight per plant (0.987), number of seeds per pod (0.285) and harvest index (0.929). But exhibited non-significant positive association with days to 50 % flowering (0.049), days to maturity (0.097) and 100 seed weight (0.106) (Table 30).

b) Association among yield components

1. Days to 50 per cent flowering

The trait recorded non-significant negative association with number of branches per plant (-0.026) and 100 seed weight (-0.005). But exhibited highly significant positive association with days to maturity (0.603).

2. Plant height (cm)

The trait exhibited highly significant positive association with days to maturity (0.309), number of pods per plant (0.189), pod weight per plant (0.193), harvest index (0.204) and seed yield per plant (0.200). Non-significant positive association with days to 50 % flowering (0.081), number of branches per plant (0.067), number of seeds per pod (0.052) and 100 seed weight (0.040) and non-significant negative association was observed with pod length (-0.038).

3. Number of branches per plant

It showed highly significant positive correlation with number of pods per plant (0.744), pod weight per plant (0.699), harvest index (0.689) and seed yield per plant (0.707). Non-significant positive association was observed with plant height (0.067), pod length (0.068), number of seeds per pod (0.020) and 100 seed weight (0.057).

Table 30. Phenotypic correlation coefficients for seed yield and its components in F_3 population of cross DSb 21 x EC 241780

Traits	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100 SW	HI	SYP
DFF	**1.000**	0.081	-0.026	0.603 **	0.031	0.110	0.068	0.051	-0.005	0.066	0.049
PH		**1.000**	0.067	0.309 **	0.189 **	-0.038	0.193 **	0.052	0.040	0.204 **	0.200**
NB			**1.000**	-0.005	0.744 **	0.068	0.699 *	0.020	0.057	0.689 **	0.707**
DM				**1.000**	0.071	0.110	0.104	0.080	0.084	0.111	0.097
NPP					**1.000**	0.207 **	0.928 **	0.037	0.084	0.868 **	0.931**
PL						**1.000**	0.246**	0.107	-0.035	0.318 **	0.237**
PWP							**1.000**	0.286 **	0.121	0.922 **	0.987**
NSP								**1.000**	0.131	0.259 **	0.285**
100 SW									**1.000**	0.060	0.106
HI										**1.000**	0.929**
SYP											**1.000**

*Significant at 5% level of probability, **Significant at 1% level of probability

DFF- Days to 50 % flowering; PH- Plant height (cm); NB- Number of branches per plant; DM- Days to maturity; NPP- Number of pods per plant; PL- Pod length (cm); PWP- Pod weight per plant (g); NSP- Number of seeds per pod; 100 SW- 100 Seed weight (g); HI- Harvest index (%); SYP- Seed yield per plant (g).

4. Days to maturity

Highly significant positive association of this trait was observed with the traits namely days to 50 % flowering (0.603) and plant height (0.309) and exhibited non-significant positive association with other traits number of pods per plant (0.071), pod length (0.110), pod weight per plant (0.104), number of seeds per pod (0.080), 100 seed weight (0.084), harvest index (0.111) and seed yield per plant (0.097). Non-significant negative association was observed with number of branches per plant (-0.005).

5. Number of pods per plant

Association of plant height (0.189), number branches per plant (0.744), pod length (0.207), pod weight per plant (0.928), harvest index (0.868) and seed yield per plant (0.931) was highly significant and positive with above trait. But exhibited non-significant positive association with days to 50 % flowering (0.031), days to maturity (0.071), number of seeds per pod (0.037) and 100 seed weight (0.084).

6. Pod length (cm)

Association of number of pods per plant (0.207), pod weight per plant (0.246), harvest index (0.318) and seed yield per plant (0.237) was highly significant and positive with this trait. The trait recorded non-significant positive association with days to 50 % flowering (0.110), number of branches per plant (0.068), days to maturity (0.110) and number of seeds per pod (0.107). But exhibited non-significant negative association with plant height (-0.038) and 100 seed weight (-0.035).

7. Pod weight per plant (g)

The trait exhibited highly significant positive association with plant height (0.193), number of branches per plant (0.699), number of pods per plant (0.928), pod length (0.246), number of seeds per pod (0.286), harvest index (0.922) and seed yield per plant (0.987). The trait recorded non-significant positive association with days to 50 % flowering (0.068), days to maturity (0.104) and 100 seed weight (0.121).

8. Number of seeds per pod

The trait recorded highly significant positive association with pod weight per plant (0.286), harvest index (0.259) and seed yield per plant (0.285). But exhibited non-significant positive association with days to 50 % flowering (0.051), plant height (0.052), number of branches per plant (0.020), days to maturity (0.080), number of pods per plant (0.037), pod length (0.107) and 100 seed weight (0.131).

9. 100 seed weight (g)

The trait recorded non-significant positive association with plant height (0.040) number of branches (0.057), days to maturity (0.084), number of pods per plant (0.084), pod weight per plant (0.121), number of seeds per pod (0.131), harvest index (0.060) and seed yield per plant (0.106). Non-significant negative association was observed with days to 50 % flowering (-0.005) and pod length (-0.035).

10. Harvest index (%)

The trait exhibited highly significant positive association with plant height (0.204), number of branches per plant (0.689), number of pods per plant (0.868), pod length (0.318), pod weight per plant (0.922), number of seeds per pod (0.259) and seed yield per plant (0.929).

4.5 Validation of molecular markers linked to rust resistance

4.5.1 Screening of parental genotypes using SSR primers for rust resistance

The two parents JS 335 (susceptible) and EC 241780 (resistant) were screened with the help of already reported 25 SSR primers mentioned in the (Table 9) for polymorphism. Out of the 25 SSR primers used for screening the parental genotypes in this study, three primers Satt 215, Satt 275 and Satt 361 showed polymorphism between the parents (Plate 9). The screening of parental genotypes with the polymorphic marker Satt 215, Satt 275 and Satt 361 has been shown in Plate 10 & 11.

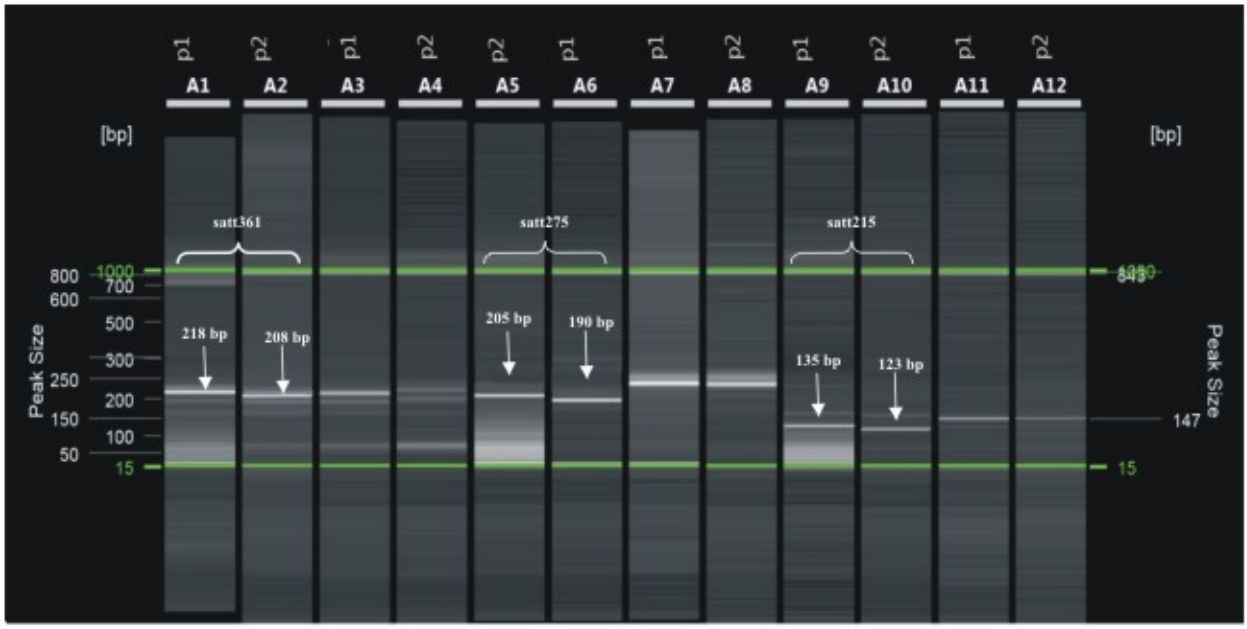


Plate 9: Parental polymorphism found with SSR primers Satt 361, Satt 275 and Satt 215 of the cross JS 335 × EC 241780 for rust resistance

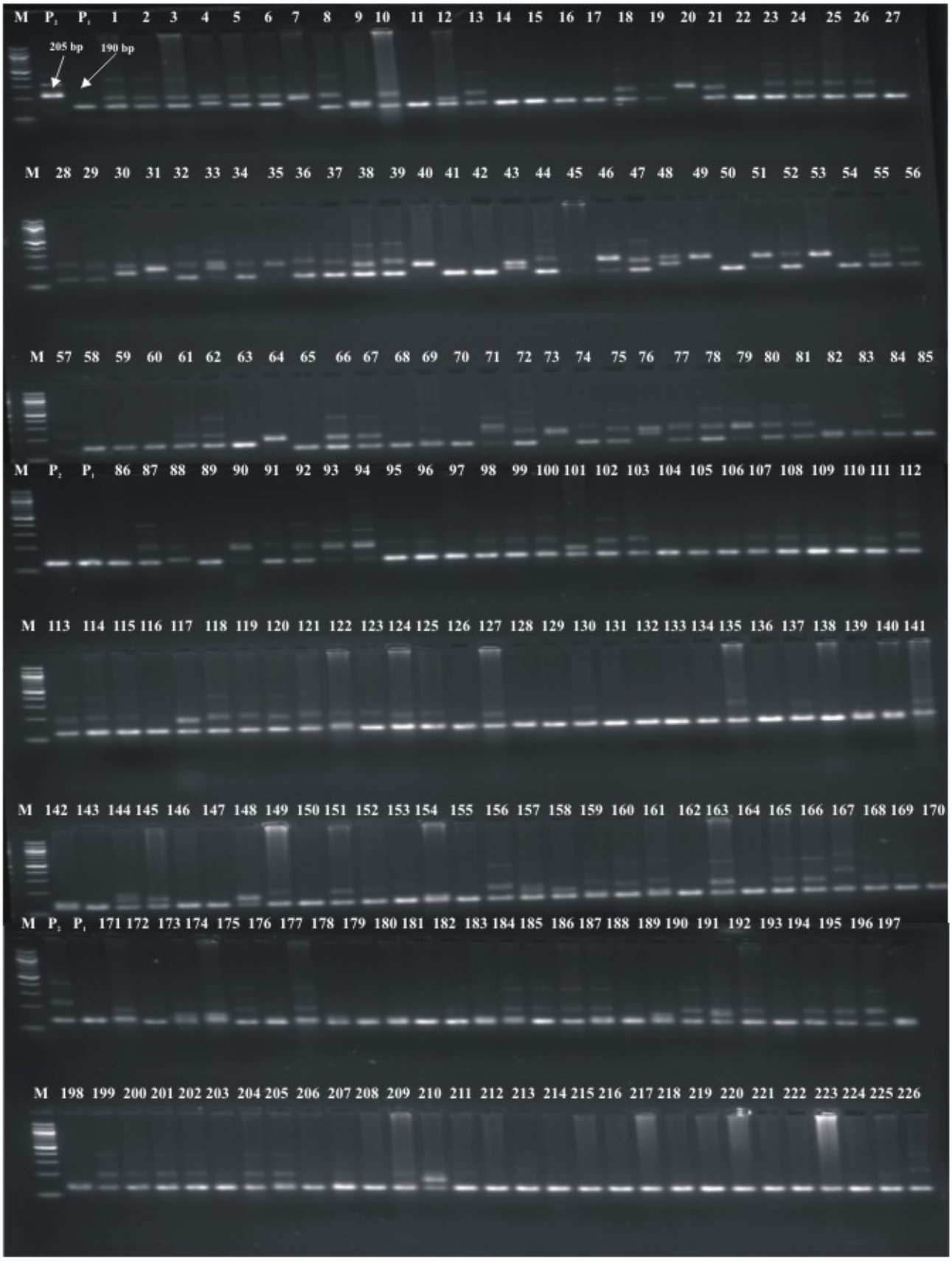


Plate 10: Validation of SSR marker Satt 275 linked to rust resistance in F_2 cross JS 335 × EC 241780

Plate 10 contd.....

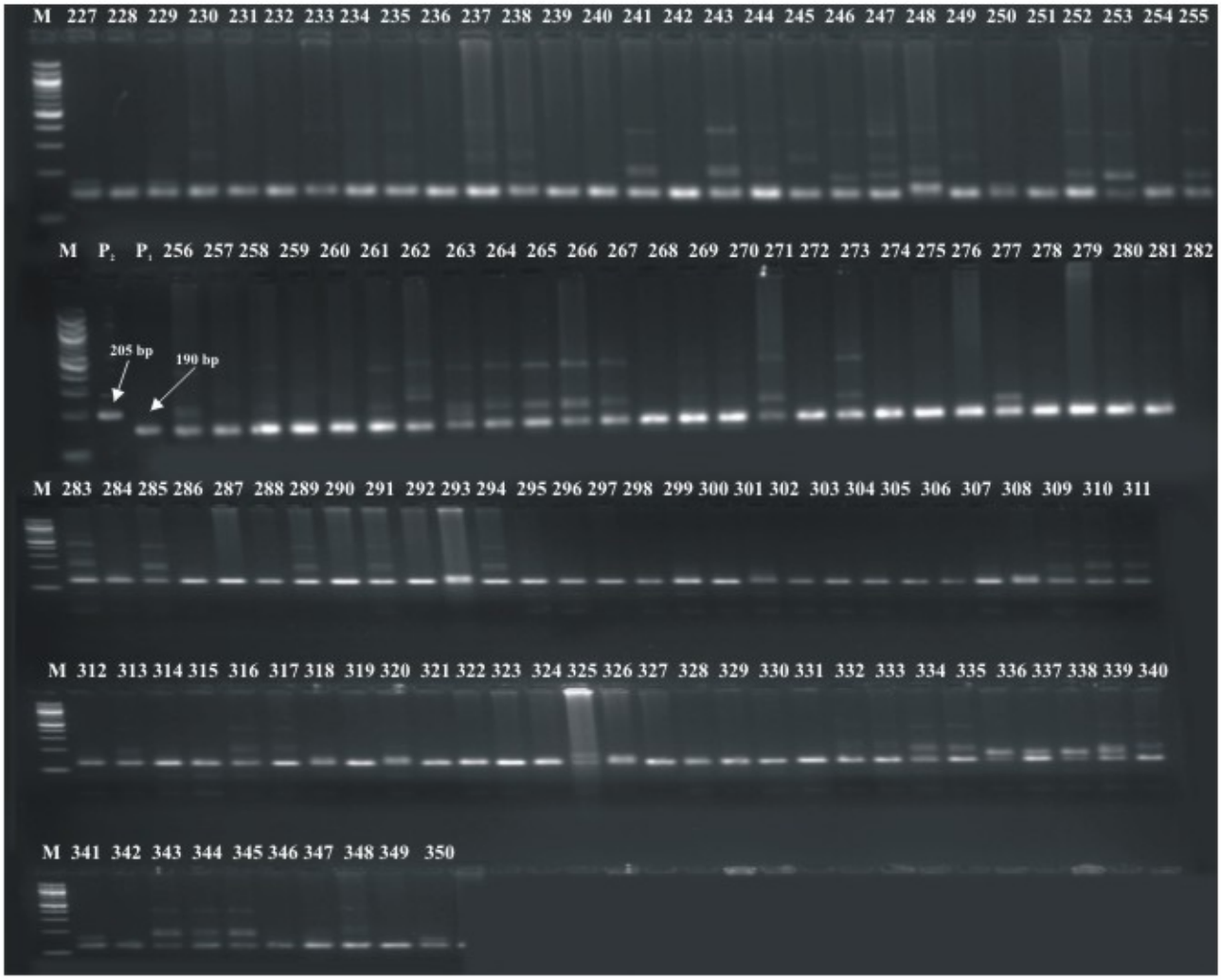


Plate 10: Validation of SSR marker Satt 275 linked to rust resistance in F_2 cross JS 335 × EC 241780

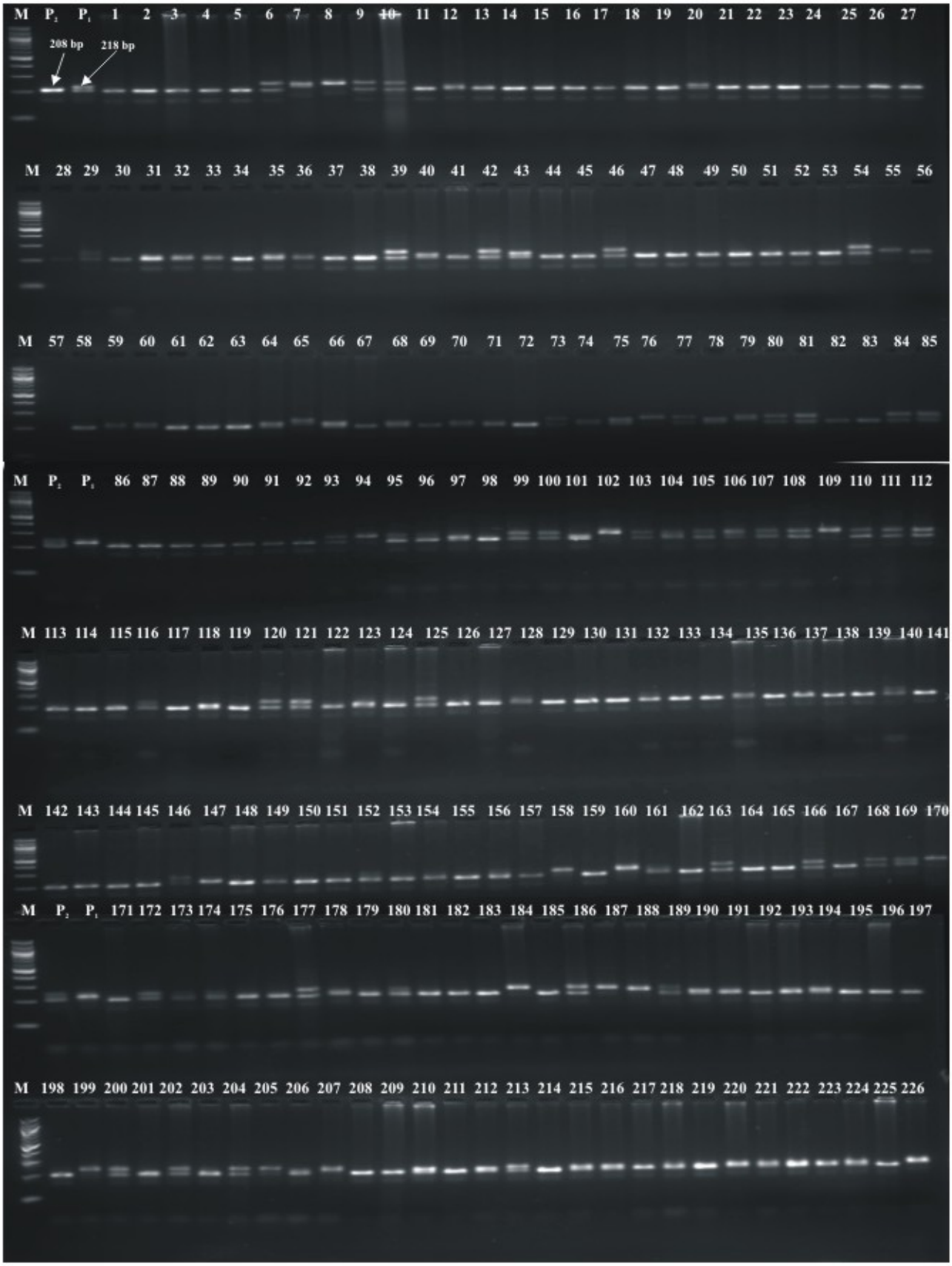


Plate 11: Validation of SSR marker Satt 361 linked to rust resistance in F_2 cross JS 335 × EC 241780

Plate 11 contd.....

