# VALIDATION OF LATE LEAF SPOT AND RUST RESISTANCE-LINKED MARKERS AND TRANSFER OF ASSOCIATED QTL TO JL 24 IN GROUNDNUT (*Arachis hypogaea* L.)

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By
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## CERTIFICATE

This is to certify that the thesis entitled "VALIDATION OF LATE LEAF SPOT AND RUST RESISTANCE-LINKED MARKERS AND TRANSFER OF ASSOCIATED QTL TO JL 24 IN GROUNDNUT (*Arachis hypogaea* L.)" submitted by SHARANABASAPPA B. YERI for the degree of DOCTOR OF PHILOSOPHY in MOLECULAR BIOLOGY AND BIOTECHNOLOGY, of college of Agriculture, University of Agricultural Sciences, Dharwad is a record of 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 for the award of any degree, diploma, associateship, fellowship or other similar titles.

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# LIST OF ABBREVIATIONS

| Particulars | Abbreviations |
|---|---|
| U | Unit |
| l | Litre (s) |
| hr | Hour (s) |
| m | Metre (s) |
| gm | Gram (s) |
| Sq | Square |
| T | Tonne (s) |
| % | Per cent |
| min | Minute (s) |
| Sec | Second (s) |
| ha | Hectare (s) |
| bp | Base pair |
| µl | Microliter (s) |
| mM | Millimolar |
| ml | Millilitre (s) |
| $^{\circ}C$ | Degree celsius |
| mg | Milligram (s) |
| kg | Kilogram (s) |
| $T_{10}E_1$ | Tris (10 mmol)-EDTA (1 mmol) |
| pmol | Picomole (s) |
| ng | Nanogram (s) |
| cm | Centimetre (s) |
| cM | Centi Morgan |
| LLS | Late leaf spot |
| Tm | Melting temperature |
| $R^2$ | Per cent phenotypic variance explained |
| QTL | Quantitative trait loci |
| Rpm | Revolutions per minute |
| PAGE | Polyacrylamide gel electrophoresis |
| CTAB | Cetyl-trimethyl-ammonium Bromide |
| EDTA | Ethylenediamine tetra-acetic acid |
| RILs | Recombinant inbred lines |
| NILs | Near isogenic lines |
| HIFs | Heterogeneous inbred families |

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# 1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed, food and forage legume crop belonging to Fabaceae. It originated in South and Central America, probably in the foothills of Andes Mountain where it was domesticated ~3,500 years ago and later spread to the tropical and sub-tropical regions of the world. The crop is cultivated over 25.4 mha with a production of about 45.2 mt globally (FAO, 2013). In India, it is grown in an area of 5.3 mha with a production of 9.5 mt (FAO, 2013). The major states cultivating groundnut include Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka and Maharashtra.

Groundnut suffers from several biotic and abiotic stresses. Most of the improved groundnut varieties belong to Spanish bunch botanical type under *A. hypogaea* ssp. *fastigiata*, and they are highly susceptible to foliar diseases namely Late Leaf Spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & Curt.) Van Arx. and rust caused by *Puccinia arachidis* Speg. LLS and rust reduce groundnut productivity by 50% in tropical and sub-tropical countries when fungicides are not applied (Subrahmanyam *et al*., 1984; Subrahmanyam *et al*., 1985; Waliyar, 1990). Co-occurrence of LLS and rust together can cause up to 70 per cent yield loss in India (Subrahmanyam *et al*., 1984; Subrahmanyam *et al*., 1985; Waliyar, 1990; Shokes and Culbreath, 1997). Management of LLS and rust using chemicals not only increases the cost of cultivation but also leads to environmental and health hazards. Hence, development of resistant cultivars is considered as the promising strategy.