Plate 11: Validation of SSR marker Satt 361 linked to rust resistance in F_2 cross JS 335 × EC 241780

4.5.2 Screening of F_2 population of cross JS 335 × EC 241780 using SSR primers for rust resistance

The SSR primers polymorphic between the parents of the F_2 population of cross JS 335 × EC 241780 were used for genotyping of the individual F_2. The F_2 genotypes were scored for the disease severity for single marker analysis. The genotypes showing the banding pattern as of the susceptible parent (JS 335) were scored as 'A', while the genotypes showing the banding pattern as of the resistant parent (EC 241780) were scored as 'B' and genotypes showing banding pattern as of the heterozygous plants were scored as 'H'.

4.5.3 Single marker analysis (SMA)

Single marker analysis was performed using the genotypic and phenotypic data of all the 350 F_2 individuals to determine the strength of association between resistance-linked marker and disease resistance by calculating *F* statistic and simple linear regression coefficient using WinQTL Cartographer version 2.5. In total, three polymorphic markers were considered for SMA namely Satt 215, Satt 275 and Satt 361.

All the three SSR markers *viz*., Satt 215, Satt 275 and Satt 361 showed significant association with rust resistance. Highest R^2 was observed for Satt 361 (8.62 %) followed by Satt 275 (3.61 %) and Satt 215 (3.19 %). The results of single marker analysis for the polymorphic markers are summarized in Table 31. The results interpret that the marker is linked to the disease severity at 5 per cent level of significance.

Table 31. Association of molecular markers with rust resistance by single marker analysis

Chromosome	Marker	F (1,n-2)	pr (F)	R^2 (%)
16	satt215	11.535	0.00076 **	3.19
3	satt275	13.108	0.00033 **	3.61
16	satt361	33.01	0.00002 **	8.62

5. DISCUSSION

Soybean [*Glycine max* (L.) Merrill] owes world wide reputation by virtue of its high quality protein (40%) and low cholesterol edible oil (20%). In view of potentiality and wide range of agricultural, industrial and medicinal values, soybean is rightly described as “nature’s unique gift” to mankind.

The observations for the last 25 years with respect to production trend revealed that increase in soybean yield per hectare has made only modest advances in the past 25 years in the United States and other countries. However, the improvement is meager in India (hovering around one t/ha). This is ascribed to narrow genetic base of soybean cultivars resulting in susceptibility to biotic (diseases and pests) and abiotic (unfavourable soils and erratic climatic conditions) stresses resulting in yield stagnation.

Most of the soybean varieties which are cultivated in India are susceptible to many diseases and pests. Among the diseases of soybean, rust caused by *Phakospora pachyrhizi* Syd. is most destructive in nature. It occurs in all parts of the world wherever soybean is cultivated resulting in yield loss of 10 to 100 per cent (Sarbhay and Pal, 1997). So screening for the disease resistance forms one of the important objective in plant breeding programme.

Breeding for higher yield is the main objective in any crop improvement programme. Since yield is polygenicaly controlled and highly influenced by environment selection based on yield alone is not effective. Therefore improvement in yield can be brought about by effecting indirect selection through yield attributes whose heritability is high and show a strong association with yield.

Variability studies provide information about the amount of variation present in the population. Correlation studies provide information about the relative contribution of the various component traits to yield and aid in identification of superior high yielding segregants from the populations.

In the absence of ample genetic variability in the existing genetic material, creation and assessment of genetic variability is a basic step in crop improvement programme. Yield being a complex character influenced by a number of yield

contributing characters controlled by polygenes and also influenced by environment. Hence, it becomes, necessary to partition the observed variability into heritable and non heritable components measured as Genotypic and Phenotypic Coefficients of Variations (PCV and GCV), heritability and genetic advance to account for created variability to be used in breeding programmes.

However, inheritance of quantitative characters is often influenced by variation in other characters which may be due to pleiotropy or genetic linkage (Harland, 1939). Hence, knowledge of association between yield and its components through estimation of genotypic and phenotypic correlations helps a great deal for effective selection.

In this context, the current study was conducted with different experiments. About 144 exotic germplasm lines including highly susceptible check JS 335 and resistant checks *viz.*, DSb 21, EC 241780 and EC 241778 were evaluated during *kharif* 2015 at Dharwad for identification of new sources for resistance to rust and genetic diversity. Based on the resistance reaction, 22 lines which exhibited resistant/moderately resistant reaction were selected. These lines were further evaluated to confirm their resistance reaction under natural epiphytotic condition at two hotspots for rust *viz.*, Dharwad and Ugarkhurd during *kharif* 2016.

Three genotypes *viz.*, DSb 21, JS 335 and EC 241780 obtained from AICRP on Soybean, UAS, Dharwad were utilized in crossing programme/hybridization during *summer* 2015 to study the inheritance pattern for rust resistance and variability. Subsequently, F_2 and F_3 populations were raised during *kharif* 2016 & 2017 respectively. In addition to this, validation of molecular markers linked to rust resistance in F_2 population of cross JS 335 x EC 241780 was carried out using already reported 25 SSR markers.

The results drawn with respect to objectives mentioned are discussed under the following sub headings.

5.1 Evaluation of exotic germplasm lines for identification of new sources for resistance to rust

5.2 Studies on genetic diversity in exotic germplasm lines

5.3 Genetic studies on inheritance pattern of rust

5.4 Genetic variability parameters for seed yield and its components using F_2 population of three different crosses

5.5. Genetic variability parameters for seed yield and its components considering F_3 population of three different crosses

5.6 Association studies among the F_3 Populations of three different crosses

5.7 Validation of molecular markers linked to rust resistance

5.8 Identification of superior segregants in F_3 families of three crosses

5.9 Future line of work

5.1 Evaluation of exotic germplasm lines for identification of new sources of resistance to rust

The important constraints for cultivation of soybean in India are outbreak of biotic stresses *viz.*, diseases and insect pests. Among the biotic stresses diseases play a major role in yield reduction. Again among the diseases, rust caused by *Phakospora pachyrhizi* Syd. is one of the devastating foliar disease of soybean. Soybean rust was first reported from Japan during 1902 and later from different soybean growing areas of the world. In India rust was first noticed at Pantnagar in September 1970, subsequently in Kalyani (West Bengal) and in low hills of Uttar Pradesh. It can cause losses from 10 to 100 per cent (Sarbhay and Pal, 1997) depending upon locality, season and cultivar. The disease appeared suddenly in epiphytotic form in past years (*kharif* 1994 and 1995) and caused substantial yield losses particularly in northern parts of Karnataka, Maharashtra and Madhya Pradesh (Anahosur *et al.*, 1995). In addition to this, most of the popular cultivars *viz.*, JS 335, JS 93-05 were highly susceptible to rust. So screening for the disease resistance forms is one of the important objective in plant breeding programme.

Breeding soybean for rust resistance requires appropriate disease screening methodologies. Most commonly, screening of genotypes has been conducted under field

conditions. However, field has limitations because it depends on natural occurrence of suitable environmental conditions and pathogen inoculums. In addition, field screening often can be conducted only once in a year.

Though triazole fungicides have been found effective for the control of rust, continuous use may pose the problem of development of resistance and environmental hazards. The management of the disease through host plant resistance has been found the best choice in all the crop improvement programmes. Utilization of resistant cultivars in farming system is the most simple, effective and economical method in the management of disease. Besides this, these resistant genotypes conserve natural resources and reduce the cost, time and energy when compared to the other methods of disease management. So identification of resistant sources and involving them in resistant breeding programme forms as one of the important criteria.

In the present study, screening of 144 exotic germplasm lines along with resistant and susceptible checks for rust disease caused by *Phakopsora pachyrhizi* under field condition was conducted at MARS, Dharwad during *kharif* 2015. Based on the resistance reaction, 22 lines which were selected. These lines were further evaluated to confirm their resistance reaction under natural epiphytotic condition at two hotspots for rust *viz.,* Ugarkhurd and Dharwad during *kharif* 2016. The exotic lines were screened for lesion colour appearance as a reaction to rust and lesions count per cm^2 on mid-vein and both the sides of mid-vein of the infected leaves were recorded. The colour of the lesion was either tan or reddish brown. The tan colour appearance of lesions signifies the susceptible reaction to rust while the reddish brown colour appearance of lesions indicated the resistant reaction to the disease.

Among 144 exotic germplasm lines including resistant and susceptible checks, only one line EC 242104 (8.89%) and resistant checks *viz.,* DSb 21 (8.89%), EC241780 (8.89%) and EC 241778 (8.89%) recorded disease grade 1 and was found to be highly resistant. Nine lines *viz.,* EC 391336 (20.00%), EC 385243 (24.44%), EC 333934 (24.44%), EC 308334 (24.44%), EC 287754 (24.44), EC 250578 (20.00%), EC 100031 (24.44%), EC 14426 (28.89%) and EC 15966 (20.00%) recorded disease grade 3 and were found to be moderately resistant and they also showed same reaction screened under natural epiphytotic condition at two hotspots for rust *viz.,* Ugarkhurd and

Dharwad during *kharif* 2016.These results are in conformity with findings of Hartman *et al.* (2005) and Verma *et al.* (2004).

In general, the lines with a low initial per cent disease index invariably resulted with a low terminal disease index. PDI status at different intervals observed in lines as EC 242104 recorded 6.67 % at 65 DAS and 8.89 % at 85 DAS, DSb 21 recorded 6.67 % at 65 DAS and 8.89 % at 85 DAS, EC 241780 recorded 6.67 % at 65 DAS and 8.89 % at 85 DAS, EC 241778 recorded 4.44 % at 65 DAS and 8.89 % at 85 DAS, EC 391336 recorded 8.89 % at 65 DAS and 20.0 % at 85 DAS and EC 15966 recorded 8.89 % at 65 DAS and 20.0 % at 85 DAS.

The apparent rate of infection was calculated by using the formula given by Van der plank (1963). This has been widely used in identification of genotypes with low rate of disease development. The range of 'r' values among 144 germplasm lines ranged from 0.009 to 0.169 indicating the importance of infection rate in spreading the rust disease. The low average 'r' values indicate less rate of infection compared to higher values.

Based on apparent rate of infection, germplasm lines EC 3251 (0.009) followed by EC 391346, EC 14426 (0.011), EC 685252 (0.017), EC 242104 (0.031), DSb 21 (0.031) and EC 241780 (0.031) recorded lower 'r' values indicating the rate of infection in these lines is very slow. Whereas germplasm lines EC 33917 (0.169) followed by EC 37937 (0.151), EC 95291 (0.124) and EC 95815 (0.114) recorded higher 'r' values indicating fast spread of disease in these lines.

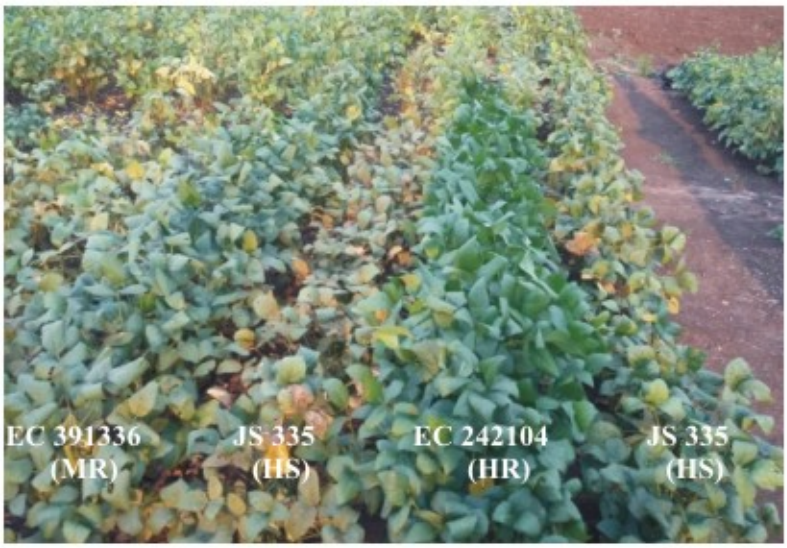
The check JS 335 (0.074) and germplasm lines *viz.*, EC 685250 (0.054), EC 39219 (0.054) and EC 242105 (0.054) having low apparent rate of infection actually recorded high disease infection at their early growth stage however infection rate was low. The germplasm lines EC 385243 (0.037), EC 391336 (0.047) and EC 308334 (0.060) having high apparent rate of infection registered very low level of disease infection at their early crop growth stage. However, once the infection started spread of the disease become faster. These results indicate the low apparent rate of infection which did not indicate the resistant level of the genotypes.

The calculated ‘r’ values varied and at times they did not remain consistent for given genotype and also did not show a particular trend in general. These observations are in agreement with that of Wilcoxson *et al.* (1975) and Nargund (1989) who have pointed out that ‘r’ values are not useful criteria for selecting the genotype; ‘r’ values indicate the progressive development of diseases and help in categorising as slow or fast rusters. However, it can be used in studying the disease development in different genetic background.

The Area Under Disease Progress Curve (AUDPC) in lines revealed a wide variation among the different lines at different intervals. Among, the lines tested, the highest average AUDPC value was observed in the lines *viz*., JS 335 (944.44) followed by EC 685250 (855.55), EC 94625, EC 685255, EC 685252 (833.33). While, the least average AUDPC value was recorded in lines EC 241778 (66.67) followed by DSb 21, EC 242104, EC 241780 (83.33) and EC 15966, EC 391336 (150.00).

The exotic lines were screened in the field conditions for lesions count per cm^2 on mid-vein and both the sides of mid-vein of the infected leaves after 65 days of sowing. The line EC 242104 (5.0) followed by DSb 21 (6.87), EC 241780 (7.56), EC 241778 (8.73), EC 287754 (8.65) and EC 391336 (9.21) recorded least number of lesions while JS 335 (42.80) followed by EC 389178 (41.53), EC 917258 (38.20), EC 457406 (37.85) and EC 389400 (37.27) recorded highest lesion count. The lines EC 242104, DSb 21, EC 241780 and EC 241778 showed resistant reaction in the form of reddish brown reaction while dark tan colour appearance of lesions was shown by JS 335 signifying high susceptibility to rust. The above results are in conformity with the findings of Miles *et al.*, (2003) and Sharadha (2015) as the reddish brown (RB) lesion type is considered to be a resistant lesion type when compared to a fully susceptible tan lesion. The data for lesions count per cm^2 on mid-vein and both the sides of mid-vein of infected leaves is presented in Table 11.

In the present study EC 242104 which exhibited highly resistant reaction during different seasons, can be utilized in future breeding programmes for development of resistant genotypes (Plate 12). In addition to rust resistance, the line EC 242104 as early in nature (matures in 90-95 days) when compared to earlier reported rust resistant lines *viz*., EC 241780 and EC 241778 which matures in 100-110 days. The growth habit of



a) *Kharif* 2015- Dharwad

b) *Kharif* 2016 - Dharwad

c) *Kharif* 2016 - Ugarkhurd

HR - Highly resistant MR - Moderately resistant HS - Highly susceptible

Plate 12: Reaction of exotic germplasm line EC 242104 during different seasons

this line is determinate as compared to those two lines which are semi determinate. These two lines are susceptible to bacterial pustule and soybean mosaic virus but EC 242104 not susceptible. These results are in conformity with the earlier reports of Patil *et al.,* (2004), Hartman *et al.,* (2005), Parameshwar (2006), Twizeyimana *et al.,* (2007) and Shivakumar *et al.* (2011). The new source of resistance can be used in combination with already identified resistance genes. Till date, six independently inherited dominant genes *viz., Rpp1, Rpp2, Rpp3* (Hartwig and Bromified, 1983), *Rpp4* (Hartwig, 1986), *Rpp5* (Garcia *et al,* 2008) and *Rpp6* (Shuxian *et al.,* 2012) have been reported as resistant genes against soybean rust. These six genes alone or in various combinations confer resistance in various degrees. *i.e.,* from moderate resistant to highly resistant reaction. Even though single gene can offer good level of resistance. However, such resistance is broken down by the pathogen through evolution of new races or non compatible host pathogen reaction. Therefore, pyramiding of different resistant genes is required to overcome historical failure of monogenic resistance.

5.2 Studies on genetic diversity in exotic germplasm lines

For any crop improvement programme, analysis of genetic diversity is the first and foremost step. Information on genetic diversity among genotypes has several important applications for crop improvement. This information can be useful to classify germplasm for identification of cultivars, assist in selection of parents for hybridization and reduce number of genotypes needed to ensure sampling of a broad range of genetic variability. Genetically diversed parent is a pre-requisite to improve the chances of selecting better segregants for various characters. When such parents are utilized in hybridization programme, they are likely to produce high heterotic effect and wide spectrum of variability (Barh *et al.,* 2014). The challenge is to select which genotype to be used in breeding programme from available germplasm, those carrying favourable rare alleles absent in elite germplasm.

The choice of parents is of paramount importance in any breeding programme. It is rather a difficult task for a plant breeder. Selection of parents on the basis of *per se* performance is good but there is a possibility of related lines being chosen resulting in limited or no advances under selection and therefore, there is a need for emphasis on a wide genetic base by the utilization of world collection on genetic criterion. Selection of

the parents on the basis of geographical diversity is another way of choosing parents and this has led to success in some cases but this need to be supplemented with genetic diversity. The measures based on genetic criteria qualifying diversity have become important in classifying material for the use by the breeders. Therefore, further study is needed to know genetic variation within available gene pool through divergence study and to make strategies for incorporating useful diversity or to facilitate the introgression of genes of interest into commercial varieties.

The precise information about the degree and direction of relationship between different genotypes is very much essential for an effective breeding programme. Genetic diversity between lines indicates the difference in gene frequencies. The multivariate analysis has been demonstrated to classify biological populations and to identify factors influencing their genetic divergence (Rao, 1960). The hybrids involving the parents with more diversity among them are expected to exhibit higher amount of heterotic expression and finally created broad spectrum of variability in segregating generations (Naik *et al.* 2006).

The k-*means* cluster analysis provides a measure of magnitude of divergence between the groups under comparison. It considers the means of the characters under study, and their consequence (Queen, 1967). The technique has been applied in several crops to select genotypes for further breeding programmes (Shabbir *et al.*, 2016).

The k-*means* cluster analysis for yield related traits in 144 germplasm lines including checks in the present study were grouped into eight clusters. The formation of distinct solitary clusters may be due to the fact that exotic germplasm lines belong to different regions which may be responsible for this type of genetic diversity. It could be seen that clusters vary much with respect to mean expression of various characters which resulted in distinct clusters. There were observed significant differences between the mean of clusters for all the traits. Maximum number of genotypes were grouped in the cluster V (39 genotypes) followed by cluster I (35 genotypes), cluster VII and cluster VIII (20 genotypes each). The genotypes from cluster II are highly divergent from the genotypes of clusters VI and of medium divergence from the genotypes of cluster VII and cluster VIII. The results of cluster mean analysis clearly indicated that the cluster VI exhibited maximum cluster mean values for the traits *viz.*, seed yield per

plant, number of pods per plant, number of branches per plant and cluster II exhibited minimum cluster mean values for the traits *viz*., days to 50 % flowering, number of branches per plant, days to maturity and number of pods per plant. Therefore, it can be concluded that the genotypes of the cluster VI exhibited maximum potentiality for yield related traits while, the genotypes from the cluster II had low potentiality for yield related traits. These findings are similar to the reports of Maharaddi (1996), Ramgiry *et al.* (1999), Ganeshmoorthy and Sheshadri (2002), Aravind (2006) and Parameshwar (2006).

The inter-cluster distance was maximum between clusters II and VI (D=55.64) followed by clusters VII and VIII (D=53.07). The minimum inter-cluster distance was observed between clusters I and III (D=7.07). The genotypes belonging to the clusters separated by high statistical distance could be used in hybridization programme for obtaining a wide spectrum of variation among the segregants. It is true that larger the divergence between genotypes, higher would be the heterosis when hybrid programme is planned to develop yield superior varieties (Bekele *et al.*, 2012). In this context, genotypes from cluster I (EC 242104) cluster V (JS 335), cluster VI (DSb 21) and cluster IV (EC 241780 and EC 241778) can be used as parents in hybridization programme.

The characters contributing maximum to the divergence should be given greater emphasis in deciding the clusters for the purpose of further selection and choice of parents for hybridization. Contribution of each character towards genetic divergence was estimated based on number of times it appeared in the first rank. The results depicted that the most important traits which contributed maximum to total genetic divergence are; number of pods per plant (41.82), seed yield per plant (18.24), number of branches per plant (17.47) and days to 50 % flowering (13.42). They accounted for about 90% of total genetic divergence in the material. Looking to these results number of pods per plant should be considered as an important trait when selecting parents for hybridization programme. Major contribution toward total genetic diversity by oil content (Bekele *et al.*, 2012), 100-seed weight (Pawar *et al.*, 2013), days to maturity (Sharma *et al.*, 2012) and protein content (Sharma *et al.*, 2005) have also been reported earlier in soybean.

Based on the cluster means, cluster VI for seed yield per plant, number of pods per plant, number of branches per plant, days to 50 % flowering and cluster II for early maturity are considered to be superior. Thus, crosses among the genotype(s) of these clusters would exhibit high heterosis and is also likely to produce new recombinants with desired traits in soybean.

The clustering pattern could be utilized in selection of parents for crossing and deciding the best cross combinations which may generate the highest possible variability for various traits. The genotypes with high values of any cluster can be used either for direct adoption or for hybridization followed by selection. In the present study, seed yield per plant, an important contributing trait to genetic diversity, is larger in the genotype DSb 21 of cluster VI. This genotype was also high in number of pods per plant. The genotype in the cluster I (EC 242104) differed from other clusters in respect of disease resistance. The genotypes grouped in the cluster IV (EC 241780 and EC 241778) were found to be rust resistant. These lines can be used in hybridization programme for breeding rust resistance. The genotype of cluster V (JS 335) was early maturing and this genotype can be utilized in development of early maturing varieties. Therefore, genotypes of these clusters may be utilized in future breeding programme for creating wide spectrum of variability for different yield contributing characters. This will facilitate to isolate superior genotypes with higher seed yield.

5.3 Genetic studies on inheritance pattern of soybean rust

Soybean rust is the most devastating disease causing significant yield losses. In India, soybean rust occurs in the highly productive zone of the soybean cultivation, *i.e.*, southern parts of Maharashtra and northern parts of Karnataka. Though effective control measures are available through fungicides for soybean rust but they are not economical and environmental friendly in addition to causing health hazards.

Information regarding the genetic mechanisms controlling the inheritance of rust resistance provides useful genetic information to the plant breeders but it is applicable to specific germplasm and range of tested environments. Therefore, further genetic studies may be useful to identify sources of resistance that are applicable to different environments.

Information on the host differential response and genetics of resistance has lead to identification of six different rust resistant genes (*Rpp*: Resistance to *P. pachyrhizi*), *viz.*, *Rpp1* to *Rpp6*, against specific isolates of *P. pachyrhizi* (Hartman *et al.* 2005; Bonde *et al.* 2006; Miles *et al.* 2011). *Rpp1* confers an immune response for which there are no visible symptoms in the plant (Miles *et al.* 2006). Resistance responses mediated by the *Rpp*2 to *Rpp*5 loci results in the formation of visible reddish-brown lesions which limit fungal growth and sporulation, there by suggesting of a hypersensitive-like response (Bonde *et al.* 2006; Garcia *et al.* 2008). The susceptible interaction with rust results in tan colored lesions and fully sporulating uredenia (Bromfield and Hartwig 1980; Bromfield, 1984; Miles *et al.* 2006). Gowtham *et al.*, (2018) reported for the first time *Rpp5* as gene confirming resistance in Indian genotypes against soybean rust.

Even though more than 110 varieties have been released in India, none of them are resistant to soybean rust. In Karnataka too, the popular cultivars JS 335, JS 93-05 and DSb 1 are highly susceptible to rust resulting in 30-80 % yield loss depending on the severity of disease. Among them, JS 335 is the most popular variety in this region and more than 80 per cent of the area is covered by this variety (monoculture) which is highly susceptible to rust. Although, several fungicides have been found to be effective in managing the disease, but not economical and also cause environmental pollution and health hazards. Under these circumstances, the best strategy would be breeding for rust resistant cultivars or incorporating resistance into popular susceptible cultivars. Keeping this in view, a long term breeding programme has been initiated at the University of Agricultural Sciences, Dharwad to develop rust resistant variety using conventional plant breeding approaches.

After rigorous screening of more than 2000 germplasm lines, two lines *viz.*, EC 241778 and EC 241780 were identified as rust resistant at hot spots *viz.*, Dharwad and Ugarkhurd (Belagavi District) during 2002-05. Immediately these two lines were utilized in hybridization programme with agronomically superior but rust susceptible varieties *viz.*, JS 335, JS 93-05 and DSb 1. This lead to the development and release of first ever highly genetic basis of rust resistant and high yielding variety DSb 21 (Basavaraja *et al.*, 2012).

Three soybean genotypes including two resistant lines *viz.*, DSb 21 and EC 241780 and one susceptible JS 335 were used for inheritance studies (Table 8). Three crosses were made in susceptible x resistant, resistant x susceptible and resistant x resistant combinations to elucidate the information regarding inheritance of rust resistance. Results of field study are presented in the Table 18.

Among three crosses, two crosses *viz.*, susceptible x resistant (JS 335 x EC 241780) and resistant x susceptible (EC 241780 x JS 335) combination, susceptible and resistant parents were used as females respectively. The F_1's of two crosses were resistant to rust in the field condition. Out of 350 F_2 plants in the cross susceptible x resistant (JS 335 x EC 241780), 272 plants were resistant and 78 plants were susceptible under field conditions and out of 456 F_2 plants in the cross resistant x susceptible (EC 241780 x JS 335), 352 plants were resistant and 104 plants were susceptible under field conditions. F_2 populations of two crosses segregated into 3:1 ratio (3 resistant: 1 susceptible) indicating single gene responsible for resistance to soybean rust with resistance being dominant over susceptibility. The above results are in conformity with the findings of Bromfield and Hartwig (1980) determined the inheritance of soybean rust resistance in two F_2 populations with PI 230970 and PI 230971 as the resistant parents. Their analysis of these F_2's showed that their rust resistance was dominant and qualitatively (simply) inherited. Other studies have reported partial to complete dominance action in the inheritance of rust resistance (Garcia *et al.*, 2008; Ray *et al.*, 2009) and few genetic studies have been conducted with the goal of understanding the genetics of soybean rust resistance, some studies have shown that rust resistance is qualitatively inherited and largely controlled by single dominant genes. Previously reported a six single dominant genes for specific resistance to *P. pachyrhizi* have been identified in different cultivars as *Rpp1*, *Rpp2*, *Rpp3*, *Rpp4* (Hartwig, 1986), *Rpp5* (Garcia *et al.*, 2008), (Gowtham *et al.*, 2018) and *Rpp6* (Shuxian *et al.*, 2012).

The F_3 families segregated in 1:2:1 ratio (1 resistant: 2 segregating: 1 susceptibility) confirming the results observed in F_2 generation of two crosses *viz.*, susceptible x resistant (JS 335 x EC 241780) and resistant x susceptible (EC 241780 x JS 335). These genotypes have not been previously studied in detail for rust resistance. The resistant genotypes reported in the present study can be used as confirmed sources for resistance and utilized in the breeding programmes.

In resistant x resistant combination (DSb 21 x EC 241780) all the F_1 plants were resistant. Out of 432 F_2 plants, 414 were resistant and 18 plants were susceptible under field conditions. The data showed 15:1 ratio with good fit. The results indicated the presence of different resistant genes in these genotypes. The F_3 family segregation also confirmed F_2 results, as the 100 families segregated into 7:8:1 ratio (7 resistant: 8 segregating: 1 susceptible). Similar results were reported by Rahangdale and Raut (2004), where in the resistance may be because of the different races used in the study and incidentally those two genes might confer complete resistance in presence of each other. These observations indicate a clue that high resistance against many races can be induced by pyramiding resistant genes in one genotype. Similar results were opined by Cheng and Chan, (1968) reported single dominant genes identified in the Indian accession PI462312 (Ankur). The name *Rpp*3 gene was assigned in the genotype PI462312 (Ankur) (Bromfield and Melching, 1982, Hartwig and Bromfield 1983).

Previous studies showed that soybean genotypes PI 200492, PI 230970, and PI 462312 each carried a single dominant gene conferring resistance to a specific soybean rust isolate. Line PI 459025 was identified as resistant to Taiwan 80-2 as well as Taiwan 72-1 and India 73-1. Line PI 459025 was crossed with each of the three previously identified sources of resistance. The F_1 plants, F_2 populations and selected F_3 lines were inoculated with each of the three rust isolates to determine their reaction. For each plant evaluated, a leaflet of a single trifoliolate leaf was inoculated with a different rust isolate. The results showed that PI 459025 carried a single dominant gene for resistance to all three rust isolates and that this gene was at a different loci from the three previously identified genes conferring resistance to specific rust isolates. The genotype assigned for rust resistance of PI 459025 is *Rpp1 Rpp1, Rpp2 Rpp2, Rpp3Rpp3,* and *Rpp4 Rpp4*. (Hartwig, 1986).

5.4 Genetic variability parameters for seed yield and its components using F_2 population of three different crosses

The estimation of genotypic and phenotypic components of variance is of primary importance to get an idea about the extent of heritable and non heritable variations.

Success of the breeding programme is largely depends on the extent of genetic variability present in the population for evolving desired genotype. A detailed study of extent of variability in different characters associated with the yield and the knowledge of their heritability in relation to the contribution toward the yield is the prime requisite for an efficient plant breeding programme.

The coefficient of variation indicated only the extent of variability existing among various characters but does not give any information about the heritable portion of it. Therefore it is essential to know about the heritability which permits the greater effectiveness of selection by separating out the environmental influence from the total variability. This indicates the accuracy with which a genotype can be identified by its phenotypic performance. In the present study broad sense heritability which includes both additive and non-additive gene effects (Hanson *et al.*, 1956) was estimated.

The estimates of heritability alone fail to indicate the amount of progress expected from selection (Johnson *et al.*, 1955a). Therefore, the heritability estimates appear to be more meaningful when accompanied by estimates of genetic advance. The results obtained on above parameters are discussed below.

The phenotypic coefficient of variability was higher than genotypic coefficient of variability in all the three crosses for the all characters studied (Table 19, 20 & 21). Segregating populations of all the three F_2 crosses exhibited wide range of variation for the traits *viz.*, days to 50 % flowering, plant height, days to maturity, number of pods per plant, pod weight per plant, 100 seed weight, harvest index and seed yield per plant except for number of branches per plant, pod length and number of seeds per pod in all the three crosses. This variation indicated the scope for selection of these traits for future breeding work. From the present study it is obvious that seed yield per plant, number of pods per plant and pod weight per plant in all the three crosses *viz.*, JS 335 x EC 241780, EC 241780 x JS 335 and DSb 21 x EC 241780 had highest PCV and GCV indicating the existence of substantial variability for these characters (Fig. 1 & 2). It also indicated greater scope for selection to improve upon these characters. Similar findings were reported by Sharma (1980), Chauhan and Singh (1982), Jagtap and Mehetre (1994) and Yadav (2006). But moderate estimates of PCV and GCV estimates were reported by Bangar *et al* (2003). In the present study moderate values of PCV and

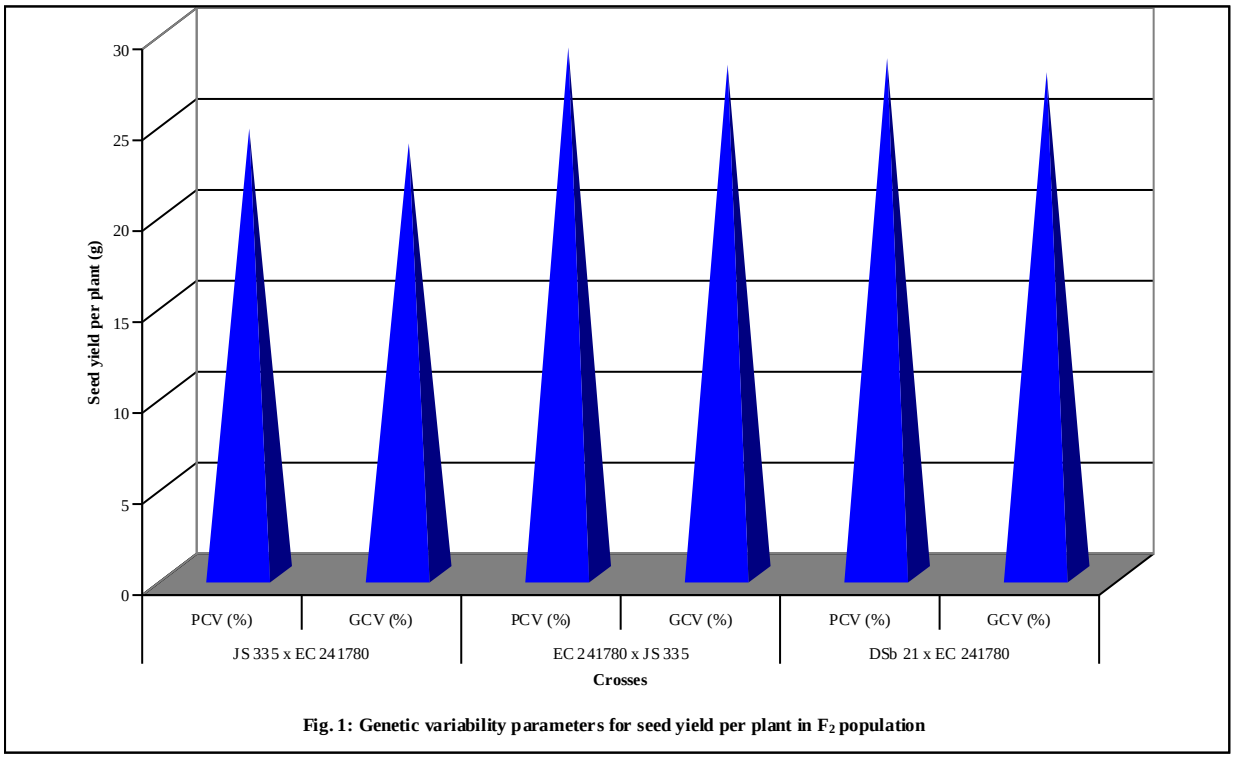


Fig. 1: Genetic variability parameters for seed yield per plant in F_2 population

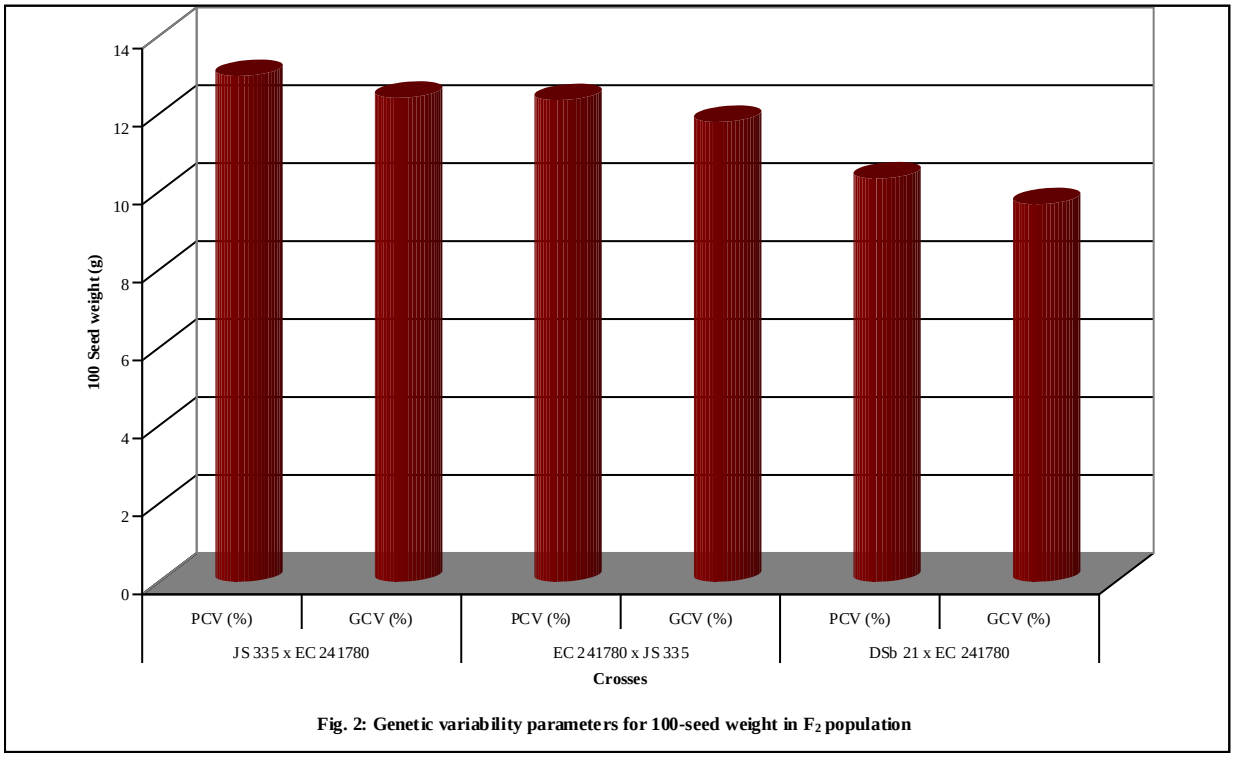


Fig. 2: Genetic variability parameters for 100-seed weight in F_2 population

GCV were noticed for plant height and number of branches per plant in all the three crosses. Moderate values of PCV and GCV were noticed for 100 seed weight in the crosses JS 335 x EC 241780 and EC 241780 x JS 335 and for harvest index in cross EC 241780 x JS 335, for pod length in DSb 21 x EC 241780. Low PCV and GCV values were noticed for days to 50 % flowering, days to maturity and number of seeds per pod. Low differences between PCV and GCV for those traits indicate the lower influence of environment and reflect on reliability of selection based on phenotypic selection.

The genotypic coefficient of variation implies the extent of genetic variability present for various characters. However, it does not indicate the extent of heritable genetic variation. Many practical decisions in breeding programmes are based on magnitude of heritable variation.

The results revealed that estimates of heritability were high for plant height, number of branches per plant, number of pods per plant, pod length, pod weight per plant, 100 seed weight and seed yield per plant in all the three crosses (Fig. 2 & 3). Similar observations were made by Nirmalakumari and Balasubramanian (1993), Shrivastava and Shukla (1998), Singh and Singh (1999) and Agarwal *et al.* (2001) for seed yield per plant. Jagtap and Mehetre (1994), Taware *et al.* (1997) and Basavaraja (2002) observed high heritability for seeds per plant. Nirmalakumari and Balasubramanian (1993), Vimaladevi (1993) and Mahajan *et al.* (1994) observed high heritability for number of pods per plant. Harvest index had high heritability in crosses JS 335 x EC 241780 and DSb 21 x EC 241780. Srivastav and Jain (1994) and Basavaraja (2002) reported high heritability for harvest index. Moderate heritability values were observed for days to maturity and number of seeds per pod in the three crosses.

Prediction of successful selection becomes more accurate if it is based on estimates of heritability coupled with genetic advance, because it gives estimates not only of genetic contribution but of expected genetic gain out of selection as well. High heritability associated with high genetic advance were observed for only one trait number of pods per plant. Natural selection based on phenotypic observations for this character would be effective. This trait appear to be controlled by additive genes. The above results are in conformity with the findings of Hina kausar (2005) for number of pods per plant.

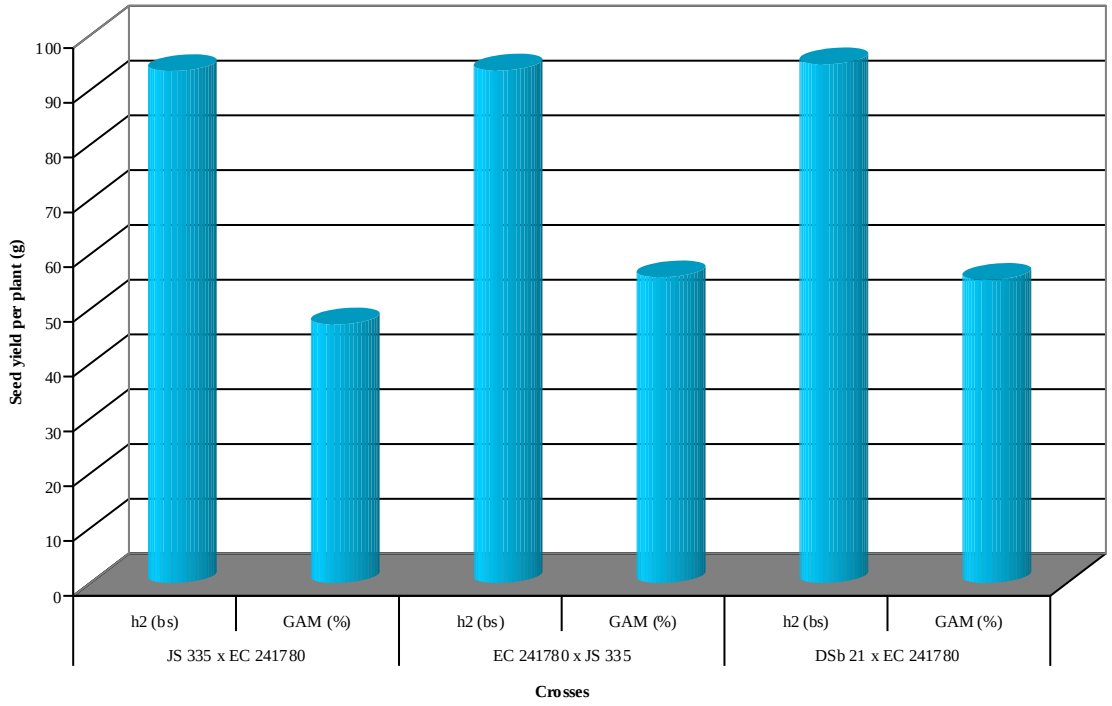


Fig. 3: Heritability and genetic advance over mean for seed yield per plant in F_2 population

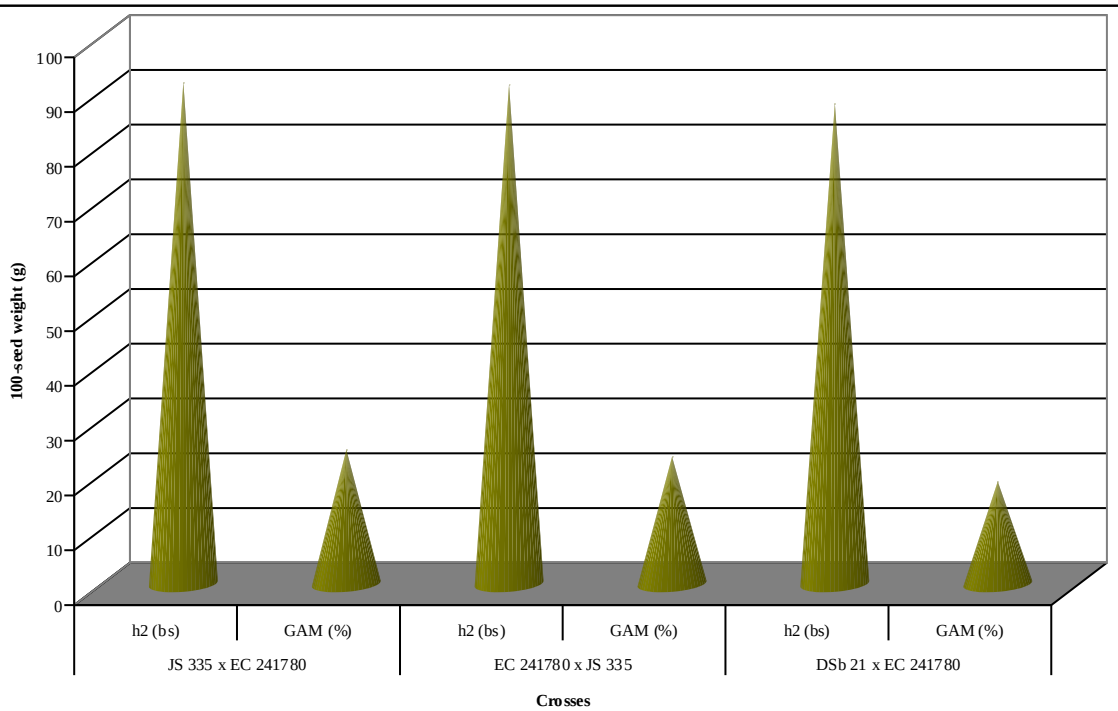


Fig. 4: Heritability and genetic advance over mean for 100-seed weight in F_2 population

High heritability associated with medium genetic advance was observed for plant height and pod weight per plant in all the three crosses. This indicates that these characters are highly influenced by environment and they may be conditioned by both additive and non additive gene actions. Hence, selection based on phenotypic observations alone may not be very effective for these traits.

From the foregone discussion, it can be concluded that high genotypic and phenotypic coefficient of variation coupled with high heritability was observed for number of pods per plant, pod weight per plant and seed yield per plant in all the three crosses indicating that there is lesser influence of environment in the expression of these characters which are amenable for phenotypic selection.

5.5 Genetic variability parameters for seed yield and its components considering F_3 population of three different crosses

The phenotypic coefficient of variability was higher than genotypic coefficient of variability in all the three crosses for the all characters studied (Table 25, 26 & 27). Segregating populations of F_3 generation of all the three crosses exhibited wide range of variation for the traits *viz.*, plant height, days to maturity, number of pods per plant, pod weight per plant, 100 seed weight, harvest index and seed yield per plant except for days to 50 % flowering, number of branches per plant, pod length and number of seeds per pod in all the three crosses (Fig. 5 & 6). This variation indicated the scope for selection of these traits for future breeding work. From the present study it is obvious that pod weight per plant in cross DSb 21 x EC 241780 had highest PCV and GCV which indicates the existence of substantial variability for this character. It also indicated greater scope for selection to improve upon these characters. Similar findings were reported by Sharma (1980), Chauhan and Singh (1982), Jagtap and Mehetre (1994) and Yadav (2006). In the present study moderate values of PCV and GCV were noticed for plant height, number of branches per plant, number of pods per plant and seed yield per plant. Similarly moderate estimates of PCV and GCV estimates were reported by Bangar *et al* (2003). Low PCV and GCV values were noticed for days to 50 % flowering, days to maturity, pod length, number of seeds per pod, 100 seed weight and harvest index. Low differences between PCV and GCV for those traits indicated the

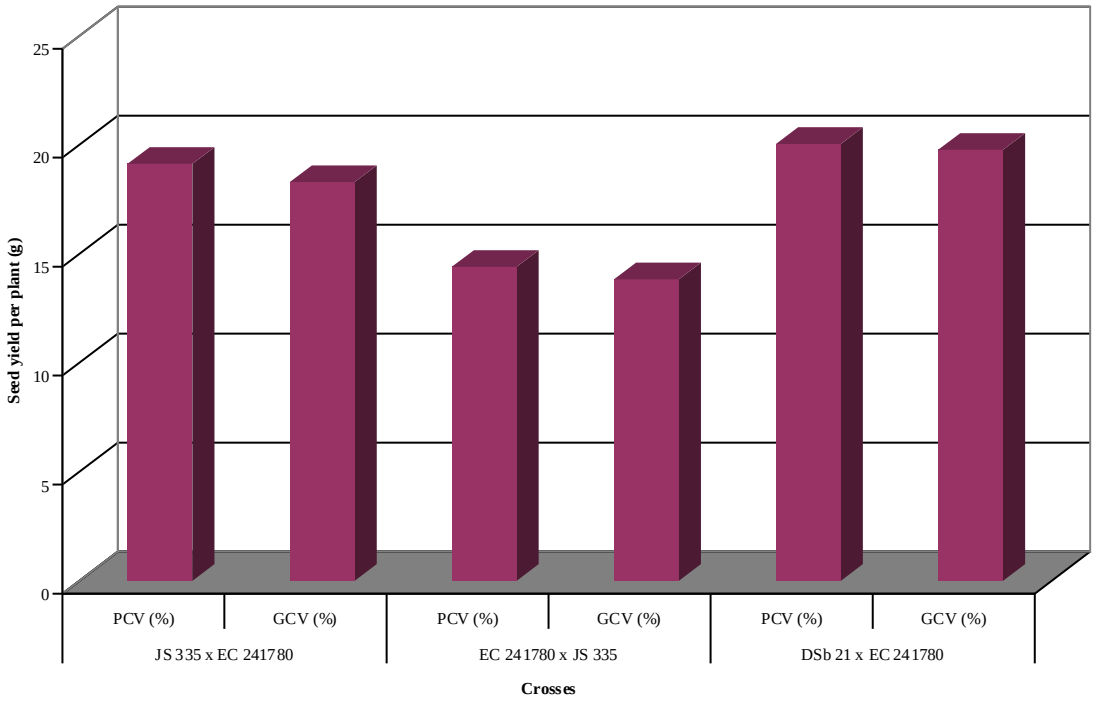


Fig. 5: Genetic variability parameters for seed yield per plant in F_3 population

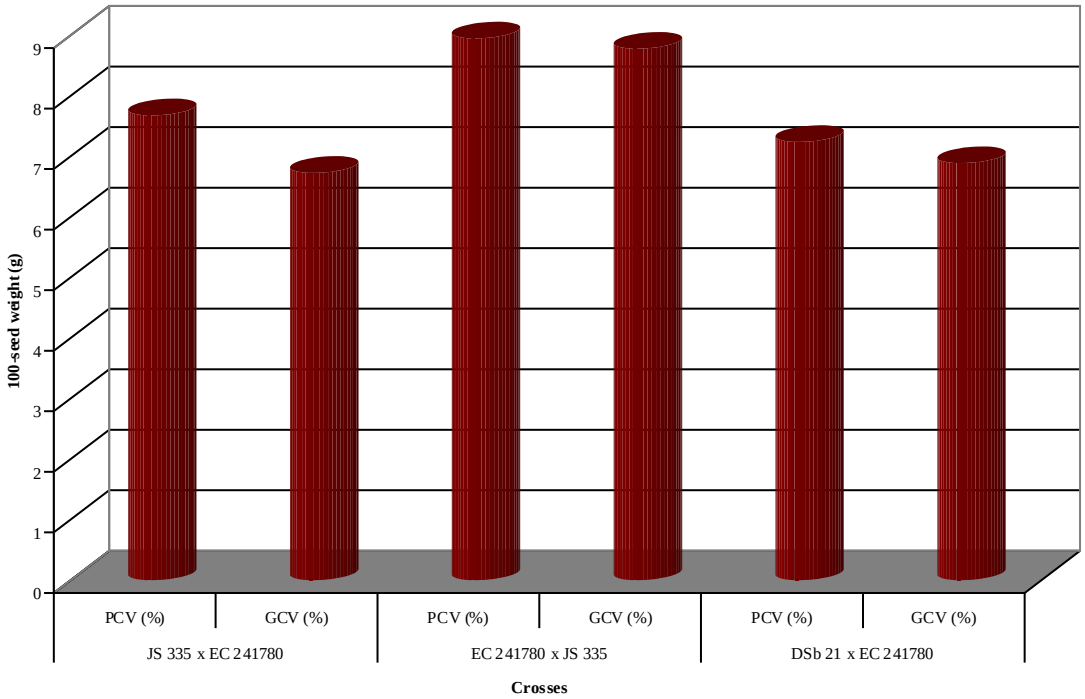


Fig. 6: Genetic variability parameters for 100-seed weight in F_3 population

lower influence of environment and reflect on reliability of selection based on phenotypic selection.

The genotypic coefficient of variation implies the extent of genetic variability present for various characters. However, it does not indicate the extent of heritable genetic variation. Many practical decisions in breeding programmes are based on magnitude of heritable variation.

The results revealed that estimates of heritability were high for plant height, number of branches per plant, number of pods per plant, pod weight per plant, 100 seed weight, harvest index and seed yield per plant in all the three crosses (Fig. 7 & 8). Similar observations were made by Nirmalakumari and Balasubramanian (1993), Shrivastava and Shukla (1998), Singh and Singh (1999) and Agarwal *et al.* (2001) for seed yield per plant. Jagtap and Mehetre (1994), Taware *et al.* (1997) and Basavaraja (2002) observed high heritability for seeds per plant. Nirmalakumari and Balasubramanian (1993), Vimaladevi (1993) and Mahajan *et al.* (1994) observed high heritability for number of pods per plant. Srivastav and Jain (1994) and Basavaraja (2002) reported high heritability for harvest index. Moderate heritability values were observed for days to 50 % flowering, pod length and number of seeds per pod in cross DSb 21 x EC 241780.

Prediction of successful selection becomes more accurate if it is based on estimates of heritability coupled with genetic advance, because it gives estimates not only of genetic contribution but of expected genetic gain out of selection as well. High heritability associated with high genetic advance was observed for only one trait number of pods per plant in the crosses JS 335 x EC 241780 and DSb 21 x EC 241780. Natural selection based on phenotypic observations for this character would be effective. This trait appear to be controlled by additive genes. The above results are in conformity with the findings of Hina kausar (2005) for number of pods per plant.

High heritability associated with moderate genetic advance was noticed for plant height in all the three crosses; number of pods per plant in cross EC 241780 x JS 335 and pod weight per plant in cross DSb 21 x EC 241780. This indicates that these characters are highly influenced by environment and they may be conditioned by both

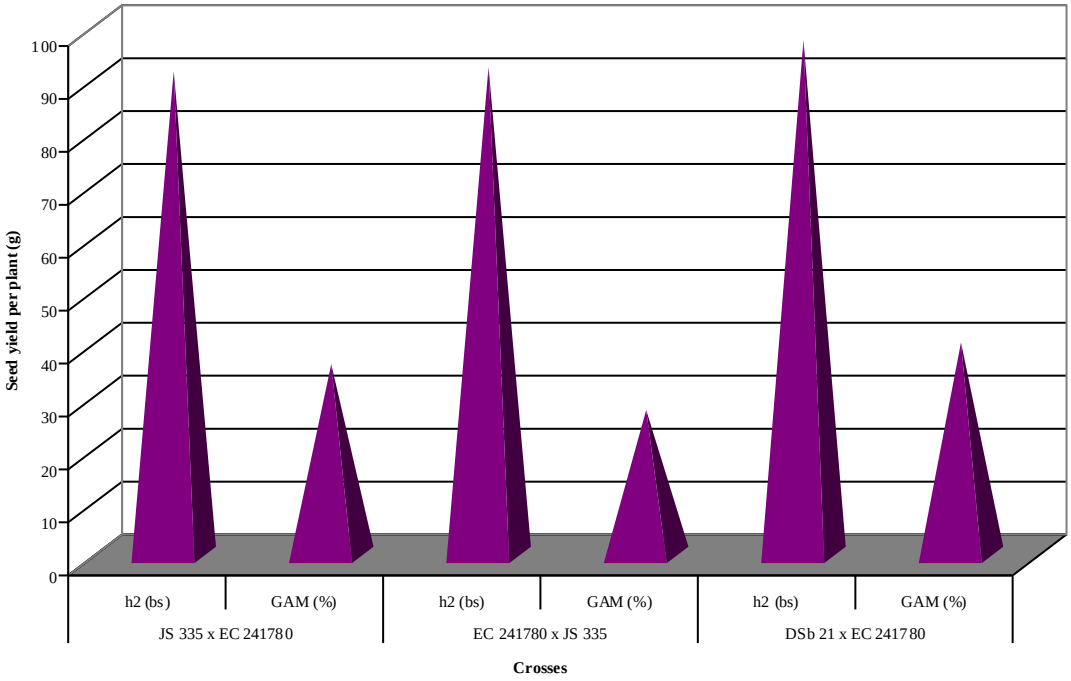


Fig. 7: Heritability and genetic advance over mean for seed yield per plant in F_3 population

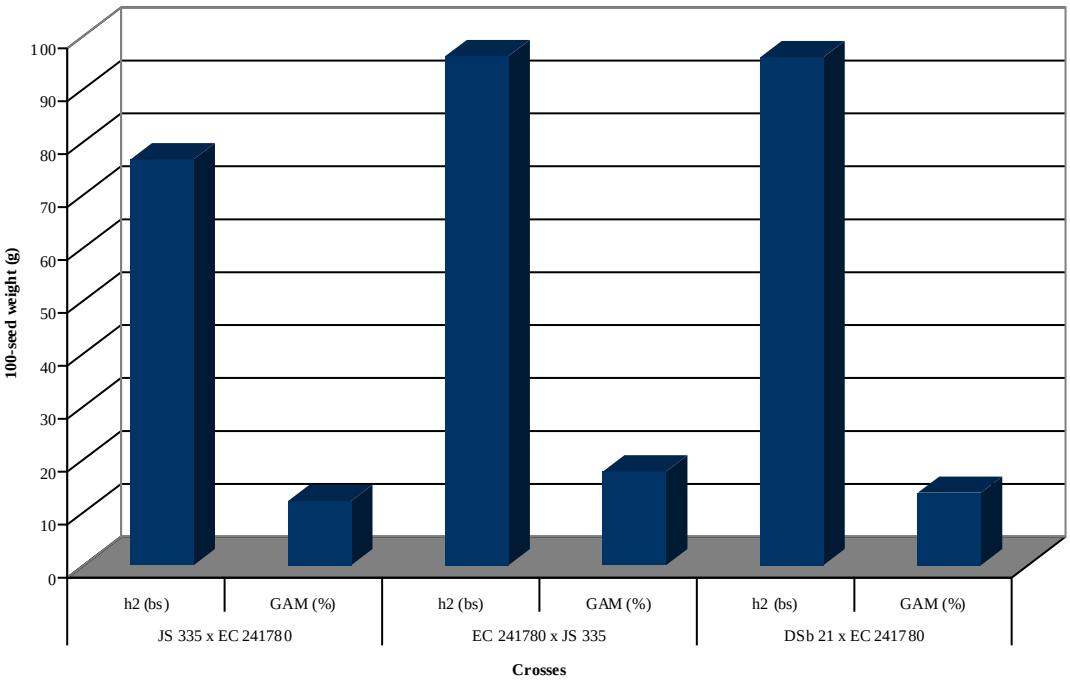


Fig. 8: Heritability and genetic advance over mean for 100-seed weight in F_3 population

additive and non additive gene actions. Hence, selection based on phenotypic observations alone may not be very effective for these traits.

From the foregone discussion, it can be concluded that high genotypic and phenotypic coefficient of variation coupled with high heritability was observed for pod weight per plant in the cross DSb 21 x EC 241780 indicating that there is lesser influence of environment in the expression of this character which is amenable for phenotypic selection.

5.6 Association studies among F_3 populations of three different crosses

The phenotype of a plant is the result of interaction of a large number of factors. Therefore yield is the sum total of the effects of several component characters and polygenicaly controlled character. The influence of these characters can be known through correlation studies. Correlation coefficients measure the magnitude and direction of association among the characters. Genetic correlation between different characters of plant often arises because of either linkage or pleiotropy (Harland, 1939).

Grafius (1959) opined that there may not be any gene for yield as such but it operates only through its components. Hence, the study of character association through correlation will help in selecting the yield attributes.

The association between two characters can be ascertained by phenotypic correlations which are determined from measurements of two characters in a number of individuals of segregating populations.

In the present study phenotypic correlations were studied for yield and its component traits. Phenotypic correlation of seed yield was positive and significant with plant height, number of branches per plant, number of pods per plant, pod weight per plant, number of seeds per pod and harvest index in all the three crosses. The results obtained from this study are in confirmation with results of Lakhani (1993), Mahajan *et al.* (1993), Singh and Yadava (2000) and Bhairav *et al.* (2006). This suggests that these characters should be considered while selecting for improvement in the seed yield.

Plant height had significant positive association with days to maturity, number of pods per plant, pod weight per plant, harvest index and seed yield per plant in all the three crosses and number of branches per plant in Cross-2. Suggesting that selection for these traits would likely to improve the seed yield in soybean. Perraju *et al.* (1982), Dixit and Patil (1982) and Kalaimagal (1991) reported the positive association of plant height with branches per plant.

Days to 50 % flowering exhibited significant positive association with days to maturity in all the three crosses, plant height in Cross-1 and Cross-2. Similar findings were reported by Sharma *et al.* (1983) and Harer and Deshmukh (1992).

Days maturity exhibited significant positive association with days to 50 % flowering and plant height in all the three crosses, number of branches per plant and harvest index in Cross-2. These results are in confirmation with the results obtained by Harer and Deshmukh (1992) and Ramana *et al.* (2000).

Number of branches per plant had significantly positive association with number of pods per plant, pod weight per plant, harvest index and seed yield per plant in all the three crosses. It had also positive association with plant height, days to 50 % flowering and days to maturity in Cross-2 (EC 241780 x JS 335) hence selection for these traits will help in yield improvement in soybean. Perraju *et al.* (1982) reported positive association of branches with pods per plant. Dixit and Patil (1982) observed positive association of branches per plant with pods per plant. Pod length exhibited highly significant and positive association with number of pods per plant, pod weight per plant, harvest index and seed yield per plant in Cross-3. In addition to these characters it had significant positive association with number of branches per plant in Cross-1 (JS 335 x EC 241780).

Number of pods per plant revealed highly significant positive association with plant height, number of branches per plant, pod weight per plant, harvest index and seed yield per plant in all the three crosses. In addition to these characters it had significant positive association with pod length in Cross-3 (DSb 21 x EC 241780). So selection for

these traits is expected to result in positive gains. Bhandarkar (1999) reported positive association of pods per plant with number of branches per plant.

Pod weight per plant exhibited highly significant and positive association with plant height, number of branches per plant, number of pods per plant, number of seeds per pod, harvest index and seed yield per plant in all the three crosses. While, it had significant positive association with pod length in Cross-3 (DSb 21 x EC 241780). So selection for these traits is expected to result in positive gains.

Number of seeds per pod exhibited positive association with pod weight per plant, harvest index and seed yield per plant in all the three crosses. This suggests that selection of these traits would improve the seed yield in soybean. Similar results were obtained by Amaranath (1986) and Dixit and Patil (1982). This indicates that though these traits are important to some extent for improvement of seed yield but their importance cannot be over emphasized.

100 seed weight recorded significant positive association with pod weight per plant, harvest index and seed yield per plant in Cross-1 and Cross-3. But it had non-significant negative association with number of seeds per pod and pod length in Cross-2 and Cross-3., This indicates that these traits are important only to some extent for improvement of seed yield. Ramana *et al.* (2000) reported negative association of 100 seed weight with seeds per pod.

Harvest index had highly significant positive association with plant height, number of branches per plant, number of pods per plant, pod weight per plant, number of seeds per pod and seed yield per plant in all the three crosses. Similar results were reported by Weilemann and Luquez (2000) and Hinakausar (2005).

It may be inferred that the characters namely plant height, number of branches per plant, number of pods per plant, pod weight per plant, number of seeds per pod and harvest index showed valuable in all the three crosses. Therefore more emphasis should be given for these traits while selecting for genetic improvement in seed yield of soybean.

5.7 Validation of molecular markers linked to rust resistance in F_2 mapping population of the cross JS 335 x EC 241780

Breeding work utilizing both phenotypic and genotypic markers are more reliable and fast. Conventional breeding methods may create resistant varieties which is time consuming and intensive task. Marker assisted selection (MAS) has been proven as a highly efficient breeding method in improvement of cultivars or lines for various biotic stresses in crop breeding programmes, because of its efficiency in selecting plants with appropriate gene combinations in segregating population (Collard and Mackill, 2008). Recent advancements made in the field of genomics have provided a varied number of molecular markers in many crop species. DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding via marker-assisted selection. Genetic markers represent genetic differences between individual organisms or species. They do not represent the target genes themselves but act as 'signs' or 'flags'. Genetic markers that are located in close proximity to genes (*i.e.* tightly linked) may be referred to as gene 'tags'. Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or 'linked' to genes controlling the trait. All genetic markers occupy specific genomic positions within chromosomes (like genes) called 'loci' (singular 'locus') (Collard *et al.*, 2005).

DNA markers are the most widely used type of marker predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Peterson *et al.*, 1996). These markers are selectively neutral because they are usually located in non-coding regions of DNA. DNA markers are practically unlimited in number and are not affected by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995). Thus MAS application in breeding programmes due to its time saving, consistency, biosafety and accuracy in selection of complex traits recognized well (Jena and Mackill, 2008).

The genetics of rust resistance of six dominant genes specific to soybean rust isolates has been described: *Rpp1, Rpp2, Rpp3, Rpp4, Rpp5* and *Rpp6* (Bromfield and Hartwig, 1980, Mclean and Byth, 1980, Hartwig and Bromfield, 1983, Hartwig, 1986,

Garcia *et al.,* 2008, Shuxian *et al.,* 2012). *Rpp1* was identified in soybean genotype PI 200492 (McLean and Byth, 1980), *Rpp2* in PI 230970 (Bromfield and Hartwig, 1980), *Rpp3* in PI 462312 (Hartwig and Bromfield, 1983) and *Rpp4* in PI 459025 (Hartwig, 1986). Resistance genes have also been described in cultivated soybean. All described *Rpp* (*Rpp*: resistance to *P. pachyrhizi*) genes have already been mapped on soybean chromosomes (Chr), *Rpp1* is mapped on chromosome 18, *Rpp2* on Chr 16, *Rpp3* on Chr 6, *Rpp4* on Chr 18, *Rpp5* on Chr 3 and *Rpp6* on Chr 16 (Hyten *et al.,* 2007, Garcia *et al.,* 2008, Silva *et al.,* 2008, Hyten *et al.,* 2009).

Soybean has a reasonably dense molecular-marker linkage map (Song *et al.,* 2004) and the association of markers to known genes has been pursued by many groups. Molecular mapping of soybean rust-resistance genes in soybean has previously been reported.

Brogin *et al.* (2004) identified Simple Sequence Repeat (SSR) markers linked to rust resistance present in cultivar FT-2 in the linkage group (LG) -C2 of the previous soybean consensus map reported by Cregan *et al.* (1999). However, the locus could not be identified in the study. As soybean rust resistance gene from the cultivar Hyuuga was mapped at 3-cM interval on LG-C2 between Satt 134 and Satt 460 (Monteros *et al.,* 2007). Hyten (2007) recently mapped the *Rpp3* locus at same interval that Monteros *et al.* (2007) mapped. The *Rpp1* locus has been mapped to 1-cM interval on LG-G between Sct_187 and Satt 064LG-G (Hyten *et al.,* 2007). Molecular tagging of soybean rust resistance can help in the process of resistance breeding.

Identification of resistant lines for soybean rust disease is a major challenge to soybean breeders. Therefore, the present study was undertaken to develop a segregating population derived from resistant and susceptible parents followed by screening with SSR markers towards their linkage with resistance. The difficulty in genotyping of all the plants in a mapping population can be reduced through screening of markers with susceptible (JS 335) and resistant (DSb 21) parents and the markers showing parental polymorphism can be used for genotyping. To know the parental polymorphism in the present study, parents were screened with twenty five reported SSR markers for rust resistance. Among the different molecular markers, SSRs are of interest for genetic mapping because each marker corresponds to a single position in the genome, but has

several alleles yielding a high degree of polymorphism (Cregan *et al.,* 1999). Further they are easy to use, yield consistent results and are accessible to all biotechnology labs.

Out of the twenty five SSR markers used for screening the parents only three markers Satt 361, Satt 275 and Satt 215 exhibiting polymorphism between JS 335 (susceptible) and EC 241780 (resistant) parents were taken as candidate markers for analysis of individuals in segregating F_2 population of the cross JS 335 x EC 241780. Total 350 F_2 plants were screened using these polymorphic markers.

The genotypes were further scored for the banding pattern. The genotypes showing the banding pattern as that of the susceptible parent were scored as 'A', while the genotypes showing the banding pattern as of the resistant parent were scored as 'B' and the genotypes showing the banding pattern as of the heterozygous plants were scored as 'H'.

The trait considered for single marker analysis was rust disease (Per cent Disease Index) and it was done with using the software WinQTLCart 2.5 version. The polymorphic markers were further analysed by single marker analysis which showed a significant association with rust resistance, explaining the highest phenotypic variance of 8.62 per cent for the marker Satt 361 followed by 3.61 per cent for the marker Satt 275 and 3.19 per cent for the marker Satt 215 at 5 per cent level of significance. SSR markers Satt 215, Satt 361 and Satt 275 reported on chromosome 16 and 3 linked with soybean rust resistance gene Rpp2 and Rpp 5, respectively.

These markers are found to be associated with rust resistance in the genotype EC 241780. Thus, these polymorphic markers can be used to distinguish between resistant and susceptible cultivars of soybean and advance the resistance cultivars through marker assisted backcross breeding in the soybean breeding programme. The marker can be used to screen a large number of germplasm for disease resistance. The present markers identified can be further used for large scale screening of germplasm and also for the identification of new sources of resistance against soybean rust. The results are in agreement with earlier reports of Deshmukh *et al.* (2015), Gowtham *et al.*, (2018) also reported Satt 275 as polymorphic marker which showed a significant relationship with the disease severity for all the elite soybean lines with R^2 value of 3.75 at 5 per cent level of significance.

Although soybean rust resistance controlled by single dominant genes such as *Rpp*1 to *Rpp*6 are introduced into elite soybean cultivars by backcrossing in a relatively short time, *P. pachyrhizi* isolates have overcome or will likely overcome any single gene resistance in the future. Therefore, it is important to continue screening to identify novel soybean rust resistance sources for breeding the right combination of resistant *Rpp* loci using the flanking markers. This may lead to increased durability of resistance to soybean rust and could be effectively used in the soybean breeding programmes.

5.8 Identification of superior segregants in F_3 families of three crosses

In the present investigation, the F_3 families of three crosses, *viz.*, JS 335 x EC 241780, EC 241780 x JS 335 and DSb 21 x EC 241780 have generated sufficient variability and also some useful segregants. Among them nine superior segregants were identified based on mean + 2 standard deviation (Plate 13).

Among the three crosses, Cross-1 (JS 335 x EC 241780) has generated five superior segregants, Cross-2 (EC 241780 x JS 335) and Cross-3 (DSb 21 x EC 241780) have generated two superior segregants each. Their yield and component traits *viz.*, days to maturity, plant height, number of branches, number of pods, 100 seed weight, harvest index and seed yield per plant are presented in Table 32.

Among the five segregants in Cross-1 (JS 335 x EC 241780), segregant No.23 of Line-8 recorded highest yield per plant (25.1 g) with more number of pods per plant (86.4) and harvest index (51.7 %) followed by segregant No.39 of Line-13 has recorded 24.4 g yield per plant with 78.3 pods and harvest index (50.4 %).

In Cross-2 (EC 241780 x JS 335), segregant No.445 of Line-97 recorded higher yield per plant (22.2 g) with more number of pods per plant (71.2) and harvest index (52.3 %) followed by segregant No.88 of Line-25 has recorded 19.1 g yield per plant with 61.6 pods per plant and harvest index (48.2 %).

In Cross-3 (DSb 21 x EC 241780), segregant No.145 of Line-29 recorded higher yield per plant (23.2 g) with more number of pods per plant (65.9) and harvest index (51.2 %) followed by segregant No.175 of Line-42 has recorded 22.6 g yield per plant with 77.0 pods per plant and harvest index (46.3 %).

Plate 13: Progeny lines showing resistant reaction in F_3 families

Table 32. Superior segregants and their salient features

Sl. No.	Cross/parents	Line No.	Salient features								
			Days to maturity	Plant height (cm)	No. of branches	No. of pods	100 seed weight (g)	Harvest index (%)	Seed yield (g)	Rust reaction	
										0-9 scale	Remarks
1	JS 335 x EC 241780	8	91.5	59.7	5.0	86.4	12.4	51.7	25.1	3	MR
		13	89.5	60.5	5.1	78.3	12.7	50.4	24.4	5	MS
		21	90.5	57.7	5.1	79.8	13.7	52.0	23.9	1	HR
		3	87.5	37.9	5.0	70.6	12.0	53.9	22.0	7	S
		70	89.0	54.7	4.5	65.3	12.3	49.7	21.9	7	S
2	EC 241780 x JS 335	97	90.5	45.2	4.4	71.2	11.9	52.3	22.2	1	HR
		25	89.5	38.1	4.5	61.6	12.4	48.2	19.1	7	S
3	DSb 21 x EC 241780	29	94.5	69.0	5.4	65.9	13.3	51.2	23.2	1	HR
		42	90.5	62.8	5.4	77.0	13.2	46.3	22.6	1	HR
4	JS 335 (Parent)	-	87.0	46.1	4.4	46.0	10.2*	42.5	12.2	9	HS
5	DSb 21 (Parent)	-	94.0	66.2	5.1	72.2	14.0	47.7	16.7	1	HR
6	EC 241780 (Parent)	-	104	65.3	4.6	54.5	13.3	44.2	13.4	1	HR

*Seed size has been reduced due to rust infection

Rust reaction: MR – Moderately resistant
MS – Moderately susceptible
S – Susceptible
HS – Highly susceptible
HR – Highly resistant

5.8.1 Reaction to rust

Cross-1 (JS 335 x EC 241780) has generated five superior segregants, among them only one segregant with highly resistant reaction and one segregant with moderately resistant reaction to rust, whereas Cross-2 (EC 241780 x JS 335) has generated only one segregant with highly resistant reaction to rust and Cross-3 (DSb 21 x EC 241780) has generated two segregants with highly resistant reaction. Among the rust resistant segregants one segregant 21 of Cross-1 and one segregant *i.e.*, 29 of Cross-3 recorded high yield with resistant reaction (Table 32).

5.9 Future line of work

1. The results of screening studies (*Kharif* 2015 & 2016) against rust under natural epiphytotic condition revealed that the line EC 242104 exhibited highly resistant reaction which can be further utilized in future breeding programmes for rust resistance

2. The results of diversity studies revealed that the clustering pattern could be utilized in selection of parents for hybridization and deciding the best cross combinations which may generate the more variability for various traits

3. Inheritance study revealed that in all the three crosses studied the rust resistance is controlled by single dominant gene. The resistant genotypes reported in the present study can be used as confirmed sources for rust resistance and can be further utilized in future breeding programmes

4. Superior segregants identified for yield and its component traits need to be further tested for their superiority and rust resistance across locations/ years.

5. Validation of molecular markers linked to rust resistance in F_2 population of cross JS 335 x EC 241780 was carried out using 25 SSR markers. The results revealed that since the phenotypic variance (R^2) value is low need to screen more markers to identify marker–trait association for rust resistance in the population.

6. SUMMARY AND CONCLUSIONS

Soybean [*Glycine max* (L.) Merrill] occupies a unique position among edible legumes. Traditional food legumes (pulses) are rich in protein but contain limited amount of oil. Soybean contains more protein (about 40 per cent) than other pulses with higher content of edible oil (about 20 per cent). It is also rich in lysine, an essential amino acid that is deficient in most of the cereals. Therefore the quality of soya protein is now recognised as similar to that of meat protein. The edible oil in soybean is approximately 85 per cent unsaturated and contains the essential fatty acids.

The present investigation was carried out during 2015-17 at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad and R & D farm, Ugar Sugar Works, Ugarkhurd. The current study was carried out with different experiments. About 144 exotic germplasm lines including highly susceptible check JS 335 and resistant checks *viz.*, DSb 21, EC 241778 and EC 241780 were evaluated during *kharif* 2015 at Dharwad for identification of new sources for resistance to rust and genetic diversity. Based on the resistance reaction, 22 lines which exhibited resistant/moderately resistant reaction were selected. These lines were further evaluated to confirm their resistant reaction under natural epiphytotic condition at two hotspots for rust *viz.*, Dharwad and Ugarkhurd during *kharif* 2016.

Three genotypes *viz.*, DSb 21, JS 335 and EC 241780 procured from AICRP on Soybean, UAS, Dharwad were utilized in crossing programme/hybridization during *summer* 2015 to study the inheritance pattern for rust resistance and variability. Subsequently, F_2 and F_3 populations were raised during *kharif* 2016 & 2017 respectively. In addition to this, validation of molecular markers linked to rust resistance in F_2 population of cross JS 335 x EC 241780 was carried out using already reported 25 SSR markers.

Among 144 exotic germplasm lines including resistant and susceptible checks, only one line EC 242104 (8.89%) and resistant checks *viz.*, DSb 21 (8.89 %), EC241780 (8.89 %) and EC 241778 (8.89%) recorded disease grade 1 and was found to be highly resistant. Nine lines *viz.*, EC 391336 (20.00%), EC 385243 (24.44%), EC 333934 (24.44%), EC 308334 (24.44%), EC 287754 (24.44), EC 250578 (20.00%), EC 100031

(24.44%), EC 14426 (28.89%) and EC 15966 (20.00%) recorded disease grade 3 and were found to be moderately resistant and they confirmed their resistance reaction during *kharif* 2016 also screened under natural epiphytotic condition at two hotspots for rust *viz.*, Dharwad and Ugarkhurd.

The range of 'r' values among 144 germplasm lines including resistant and susceptible checks ranged from 0.009 to 0.169 indicating the importance of infection rate in spreading rust disease. Based on apparent rate of infection, germplasm line EC 3251 (0.009) followed by EC 391346, EC 14426 (0.011), EC 685252 (0.017) EC 242104 (0.031), DSb 21 (0.031) and EC 241780 (0.031) recorded lower 'r' values indicating the rate of infection in these lines is very slow. Whereas germplasm line EC 33917 (0.169) followed by EC 37937 (0.151), EC 95291 (0.124) and EC 95815 (0.114) recorded higher 'r' values indicating faster spread of disease in these lines.

The Area Under Disease Progress Curve (AUDPC) in lines revealed a wide variation among different lines at different intervals. Among, the lines tested, the highest average AUDPC value was observed in the lines JS 335 (944.44) followed by EC 685250 (855.55), EC 94625, EC 685255, EC 685252 (833.33). While, the least average AUDPC value was recorded in lines EC 241778 (66.67) followed by DSb 21, EC 242104, EC 241780 (83.33) and EC 15966, EC 391336 (150.00).

The k-*means* cluster analysis for yield related traits in 144 germplasm lines including checks in the present study were grouped into eight clusters. The formation of distinct solitary clusters may be due to the fact that exotic germplasm lines belong to different regions which may be responsible for this type of genetic diversity. It could be seen that clusters vary much with respect to mean expression of various characters which resulted in distinct clusters. There were significant differences between the mean of clusters for all the traits. Maximum number of genotypes were grouped in the cluster V (39 genotypes) followed by cluster I (35 genotypes), cluster VII and cluster VIII (20 genotypes each).

Based on the cluster means, cluster VI for seed yield per plant, number of pods per plant, number of branches per plant, days to 50 % flowering and cluster II for early maturity are considered to be superior. Thus, crosses among the genotype(s) of these

clusters would exhibit high heterosis and likely to produce new recombinants with desired traits in soybean.

In two crosses *viz.*, susceptible x resistant (JS 335 x EC 241780) and resistant x susceptible (EC 241780 x JS 335) combination, susceptible and resistant parents were used as females respectively. The F_1's of two crosses were resistant to rust in the field condition. Out of 350 F_2 plants in the cross susceptible x resistant (JS 335 x EC 241780), 272 plants were resistant and 78 plants were susceptible under field conditions and out of 456 F_2 plants in the cross resistant x susceptible (EC 241780 x JS 335), 352 plants were resistant and 104 plants were susceptible under field conditions. F_2 populations of two crosses segregated into 3:1 ratio (3 resistant: 1 susceptible) indicating single gene responsible for resistance to soybean rust with resistance being dominant over susceptibility. The F_3 families segregated in 1:2:1 ratio (1 resistant: 2 segregating: 1 susceptibility) confirming the results observed in F_2 generation of two crosses *viz.*, susceptible x resistant (JS 335 x EC 241780) and resistant x susceptible (EC 241780 x JS 335).

In resistant x resistant combination (DSb 21 x EC 241780) all the F_1 plants were resistant. Out of 432 F_2 plants, 414 were resistant and 18 plants were susceptible under field conditions. The data showed 15:1 ratio with good fit. The results indicated the presence of different resistant genes in these genotypes. The F_3 family segregation also confirmed F_2 results, as the 100 families segregated into 7:8:1 ratio (7 resistant: 8 segregating: 1 susceptible). These genotypes have not been previously studied in detail for rust resistance. The resistant genotypes reported in the present study can be used as confirmed sources for resistance and utilized in the breeding programmes.

The phenotypic coefficient of variation was higher than genotypic coefficient of variation in all the three crosses for the all characters studied. Segregating populations of all the three F_2 crosses exhibited wide range of variation for the traits *viz.*, days to 50 % flowering, plant height, days to maturity, number of pods per plant, pod weight per plant, 100 seed weight, harvest index and seed yield per plant except number of branches per plant, pod length and number of seeds per pod in all the three crosses.

Analysis of variance revealed that highly significant variations among the lines in all the three crosses for all the eleven characters studied. Phonotypic coefficient of

variation was higher in magnitude than corresponding genotypic coefficient of variation in respect of all the characters. Pod weight per plant in cross DSb 21 x EC 241780 had highest PCV and GCV which indicates the existence of substantial variability for this character. Moderate values of PCV and GCV were noticed for plant height, number of branches per plant, number of pods per plant and seed yield per plant.

High heritability associated with high genetic advance was observed for only one trait number of pods per plant in all the three F_2 crosses. Natural selection based on phenotypic observations for this character would be effective. This trait appear to be controlled by additive genes.

High heritability associated with moderate genetic advance was noticed for plant height in all the three F_3 crosses; number of pods per plant in cross EC 241780 x JS 335 and pod weight per plant in cross DSb 21 x EC 241780. This indicates that these characters are highly influenced by environment and they may be conditioned by both additive and non additive gene actions. Hence, selection based on phenotypic observations alone may not be very effective for these traits.

The correlation studies revealed highly significant positive association of seed yield with plant height, number of branches per plant, number of pods per plant, pod weight per plant and harvest index. It exhibited non-significant positive association with days to 50 % flowering and days to maturity in all the F_3 three crosses studied. Therefore emphasis may be laid on these characters for improving seed yield.

Out of the twenty five SSR markers used for screening the parents, only three markers Satt 361, Satt 275 and Satt 215 exhibiting polymorphism between JS 335 (susceptible) and EC 241780 (resistant) parents were taken as candidate markers for analysis of individuals in segregating F_2 population of the cross JS 335 x EC 241780. Total 350 F_2 plants were screened using these polymorphic markers. The trait considered for single marker analysis was rust disease (Per cent Disease Index) and it was done with using the software WinQTLCart 2.5 version. The polymorphic markers were further analysed by single marker analysis which showed a significant association with rust resistance, explaining the highest phenotypic variance of 8.62 per cent for the marker Satt 361 followed by 3.61 per cent for the marker Satt 275 and 3.19 per cent for the marker Satt 215 at 5 per cent level of significance.

These markers are found to be associated with rust resistance in the genotype EC 241780. Thus, these polymorphic markers can be used to i) distinguish between resistant and susceptible cultivars of soybean and ii) advance the resistance lines through marker assisted backcross breeding programme in soybean.

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* - *Original not seen*

Appendix I. The meteorological data for the year 2015 at the Main Agricultural Research Station, Dharwad

Months	Temperature		RH%	Rainfall (mm)	No. of rainy days
	Maximum°C	Minimum°C			
January	28.6	13.3	52	000.2	-
February	31.8	14.6	40	-	-
March	33.2	19.3	55	105.2	3
April	35.1	20.3	51	13.2	1
May	34.7	21.9	63	129.4	7
June	28.8	21.2	80	160.2	11
July	28.7	21.0	79	42.8	6
August	28.7	20.6	79	34.4	5
September	29.9	20.6	78	22.2	3
October	31.2	19.6	65	179.8	5
November	30.0	18.0	70	28.6	2
December	30.6	15.7	56	0.00	-
Total				**716.0**	**43**

Appendix II. The meteorological data for the year 2016 at the Main Agricultural Research Station, Dharwad

Months	Temperature		RH%	Rainfall (mm)	No. of rainy days
	Maximum°C	Minimum°C			
January	30.1	14.0	44	0.4	-
February	33.6	17.9	46	0.2	-
March	36.1	20.6	41	2.4	-
April	38.0	21.6	50	20.4	3
May	36.0	22.1	58	82.8	4
June	29.2	21.3	78	75.6	10
July	26.3	21.0	86	150.2	18
August	26.4	20.6	85	112.2	11
September	27.1	20.0	80	73.4	6
October	29.7	18.8	61	44.8	2
November	30.8	14.4	44	5.8	1
December	30.0	14.0	45	0	0
Total				**568.2**	**55**

Appendix III: The meteorological data for the year 2017 at the Main Agricultural Research Station, Dharwad

Months	Temperature		RH%	Rainfall (mm)	No. of rainy days
	Maximum°C	Minimum°C			
January	30.2	13.9	48.0	000.0	0
February	33.5	16.3	35.1	000.0	0
March	35.1	18.4	37.9	000.0	0
April	37.7	21.2	48.4	012.6	2
May	35.4	21.9	62.5	101.8	8
June	29.5	21.5	79.0	031.4	5
July	27.6	21.0	83.4	117.8	10
August	28.6	20.8	85.4	32.4	5
September	28.7	20.7	90.9	197.6	13
October	29.5	19.9	89.1	72.6	7
November	29.8	15.8	76.0	16.2	1
December	28.7	14.1	77.0	0.4	0
Total				**582.8**	**51**

Appendix IV. *Per se* performance of 140 exotic germplasm lines and four checks for yield related traits in soybean during *kharif* 2015

Sl. No.	Genotypes	DFF	NB	DM	NPP	SYP
1	EC 1028	34	5.4	85	64.2	13.50
2	EC 10027	29	5.4	80	37.2	9.30
3	EC 100031	34	5.4	90	52.6	10.50
4	EC 100772	33	5.2	91	44.2	11.05
5	EC 104817	33	5.0	87	40.2	10.05
6	EC 107416	35	4.0	89	47.4	11.85
7	EC 114520	33	5.0	90	65.4	12.35
8	EC 114573	33	4.8	88	43.6	10.90
9	EC 116343	31	5.2	86	60.0	12.69
10	EC 118420	35	4.8	87	53.0	10.24
11	EC 118443	34	5.6	90	67.0	12.45
12	EC 12570	33	4.6	91	48.2	12.05
13	EC 14426	36	5.8	81	78.6	12.35
14	EC 242091	37	5.2	89	68.8	12.47
15	EC 14476	36	5.2	91	72.6	12.69
16	EC 14573	32	5.6	81	39.8	9.95
17	EC 149988	33	5.2	89	60.6	10.65
18	EC 15966	36	4.8	81	45.2	11.30
19	EC 16119	38	4.8	89	42.0	10.50
20	EC 16738	35	5.6	90	65.6	13.23
21	EC 172607	39	4.6	92	38.8	9.70
22	EC 175529	40	4.2	90	41.0	10.25
23	EC 177744	40	4.0	81	48.6	12.15
24	EC 187456	34	4.4	90	57.8	12.56
25	EC 184337	33	4.2	91	45.6	11.40
26	EC 19923	34	4.4	90	66.0	13.25
27	EC 225114	36	5.4	92	43.2	10.80
28	EC 221329	37	4.6	91	45.4	10.23
29	EC 2388	36	3.6	91	46.0	11.50
30	EC 232019	34	3.4	93	50.2	12.55

Contd……..

Sl. No.	Genotypes	DFF	NB	DM	NPP	SYP
31	EC 241309	34	5.2	91	47.2	11.80
32	EC 241761	37	5.6	91	35.0	8.75
33	EC 241766	35	4.2	90	39.2	9.80
34	EC 242018	36	3.4	89	41.6	10.40
35	EC 242038	37	5.0	88	59.4	13.85
36	EC 242104	39	5.2	93	62.0	13.25
37	EC 242105	34	3.8	90	47.0	11.75
38	EC 245984	37	3.8	87	52.0	10.35
39	EC 245989	32	5.6	88	47.4	11.85
40	EC 2581	33	5.8	91	76.4	12.56
41	EC 25269	32	3.8	92	53.6	12.36
42	EC 250578	34	3.4	90	41.8	10.45
43	EC 250588	33	4.8	81	33.4	8.35
44	EC 250607	40	5.2	93	61.0	13.10
45	EC 250608	40	4.6	94	49.4	12.35
46	EC 250619	39	4.8	83	58.4	13.26
47	EC 251329	33	5.2	81	48.2	12.05
48	EC 251334	37	4.0	82	58.4	13.25
49	EC 251341	33	3.8	93	54.4	13.60
50	EC 251358	33	4.2	90	52.0	10.53
51	EC 251401	31	4.0	81	34.6	9.64
52	EC 251409	33	4.0	90	40.0	10.00
53	EC 251411	32	5.0	93	58.8	13.56
54	EC 251 456	38	4.2	90	42.4	10.60
55	EC 251501	40	5.2	93	56.0	14.00
56	EC 251516	40	4.6	95	59.0	13.25
57	EC 251762	36	5.0	86	61.0	13.25
58	EC 274755	37	5.6	89	59.2	13.80
59	EC 287754	36	4.6	87	45.6	11.40
60	EC 30832	37	3.6	86	53.2	13.30
61	EC 308334	36	4.4	81	46.0	11.50
62	EC 309512	38	5.0	87	51.6	12.90

Contd……..

Sl. No.	Genotypes	DFF	NB	DM	NPP	SYP
63	EC 309538	40	5.4	91	45.0	11.25
64	EC 309545	39	5.4	90	37.8	9.45
65	EC 315213	38	3.4	91	51.8	12.95
66	EC 3251	41	3.6	95	38.2	9.55
67	EC 325092	42	3.6	96	33.8	8.45
68	EC 325099	38	4.2	81	38.0	9.50
69	EC 325101	35	3.8	87	30.6	8.96
70	EC 325102	30	3.8	83	24.4	9.25
71	EC 329158	32	3.2	89	30.2	7.55
72	EC 33875	34	5.4	87	64.8	13.52
73	EC 33917	34	3.8	81	39.0	9.75
74	EC 33922	37	5.4	91	54.2	11.23
75	EC 33940	38	4.6	90	59.8	13.25
76	EC 333868	40	5.8	92	60.0	13.60
77	EC 333875	39	4.6	91	55.8	13.95
78	EC 333881	38	4.6	90	35.6	8.90
79	EC 333886	38	4.0	81	26.4	9.60
80	EC 333891	36	5.4	87	49.4	12.35
81	EC 333904	34	5.2	90	32.8	8.20
82	EC 333909	38	4.6	83	35.6	10.63
83	EC 333920	35	4.6	89	61.0	13.25
84	EC 333934	38	4.2	87	58.0	10.50
85	EC 338597	39	4.2	86	47.4	11.85
86	EC 34057	37	4.8	87	59.8	13.25
87	EC 34078	38	4.2	90	32.8	8.20
88	EC 34079	36	4.3	86	36.0	9.00
89	EC 34092	38	6.0	87	59.2	12.35
90	EC 34500	38	3.8	87	36.0	9.00
91	EC 340924	33	6.0	90	45.0	11.25
92	EC 36816	32	5.0	91	48.8	12.20
93	EC 37937	33	3.8	93	32.4	8.10
94	EC 376065	37	3.8	94	37.6	9.40

Contd……

Sl. No.	Genotypes	DFF	NB	DM	NPP	SYP
95	EC 377552	33	6.0	81	55.2	13.80
96	EC 380322	33	5.6	89	69.2	12.20
97	EC 383165	34	6.0	81	76.4	13.25
98	EC 385243	33	5.2	90	88.8	11.20
99	EC 389148	34	4.6	92	40.0	10.00
100	EC 389151	32	5.2	90	46.8	11.70
101	EC 389178	38	4.4	87	53.2	13.30
102	EC 389400	37	5.6	90	70.2	12.56
103	EC 39219	33	5.0	89	56.2	12.35
104	EC 39362	37	4.8	93	31.4	8.52
105	EC 39491	37	4.8	94	49.2	12.30
106	EC 39516	36	3.8	91	60.4	13.25
107	EC 39536	38	4.2	83	52.2	12.50
108	EC 390981	39	4.0	91	52.2	12.35
109	EC 391158	40	4.0	96	43.4	10.85
110	EC 391336	38	4.4	91	45.2	11.30
111	EC 391346	37	5.0	87	37.2	9.30
112	EC 392532	38	3.8	81	39.6	9.90
113	EC 392580	33	3.8	87	37.8	9.45
114	EC 394839	33	5.2	90	58.2	13.56
115	EC 396052	33	4.2	91	42.8	10.70
116	EC 396053	37	5.6	81	67.6	12.56
117	EC 397158	38	3.4	90	39.6	9.90
118	EC 4435	39	5.6	87	74.4	13.25
119	EC 42081	36	4.8	89	59.6	12.35
120	EC 457161	37	4.8	90	55.6	12.90
121	EC 457175	38	4.4	91	46.2	11.55
122	EC 457286	39	4.6	90	52.2	12.05
123	EC 457406	29	5.2	87	45.0	11.25
124	EC 457419	36	4.6	86	54.0	12.52
125	EC 49393	37	5.4	87	49.6	12.40
126	EC 65772	37	5.6	91	53.2	12.30

Contd……..

Sl. No.	Genotypes	DFF	NB	DM	NPP	SYP
127	EC 685246	33	4.2	90	43.4	10.85
128	EC 685250	30	4.8	81	42.6	10.65
129	EC 685251	33	4.6	91	38.0	9.50
130	EC 685252	33	5.4	81	54.2	12.55
131	EC 685255	29	4.8	87	50.0	12.50
132	EC 685256	30	4.4	90	40.8	10.20
133	EC 685258	33	4.6	81	39.0	9.75
134	EC 7048	33	5.6	91	69.6	12.35
135	EC 85705	32	5.6	93	69.8	12.36
136	EC 9172587	36	5.0	94	65.0	12.25
137	EC 93413	36	4.4	90	70.2	13.24
138	EC 94625	36	5.6	89	64.4	11.65
139	EC 95291	29	5.8	80	61.6	12.69
140	EC 95815	33	5.2	83	68.2	12.86
141	EC 241780 (C)	47	6.0	104	79.0	11.20
142	EC 241778 (C)	45	5.6	103	74.0	10.53
143	DSb 21 (C)	42	7.2	93	88.0	15.78
144	JS 335 (C)	35	4.8	84	48.0	8.25
	Mean	**35.6**	**4.7**	**88.5**	**51.1**	**11.47**
Range	Min	29	3.2	80	24.4	7.55
	Max	47	7.2	104	88.8	15.78

Appendix V. *Per se* performance of F_3 progeny lines of cross JS 335 x EC 241780

Progeny No.	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100SW	SYP	HI
1	41.0	59.5	5.3	89.5	52.9	4.0	21.9	2.7	13.7	14.1	45.1
2	36.5	53.9	2.5	88.5	30.7	4.2	12.8	2.7	13.2	9.6	40.7
3	38.5	37.9	5.4	87.5	70.6	3.8	34.2	2.9	12.0	22.0	53.0
4	36.5	45.6	4.0	85.5	66.2	3.7	29.2	2.7	13.9	18.7	47.2
5	37.5	51.5	4.3	86.5	58.5	3.5	25.5	2.7	12.8	16.6	46.4
6	40.0	62.3	2.8	90.0	53.3	3.8	25.4	2.9	13.3	16.3	44.6
7	39.5	57.3	3.8	89.0	47.7	3.8	21.0	2.7	12.4	14.0	45.5
8	39.5	59.7	5.0	91.5	86.0	3.9	36.9	2.9	12.4	24.9	51.7
9	40.5	59.5	3.1	91.0	48.3	3.9	24.1	3.1	13.0	15.5	45.7
10	38.5	60.9	3.6	87.5	59.3	3.6	27.4	3.0	12.0	17.9	47.0
11	37.5	49.6	2.9	89.5	62.6	3.9	25.8	2.8	12.4	17.1	47.0
12	36.5	51.9	3.9	89.5	44.6	3.8	19.8	2.8	11.8	13.1	43.0
13	39.5	60.5	5.1	89.5	78.3	4.1	36.7	2.9	12.7	24.4	50.4
14	38.5	34.8	4.1	90.5	44.5	4.4	20.7	2.9	11.4	13.9	42.0
15	36.5	49.6	4.7	89.0	66.9	3.7	29.6	2.8	13.7	19.9	48.1
16	37.5	40.2	4.9	89.5	41.8	3.7	19.8	2.9	14.1	13.8	40.7
17	39.0	44.5	4.4	89.5	46.5	4.2	21.5	2.9	12.3	14.3	44.7
18	37.5	59.7	4.8	89.5	46.5	4.2	20.7	2.8	13.9	14.5	43.9
19	39.5	63.9	3.5	92.0	48.1	3.5	20.0	2.8	12.9	14.1	44.9
20	37.5	55.7	4.1	87.5	53.9	3.7	25.8	3.0	12.4	17.1	46.8
21	38.5	57.7	5.4	90.5	79.8	3.9	35.6	2.9	13.7	23.9	52.0
22	37.5	41.7	4.5	88.0	51.9	4.1	23.4	2.9	10.7	15.7	45.8
23	39.5	57.1	4.1	89.5	44.0	3.8	19.1	2.8	12.3	13.4	41.9
24	40.5	45.6	5.9	89.5	63.7	4.1	29.5	3.0	12.5	20.6	48.1
25	38.5	39.8	3.6	88.5	37.9	4.0	15.0	2.7	11.8	10.9	39.1
26	38.5	58.5	2.5	89.5	51.7	4.1	20.3	2.7	13.9	14.1	44.9
27	37.5	43.1	3.7	88.5	39.9	3.7	18.0	2.8	13.2	12.1	41.7
28	36.5	45.9	3.2	85.5	48.2	3.8	20.3	2.7	12.1	13.2	42.7
29	39.5	51.8	3.5	89.5	46.2	4.0	21.7	2.9	13.8	14.4	46.4
30	35.5	31.7	3.5	88.5	55.7	3.8	22.1	2.7	12.2	14.8	45.0
31	41.5	44.9	2.6	91.0	54.8	3.6	24.0	2.8	12.2	15.6	45.0
32	37.0	39.1	3.7	86.5	45.4	3.7	21.9	2.9	12.9	15.0	44.2
33	39.5	48.0	3.8	90.0	39.1	3.9	18.1	2.9	14.0	13.0	42.7
34	39.0	44.8	4.8	89.0	53.9	4.2	23.0	2.8	12.1	15.0	44.6
35	37.5	54.6	5.4	87.0	51.8	4.1	22.5	2.9	13.9	14.6	44.6
36	38.5	63.1	4.8	90.5	64.6	4.0	28.1	2.8	12.9	18.7	47.9
37	39.5	49.6	4.8	90.0	61.8	3.8	29.5	2.7	12.9	19.3	48.6
38	37.0	43.4	5.1	86.5	57.6	4.1	27.1	2.9	12.2	17.9	47.0
39	39.0	44.8	5.4	90.0	49.2	3.8	20.2	2.7	12.5	14.0	44.1
40	39.5	42.8	4.5	89.5	52.8	3.5	22.8	2.9	12.8	14.9	44.2
41	37.5	40.8	3.8	88.5	61.7	3.9	29.2	2.9	12.3	19.0	47.5
42	39.0	46.2	5.1	89.0	51.2	3.7	22.7	2.8	13.2	14.8	43.6
43	38.5	54.6	4.3	88.5	46.3	3.5	21.3	2.9	13.9	15.0	44.0
44	38.0	45.4	3.4	90.0	39.8	3.8	19.5	2.9	13.2	13.3	42.6
45	37.5	39.1	3.6	88.5	37.0	3.9	16.4	2.9	13.0	12.4	39.8
46	39.0	52.7	4.4	90.0	51.9	3.7	24.1	2.9	13.0	16.9	46.0
47	37.0	45.0	4.8	85.5	52.2	4.2	21.9	2.7	13.8	14.7	44.9
48	37.5	42.9	4.1	87.0	53.0	3.6	23.0	2.8	12.4	16.0	45.9
49	38.5	48.6	4.6	89.5	52.0	4.2	23.0	2.9	11.7	15.7	45.0
50	37.5	42.7	3.7	88.5	36.8	4.2	16.9	2.9	12.4	12.1	40.8
51	38.5	43.8	4.7	89.5	62.5	4.3	27.3	2.8	11.5	18.0	46.8

Contd..

Progeny No.	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100SW	SYP	HI
52	36.5	48.8	4.1	88.0	42.9	3.9	20.7	3.0	13.2	14.0	43.0
53	37.5	35.5	5.4	88.5	51.7	3.9	22.9	2.9	11.7	14.7	44.8
54	39.5	44.6	4.8	89.5	40.6	4.3	16.2	2.7	10.6	11.8	40.7
55	39.0	40.6	5.1	88.5	55.1	4.4	25.9	2.9	10.8	16.9	45.5
56	40.0	45.0	5.0	90.0	43.7	4.2	20.8	2.9	14.0	13.9	42.1
57	37.5	55.9	4.4	88.5	44.1	4.0	19.0	2.8	13.4	13.7	42.2
58	37.5	51.7	4.1	88.5	45.9	3.6	20.0	2.8	12.8	13.8	43.0
59	38.5	45.7	5.4	88.0	51.7	4.6	25.1	3.1	13.0	17.7	46.9
60	39.5	50.9	4.5	89.5	49.1	3.8	22.9	2.9	13.1	14.9	45.0
61	39.0	59.2	5.8	89.0	72.1	4.1	31.3	2.7	12.8	20.9	49.7
62	38.5	46.9	5.9	89.5	59.1	3.9	25.9	2.8	11.7	17.0	45.9
63	36.5	46.6	4.6	87.0	51.8	4.3	24.1	2.9	12.3	15.9	45.0
64	39.5	35.7	4.4	89.0	45.3	3.8	23.1	3.0	11.9	15.6	45.0
65	40.5	54.8	3.5	89.5	38.8	3.7	16.9	2.8	10.6	12.0	40.8
66	37.5	53.9	5.3	87.0	47.8	4.4	21.8	2.8	12.5	14.7	44.1
67	38.5	33.7	5.4	88.5	53.0	3.9	26.0	3.1	11.5	17.0	46.8
68	39.0	35.1	4.1	89.5	42.3	4.1	22.9	2.9	11.9	15.1	44.1
69	41.0	57.8	4.8	89.5	51.4	4.2	22.7	2.8	12.2	14.4	44.2
70	39.5	54.7	4.5	89.0	65.3	3.9	32.9	3.0	12.3	21.9	49.7
71	40.5	43.9	5.7	89.5	59.4	4.2	27.7	2.9	12.5	18.3	46.9
72	38.5	35.6	4.5	88.0	52.0	3.6	21.7	2.8	11.3	14.7	44.0
73	37.5	50.9	4.1	89.5	48.6	3.9	23.1	3.0	12.3	15.8	45.2
74	38.5	40.9	4.5	86.5	46.2	4.0	22.6	2.9	12.4	15.1	45.8
75	39.5	41.9	3.6	88.5	52.8	4.4	22.4	2.8	12.4	14.9	44.7
76	36.5	48.7	3.3	89.5	50.8	3.5	22.0	2.8	11.1	14.7	44.1
77	37.5	46.1	3.2	86.5	42.8	3.6	18.1	2.9	11.4	12.1	42.7
78	40.0	42.9	4.4	88.5	54.0	4.1	18.3	2.7	12.2	12.1	42.3
79	38.5	49.7	3.3	89.5	36.2	3.8	15.9	2.8	11.2	10.5	39.4
80	38.5	48.9	3.6	89.5	46.9	4.4	22.6	3.0	12.4	15.2	45.0
81	38.5	45.9	4.7	89.5	51.9	4.2	26.1	3.1	11.3	17.9	49.3
82	36.5	58.1	4.5	87.0	57.9	4.4	27.7	2.9	12.4	18.9	48.0
83	41.0	52.7	3.7	89.0	44.7	3.7	21.1	2.9	11.3	14.1	44.1
84	36.5	44.6	5.3	87.5	65.0	3.4	30.0	2.9	11.4	20.1	50.4
85	38.5	40.8	4.1	89.5	44.0	3.9	21.0	3.0	11.3	14.2	45.7
86	37.5	40.0	3.3	88.0	42.1	3.6	17.8	2.7	10.8	12.3	40.4
87	38.5	54.7	3.6	88.5	45.0	4.3	20.7	2.9	11.4	14.0	43.5
88	38.5	37.0	3.3	86.5	43.6	3.7	20.2	2.9	10.9	13.2	43.0
89	37.5	49.0	3.0	88.5	52.6	4.3	22.8	2.9	13.1	14.9	44.8
90	40.5	59.7	4.5	89.5	61.9	4.3	27.1	2.8	13.1	17.8	46.7
91	38.5	41.9	4.7	88.0	43.0	3.9	20.9	2.9	12.4	14.0	43.4
92	36.5	53.0	5.1	88.5	66.8	4.3	27.0	2.7	12.5	17.6	46.6
93	37.5	50.8	4.6	87.5	45.0	4.2	20.5	2.8	13.1	14.2	44.3
94	38.5	56.4	3.6	87.5	51.7	3.9	24.7	2.9	13.1	16.9	46.0
95	39.5	43.8	3.7	89.5	42.9	4.1	18.1	2.8	13.4	12.9	41.8
96	37.5	52.6	4.5	89.5	45.6	4.0	20.1	2.9	13.3	13.9	43.0
97	40.5	35.6	4.1	90.5	36.7	3.7	14.9	2.7	11.3	11.8	39.2
98	39.5	60.7	3.3	89.5	51.9	3.9	22.7	2.8	14.2	15.4	45.7
99	38.5	61.2	5.6	89.5	72.7	4.1	31.2	2.8	13.5	20.8	49.1
100	37.5	40.0	4.3	87.5	56.0	4.3	24.0	2.8	14.0	16.2	46.0

DFF- Days to 50 % flowering; PH- Plant height (cm); NB- Number of branches per plant; DM- Days to maturity; NPP- Number of pods per plant; PL- Pod length (cm); PWP- Pod weight per plant (g); NSP- Number of seeds per pod; 100 SW- 100 Seed weight (g); HI- Harvest index (%); SYP- Seed yield per plant (g).

Appendix VI: *Per se* performance of F_3 progeny lines of cross EC 241780 x JS 335

Progeny No.	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100SW	SYP	HI
1	39.5	50.9	3.3	89.5	40.0	3.7	17.9	2.7	12.2	11.9	41.8
2	37.5	39.0	3.5	87.5	48.9	3.6	24.9	2.9	14.0	15.8	43.9
3	36.5	46.4	4.3	86.5	60.3	3.8	27.8	2.7	11.4	17.1	44.0
4	40.5	39.0	4.7	89.5	54.3	3.4	27.4	2.9	13.8	17.1	44.4
5	37.5	46.0	4.2	87.5	55.7	3.4	25.7	2.8	11.3	16.2	44.7
6	38.5	35.2	4.3	88.5	39.6	3.4	20.0	2.9	10.6	12.9	40.5
7	37.5	52.1	3.2	89.5	46.0	3.7	22.0	2.9	13.4	13.9	42.2
8	36.5	51.9	4.6	89.5	47.8	3.5	24.7	2.9	14.1	15.7	46.0
9	39.5	33.5	3.2	89.5	32.9	3.5	18.4	2.9	14.2	11.8	40.7
10	36.5	49.7	4.7	89.5	40.9	3.7	21.0	3.0	12.7	13.1	41.2
11	39.5	50.7	4.2	89.5	38.0	3.4	17.9	2.8	14.6	12.4	40.5
12	40.5	54.9	4.4	89.5	43.0	4.0	19.9	2.7	11.3	12.4	40.0
13	38.5	32.1	3.3	89.5	37.6	3.5	19.3	2.9	13.4	12.1	38.3
14	38.5	43.0	3.3	87.5	40.9	3.8	19.0	2.7	12.6	12.2	38.2
15	41.5	54.9	5.2	89.5	64.8	3.7	32.8	2.9	11.9	18.0	46.9
16	37.5	43.1	4.7	86.5	56.0	3.3	27.7	2.9	10.9	16.7	44.8
17	38.5	52.1	5.1	89.5	48.8	3.6	21.8	2.7	11.3	13.8	41.9
18	39.5	44.6	3.3	87.5	41.8	3.4	21.7	2.9	11.6	13.7	39.9
19	37.5	36.9	3.6	87.5	46.2	3.4	23.8	2.9	13.6	14.9	44.2
20	39.5	57.7	4.7	89.5	52.9	3.4	24.9	2.7	14.4	15.2	42.9
21	38.5	27.8	3.1	89.5	39.0	3.7	19.0	2.9	11.4	12.0	38.7
22	37.5	35.2	4.6	87.5	44.8	3.8	23.1	3.0	12.5	14.7	43.2
23	40.5	44.9	3.6	89.5	44.7	3.5	21.1	2.7	13.2	13.2	41.3
24	37.5	39.1	4.1	87.5	42.6	3.6	19.8	2.9	14.4	12.7	40.5
25	37.0	38.1	4.5	89.5	61.6	3.6	32.2	3.0	12.4	19.1	48.2
26	38.5	39.6	4.3	89.0	50.6	3.5	22.7	2.7	11.3	14.0	43.8
27	37.5	32.9	3.7	88.5	38.1	3.6	19.7	2.9	10.7	12.6	40.5
28	40.5	37.8	3.9	89.5	48.8	3.6	22.9	2.8	13.4	14.3	42.8
29	39.5	55.0	4.3	89.0	46.6	3.3	23.8	2.9	13.1	14.9	42.7
30	38.5	42.7	2.6	89.0	40.7	3.4	19.7	2.9	11.8	12.4	40.4
31	37.5	37.6	3.0	88.5	32.0	3.8	14.7	2.7	13.1	10.8	38.4
32	36.5	32.7	2.5	87.5	38.9	3.4	19.8	3.0	12.1	12.9	41.3
33	40.5	40.8	3.8	90.5	46.0	3.5	21.7	2.9	13.1	13.9	43.2
34	38.5	56.9	2.9	89.5	39.6	3.6	19.2	2.9	13.2	12.6	40.9
35	37.5	39.0	4.4	88.5	48.2	3.8	25.0	2.9	11.6	15.9	44.0
36	38.5	38.0	3.5	90.5	39.1	3.8	19.0	2.9	11.9	12.4	40.4
37	39.5	37.2	2.9	89.5	44.0	3.6	23.0	2.9	11.4	14.3	43.2
38	39.5	43.9	4.6	89.5	60.1	3.8	27.1	2.8	13.3	16.8	44.9
39	38.5	57.1	5.3	88.5	52.4	3.4	26.2	2.9	10.9	16.0	44.1
40	40.5	41.1	3.9	90.5	41.6	3.6	18.3	2.7	14.2	11.9	43.1
41	40.5	48.8	5.3	89.5	55.5	3.4	28.8	2.9	13.4	17.3	48.3
42	38.5	48.8	4.9	89.5	49.6	4.1	23.8	2.9	14.5	14.9	46.0
43	38.5	49.0	3.1	89.5	38.7	3.6	19.8	2.9	13.5	12.9	43.9
44	39.5	51.8	5.1	90.0	39.7	3.7	19.2	2.9	12.4	12.0	44.0
45	40.5	42.1	3.7	90.5	41.9	3.9	19.3	2.8	13.7	12.3	44.2
46	40.5	60.1	5.8	90.0	40.0	3.6	18.8	2.8	14.1	13.4	41.7
47	41.0	39.9	4.3	90.5	44.0	3.5	20.7	2.7	10.7	13.2	43.9
48	39.5	48.0	3.9	90.0	45.6	3.4	20.0	2.7	13.0	13.3	43.2
49	39.0	38.5	4.9	90.0	53.9	3.8	26.0	2.9	10.7	16.3	47.1
50	37.5	40.9	3.8	88.5	49.8	3.8	24.1	2.9	10.5	15.0	46.5
51	37.5	32.2	4.5	88.5	44.8	3.6	19.9	2.7	14.1	12.8	42.0

Contd..

Progeny No.	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100SW	SYP	HI
52	37.5	43.0	4.7	89.5	50.1	4.0	24.2	2.9	13.6	15.1	44.6
53	40.5	66.0	5.1	90.5	46.9	3.5	21.3	2.7	12.6	13.2	43.2
54	40.5	58.0	5.3	91.5	50.4	3.6	25.9	3.0	14.2	16.2	45.7
55	38.5	50.4	4.1	87.5	49.6	3.7	22.0	2.7	12.3	14.0	45.0
56	37.5	36.9	3.9	88.5	48.1	3.6	22.2	2.8	13.4	14.1	44.9
57	37.5	44.9	3.7	88.0	40.3	3.5	20.8	2.9	12.5	13.1	43.7
58	39.5	49.8	3.9	90.0	51.4	3.6	24.9	2.9	13.4	15.8	45.6
59	40.5	53.9	4.5	90.5	58.2	3.6	29.9	2.9	13.4	16.7	48.6
60	37.0	47.4	4.1	89.0	49.1	3.9	23.2	2.9	11.3	14.2	45.2
61	38.5	45.0	5.2	89.5	52.6	3.7	25.2	2.9	12.7	15.9	47.1
62	37.5	39.0	3.7	89.5	41.9	3.8	21.7	2.9	12.5	13.8	43.6
63	40.5	46.1	5.3	90.5	58.0	4.1	27.9	2.9	11.3	17.6	47.5
64	40.5	37.0	3.5	90.0	31.8	3.5	15.1	2.7	12.3	9.8	38.9
65	40.5	52.6	4.9	90.5	42.0	3.3	18.1	2.7	13.4	12.0	43.9
66	38.5	36.6	2.9	88.5	43.6	3.5	20.0	2.7	11.3	12.8	42.1
67	39.5	42.6	2.7	89.0	33.1	3.9	14.8	2.6	11.6	10.3	38.5
68	38.5	37.7	3.3	88.5	39.2	3.9	20.0	2.9	13.2	12.8	43.3
69	37.5	49.8	5.3	88.5	54.1	3.8	28.0	2.9	12.3	17.7	45.5
70	37.5	39.5	4.7	88.5	45.9	3.6	22.1	2.9	11.9	13.9	44.1
71	37.5	44.4	4.8	88.5	40.8	4.9	19.7	2.8	11.9	12.6	43.4
72	38.5	49.0	5.3	89.5	53.6	3.6	25.8	2.8	11.6	16.0	46.8
73	40.5	44.8	4.1	90.5	51.3	3.4	25.0	2.9	13.3	15.8	46.2
74	39.5	38.9	4.0	90.0	41.9	3.5	20.0	2.7	13.6	12.5	41.9
75	39.0	32.7	3.9	88.5	41.8	3.7	19.9	2.8	11.9	12.7	42.2
76	39.5	41.9	3.9	89.5	48.1	3.8	22.2	2.8	10.9	14.0	44.5
77	39.5	43.7	4.3	90.5	39.1	3.5	17.8	2.7	11.3	11.1	40.5
78	37.5	41.9	3.9	89.0	47.7	3.8	18.0	2.6	12.4	11.6	41.4
79	38.5	48.1	5.1	89.5	52.1	3.5	25.2	2.8	11.5	15.8	46.3
80	38.5	44.7	4.5	89.5	40.2	3.6	20.3	2.8	12.2	12.7	42.4
81	37.5	35.0	4.1	88.5	38.1	3.6	20.9	3.3	10.6	13.0	43.4
82	37.5	38.9	3.5	88.5	43.7	3.8	20.9	2.7	11.2	13.1	42.9
83	37.5	41.7	4.3	87.5	44.0	3.7	18.2	2.6	13.6	12.0	41.8
84	40.5	48.0	5.1	90.5	52.8	3.5	24.2	2.7	12.6	14.9	44.3
85	37.5	44.9	3.4	88.5	41.8	3.7	21.6	2.9	11.1	13.7	44.5
86	38.5	38.8	4.4	88.5	38.2	3.8	19.8	2.9	13.7	12.8	41.3
87	39.5	42.9	4.8	89.0	43.2	3.8	20.7	2.8	13.5	13.1	42.4
88	37.5	43.7	4.3	89.5	40.4	3.5	20.7	2.9	13.6	13.1	42.4
89	41.5	46.7	4.7	90.5	40.4	3.2	19.0	2.7	13.4	12.2	41.5
90	40.5	41.8	3.9	89.5	48.1	3.9	23.2	2.8	12.5	14.7	45.3
91	38.5	43.8	3.5	88.0	46.9	3.6	21.9	2.7	13.4	13.8	43.2
92	37.5	60.4	5.7	89.5	40.9	3.5	19.3	2.7	12.6	11.9	41.1
93	40.5	46.5	4.1	88.0	44.1	3.9	23.2	2.9	12.3	14.1	44.9
94	38.5	37.8	4.4	89.5	58.7	3.6	26.8	2.7	11.6	16.7	46.2
95	37.5	35.0	4.3	88.0	50.2	3.7	24.3	2.8	12.8	15.0	46.1
96	37.5	55.8	5.3	88.0	52.9	3.4	23.0	2.7	10.9	14.1	44.6
97	40.5	45.2	5.7	90.5	71.0	3.5	35.0	2.9	11.0	21.7	51.6
98	41.0	48.9	4.3	90.5	54.0	3.4	22.9	2.7	11.4	14.5	44.1
99	40.5	43.0	4.3	90.0	45.7	3.4	22.3	2.8	12.9	13.8	44.2
100	42.5	36.1	3.7	92.0	45.5	3.5	21.3	2.7	13.4	13.1	45.0

DFF- Days to 50 % flowering; PH- Plant height (cm); NB- Number of branches per plant; DM- Days to maturity; NPP- Number of pods per plant; PL- Pod length (cm); PWP- Pod weight per plant (g); NSP- Number of seeds per pod; 100 SW- 100 Seed weight (g); HI- Harvest index (%); SYP- Seed yield per plant (g).

Appendix VII: *Per se* performance of F_3 progeny lines of cross DSb 21 x EC 241780

Progeny No.	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100SW	SYP	HI
1	40.5	57.0	4.6	91.0	44.7	3.4	25.3	2.9	13.4	14.7	43.5
2	42.5	58.7	5.4	93.0	66.3	3.5	33.7	2.8	12.3	19.3	46.0
3	42.5	65.8	5.3	95.5	72.8	3.8	40.4	3.0	14.3	20.7	47.9
4	40.5	70.9	4.5	91.5	52.2	3.8	28.2	2.9	11.3	17.5	46.5
5	40.5	56.8	4.3	93.5	42.2	3.7	23.7	2.9	13.2	14.0	43.7
6	40.5	62.7	4.5	91.5	52.8	3.4	27.3	2.9	12.6	16.1	45.1
7	41.5	51.9	4.9	91.0	45.0	3.7	24.0	2.9	12.5	14.4	41.0
8	42.5	61.9	4.6	94.0	66.3	3.7	31.3	2.9	13.1	18.7	45.7
9	41.5	64.9	4.5	92.0	67.1	3.7	37.2	2.9	12.4	21.7	49.0
10	40.5	61.8	4.4	91.5	41.6	3.7	21.5	2.9	14.0	13.1	42.6
11	42.0	50.9	4.0	92.5	40.7	3.4	23.3	2.9	11.3	13.0	41.7
12	40.5	53.9	4.1	93.0	46.9	3.8	24.6	2.9	14.2	14.5	43.1
13	41.0	59.3	5.6	93.0	61.5	3.6	29.9	2.7	12.5	17.2	45.5
14	42.0	44.6	5.0	91.5	65.4	3.7	32.9	2.7	13.3	19.1	47.4
15	43.5	47.4	3.4	92.5	48.6	3.6	26.4	2.7	13.2	15.0	43.6
16	41.5	65.8	4.0	91.0	43.6	3.3	20.2	2.7	13.4	12.1	40.8
17	42.5	42.2	3.9	90.5	43.0	4.1	22.1	2.7	12.2	13.0	43.3
18	42.5	64.8	5.1	93.5	58.1	3.7	27.8	2.7	13.2	16.3	44.6
19	41.5	50.8	3.7	91.5	52.6	3.9	32.7	3.5	13.2	19.1	45.7
20	41.0	61.7	4.6	92.0	50.4	3.5	25.8	2.8	14.1	15.1	44.7
21	43.5	54.8	3.5	94.5	34.2	3.4	18.3	2.7	13.4	11.8	38.8
22	41.5	59.0	5.1	94.5	55.0	3.6	26.2	2.7	13.3	15.9	44.7
23	42.5	51.2	3.4	92.5	33.6	3.4	19.0	3.1	13.2	11.0	39.7
24	40.5	62.0	5.1	93.5	59.0	3.5	28.3	2.7	13.8	16.9	45.7
25	42.5	59.5	5.3	94.5	61.5	3.9	29.4	2.7	12.6	17.7	45.4
26	41.5	65.7	5.9	92.0	62.2	3.6	31.9	2.9	13.7	18.9	46.3
27	42.5	52.3	4.4	93.5	49.0	3.8	27.7	2.9	12.6	16.1	44.2
28	40.5	62.8	4.5	92.0	51.9	3.4	24.8	2.7	11.4	14.9	43.5
29	41.5	69.0	6.1	94.5	65.9	3.6	39.1	2.9	13.3	22.8	49.9
30	40.5	59.7	4.1	94.5	42.6	3.6	22.3	2.8	11.3	13.1	41.4
31	41.5	51.4	5.2	95.5	56.6	3.6	32.8	3.1	14.3	19.3	46.1
32	41.5	51.8	3.7	94.5	53.9	3.3	29.0	2.9	13.2	17.0	43.0
33	41.5	64.9	4.7	94.0	48.3	3.7	24.2	2.8	11.6	14.7	42.8
34	40.5	61.7	5.3	91.5	52.9	3.6	29.5	2.9	13.7	17.2	44.1
35	40.5	46.1	4.7	92.0	54.8	3.8	26.1	2.7	12.7	15.6	44.2
36	41.5	56.8	3.7	92.0	41.1	3.8	21.3	2.8	10.6	12.3	39.9
37	41.0	58.1	3.9	92.5	58.2	3.7	27.8	2.7	12.7	16.1	42.8
38	40.5	59.9	4.3	92.0	61.6	3.7	31.6	2.9	13.6	18.9	46.3
39	42.5	63.8	3.4	92.5	37.7	3.5	18.9	2.7	12.0	11.4	37.6
40	42.5	70.0	5.7	95.0	58.2	3.6	34.1	3.1	12.3	20.0	47.1
41	42.5	57.9	4.6	91.5	60.7	3.4	30.6	2.8	13.4	17.9	44.0
42	41.5	62.8	6.0	90.5	76.3	3.5	38.2	2.8	13.2	22.6	46.3
43	40.5	65.7	4.1	92.5	60.9	3.7	35.7	3.1	11.6	21.0	48.3
44	40.5	64.9	3.1	92.5	40.9	3.7	21.0	2.7	12.2	12.4	41.3
45	41.5	55.8	5.3	95.5	52.0	3.6	28.8	3.0	13.5	17.1	44.0
46	40.5	57.7	5.7	90.5	74.8	3.7	37.5	2.7	13.4	21.9	47.2
47	40.5	69.6	3.3	92.5	43.0	3.8	20.9	2.7	13.7	12.2	40.4
48	40.5	63.8	5.1	90.5	51.0	3.5	26.0	2.7	12.2	15.0	42.2
49	40.5	55.6	4.7	91.5	65.6	3.8	33.8	2.8	13.2	19.8	47.0
50	40.5	63.8	6.3	91.5	58.1	3.4	31.7	2.9	13.7	18.2	44.9
51	41.5	63.6	4.8	92.5	51.9	3.6	24.1	2.7	12.3	14.1	42.1

Contd..

Progeny No.	DFF	PH	NB	DM	NPP	PL	PWP	NSP	100SW	SYP	HI
52	40.5	49.2	3.5	91.5	38.1	3.5	18.2	2.7	12.2	10.9	39.0
53	41.5	43.9	3.7	92.5	44.8	3.5	22.7	2.8	12.4	13.4	40.7
54	42.5	58.0	4.6	94.5	43.7	3.4	20.2	2.7	12.3	12.2	40.3
55	41.5	37.4	4.7	91.5	39.8	3.7	21.2	2.9	12.4	12.2	40.2
56	41.5	64.6	3.3	91.5	38.2	3.5	20.8	2.9	14.1	11.8	38.1
57	40.5	50.7	4.8	91.0	45.2	3.6	23.2	2.9	14.1	13.8	43.0
58	40.5	45.9	4.1	91.0	40.1	3.4	20.2	2.7	12.1	11.8	38.0
59	41.5	51.8	3.7	91.5	37.4	3.6	21.0	2.9	13.3	12.1	39.2
60	42.5	61.9	4.7	94.0	51.7	3.4	25.2	2.7	12.5	14.7	42.6
61	40.5	72.9	4.1	91.5	43.6	3.4	22.1	2.7	12.2	13.1	42.3
62	40.5	53.6	4.5	91.0	44.2	3.5	21.9	2.8	13.2	12.9	41.7
63	41.5	50.8	5.9	92.0	74.2	3.6	35.2	2.7	11.3	20.8	48.3
64	40.5	42.9	3.6	91.5	37.2	3.5	17.1	2.7	11.4	11.2	39.2
65	40.5	62.9	4.7	91.0	49.0	3.4	27.0	2.9	10.4	15.9	43.7
66	41.0	45.4	4.8	90.0	48.8	3.7	25.3	2.8	10.7	14.8	43.4
67	41.5	42.1	4.8	91.0	50.5	3.7	25.7	2.7	11.2	14.8	43.8
68	40.5	56.8	4.9	91.0	45.4	3.6	24.0	2.8	13.5	13.8	43.6
69	42.0	54.2	4.2	92.0	47.6	3.4	23.6	2.7	13.8	13.3	41.2
70	42.5	62.0	5.3	92.5	57.9	3.7	34.5	3.1	12.7	20.0	47.6
71	43.5	63.8	3.7	94.5	49.8	3.5	27.5	3.0	11.9	16.1	44.1
72	41.0	49.5	4.1	91.5	47.6	3.6	28.0	2.9	13.1	16.2	43.4
73	39.0	38.3	4.5	90.5	62.3	3.6	32.2	2.8	13.4	19.0	46.1
74	41.0	48.4	3.5	91.5	41.2	3.5	21.3	2.7	11.4	12.1	41.0
75	40.0	65.8	4.7	92.0	58.9	3.6	35.0	3.1	11.9	20.7	47.6
76	42.5	52.6	3.7	94.5	46.0	3.7	24.4	2.8	12.0	13.9	42.7
77	43.5	51.5	5.9	94.0	49.1	3.6	26.9	2.9	11.3	16.0	43.4
78	42.5	59.4	4.1	92.5	51.8	3.5	28.2	2.9	13.3	17.0	44.3
79	42.0	46.7	5.8	92.0	63.5	3.6	35.3	2.9	12.3	20.7	47.9
80	43.0	58.6	3.8	93.0	55.0	3.6	29.0	2.9	12.5	17.0	45.3
81	41.0	49.3	5.3	91.0	54.0	3.4	28.2	2.7	13.2	16.6	44.7
82	40.0	38.8	5.6	90.5	66.0	3.5	34.2	2.8	12.1	19.8	47.3
83	41.0	74.5	3.7	92.0	45.9	3.4	23.7	2.8	11.4	13.7	42.0
84	44.0	66.0	2.5	96.0	39.9	3.5	19.4	2.7	12.7	11.7	40.1
85	41.0	38.8	3.4	92.5	37.7	3.4	19.0	2.7	13.2	11.3	37.2
86	41.0	51.3	5.3	91.0	54.8	3.6	32.5	3.1	13.1	18.9	47.2
87	41.0	53.6	4.1	91.5	41.1	3.5	22.2	2.9	11.2	13.0	43.4
88	40.0	50.3	3.8	90.0	48.2	3.7	25.3	2.8	11.5	14.7	43.5
89	41.0	54.5	5.3	91.5	55.7	3.6	29.1	2.7	13.3	16.8	44.4
90	42.0	54.2	3.3	92.5	34.3	3.6	19.2	2.7	12.7	11.2	39.2
91	43.0	34.7	3.3	92.0	30.4	3.5	21.4	3.1	13.4	12.3	40.4
92	40.5	53.0	3.5	91.0	42.9	3.4	22.2	2.8	13.7	13.2	41.5
93	42.0	44.0	5.6	91.0	60.5	3.5	29.2	2.7	12.8	16.8	44.2
94	40.0	55.0	4.9	91.0	47.0	3.5	26.2	2.9	12.6	15.0	41.5
95	41.0	54.5	2.9	92.0	37.9	3.7	19.7	2.7	12.3	11.7	37.9
96	42.5	56.6	5.3	91.0	59.1	3.8	32.6	2.9	13.2	18.9	47.5
97	41.0	42.5	5.6	91.5	57.1	3.6	27.2	2.7	12.8	16.0	45.1
98	42.0	53.0	5.7	92.5	69.9	3.7	33.6	2.7	10.6	19.8	48.1
99	41.0	40.4	5.9	91.5	57.4	3.6	27.3	2.7	12.3	16.0	43.4
100	39.0	41.3	3.1	90.5	32.3	3.6	19.4	2.9	13.2	11.1	39.5

DFF- Days to 50 % flowering; PH- Plant height (cm); NB- Number of branches per plant; DM- Days to maturity; NPP- Number of pods per plant; PL- Pod length (cm); PWP- Pod weight per plant (g); NSP- Number of seeds per pod; 100 SW- 100 Seed weight (g); HI- Harvest index (%); SYP- Seed yield per plant (g).

GENETIC ANALYSIS FOR RESISTANCE TO RUST IN SOYBEAN [*Glycine max* (L.) Merrill]

SURESHA, P. G. **2019** **Dr. G. T. BASAVARAJA**
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ABSTRACT

The present investigation was carried out during 2015-17 at the MARS, UAS, Dharwad and R & D Farm, Ugar Sugar Works, Ugarkhurd with different experiments. Evaluation of 144 exotic germplasm lines including highly susceptible check JS335 and resistant checks *viz.*, DSb21, EC241780 and EC241778 were evaluated during *kharif* 2015 at Dharwad for identification of new sources for resistance to rust and genetic diversity. Based on the resistance reaction, 22 lines which exhibited resistant/moderately resistant reaction were selected and further evaluated to confirm their resistant reaction under natural epiphytotic condition at two hotspots for rust *viz.*, Dharwad and Ugarkhurd during *kharif* 2016. Three genotypes *viz.*, DSb21, JS 335 and EC241780 were utilized for hybridization during summer 2015 to study the inheritance pattern for rust resistance and variability. Subsequently, F_2 and F_3 populations were raised during *kharif* 2016 & 2017 respectively. In addition to this, validation of molecular markers linked to rust resistance in F_2 population of cross JS335xEC241780 was carried out.

Among 144 exotic germplasm lines including resistant and susceptible checks, only one line EC242104 and resistant checks recorded disease grade 1 with highly resistant reaction. The k-*means* cluster analysis for yield related traits in 144 germplasm lines were grouped into eight clusters.

Inheritance study revealed that rust resistance is controlled by single dominant gene in all the crosses. The resistant genotypes can be used as a confirmed source of resistance and utilized in future resistance breeding programmes. The F_3 families of three crosses, *viz.*, JS335 x EC241780, EC241780 x JS335 and DSb21 x EC241780 have generated sufficient variability and also nine superior segregants.

Study on validation of molecular markers, only three markers Satt 361, Satt 275 and Satt 215 exhibited polymorphism. The polymorphic markers were further analysed by single marker analysis which showed a significant association with rust resistance.