Several sources of resistance have been identified both in cultivated and wild types. However, breeding for foliar disease resistant genotypes has met with limited success due to various factors. Genetic studies have shown that resistance to LLS and rust was mostly controlled by recessive genes. Also, foliar disease resistance is generally associated with low productivity, delayed maturity and unacceptable pod and kernel features. Overcoming these challenges requires raising a large segregating population. Since LLS and rust occur together and interfere with each other, a precise selection strategy is necessary. Under these circumstances, development and use of genomic resources could complement the conventional breeding methods.

Substantial efforts have been made in groundnut to develop genomic resources such as identification of diverse parent(s), development of mapping populations, development of molecular markers, identification of Quantitative Trait Loci (QTL), and markers linked to several important traits (Bhat *et al*., 2012; Pandey *et al*., 2012a; Pandey *et al*., 2012b; Varshney *et al*., 2013; Pandey *et al*., 2014). In the recent past, molecular marker technology has witnessed tremendous advancement in groundnut genomics by integrating genomic tools with conventional breeding approaches to enhance the precision and speedy development of improved cultivars (Varshney *et al*., 2005). Linkage maps have been developed for diploids involving A-genome (*Arachis duranensis*) (Halward *et al*., 1993; Milla, 2003; Garcia *et al*., 2005; Moretzsohn *et al*., 2005; Leal-Bertioli *et al*., 2009; Nagy *et al*., 2010), K genome (*Arachis batizocoi*) (earlier classified as B genome) (Gobbi *et al*., 2006; Moretzsohn *et al*., 2009) and tetraploid (cultivated) species (Fonceka *et al*., 2009; Varshney *et al*., 2009b; Hong *et al*., 2010; Khedikar *et al*., 2010; Ravi *et al*., 2011; Sarvamangala *et al*., 2011; Gautami *et al*., 2012; Sujay *et al*., 2012). Some of these maps were subsequently used to identify QTL governing abiotic stress tolerance, productivity, oil quality and resistance to LLS, rust, *Asperigillus* and aphid.

At University of Agricultural Sciences (UAS), Dharwad, a considerable progress has been made in developing the genomic resources in groundnut for improving late leaf spot and rust resistance. In this regard, two Recombinant Inbred Line (RIL) populations (TAG 24 × GPBD 4 and TG 26 × GPBD 4) were developed (Bhat *et al*., 2012). QTL analysis with extensive marker data and phenotypic data identified a common genomic region on linkage group AhXV governing both LLS and rust resistance (Khedikar *et al*., 2010; Sujay *et al*., 2012). Two QTL flanked by GM2009-GM1536 and IPAHM103-GM1954 were detected within this region. These two major QTL explained up to 82.27% and 82.96% phenotypic variance for rust, respectively. In addition, a third QTL flanked by GM1536-GM2301/GM2079 was detected only in TAG 24 × GPBD 4, which alone could explain 62.35% of phenotypic variance for rust. GM2009-GM1536, IPAHM103-GM1954 and GM1536-GM2301/GM2079 were also linked to LLS resistance and contributed up to 67.98%, 42.66% and 17.37% of phenotypic variance, respectively. Again, GM1536-GM2301/GM2079 was detected only in TAG 24 × GPBD 4. A second

genomic region governing LLS was mapped on linkage group AhXII. This region carried a major QTL (GM1573/GM1009-pPGPseq8D09) contributing up to 10.27–62.34% phenotypic variance (Sujay *et al.*, 2012).

A large number of mutants differing for LLS resistance were also developed at UAS, Dharwad (Gowda *et al.*, 1996). These mutants were analyzed with a new class of transposable element-specific marker (*AhMITE1*-PCR). Origin of LLS resistant mutants at very high frequency was associated with the transposition of *AhMITE1* as detected by *AhMITE1*-PCR (Gowda *et al.*, 2010).

The markers linked to LLS and rust resistances were validated using diverse germplasm and other mapping populations. Six LLS and rust resistance-linked markers (IPAHM103, GM1536, GM1954, GM2009, GM2301 and GM2079) and three markers linked to only LLS (GM1009, GM1573 and pPGPseq8D09) were validated using the RILs of TG 19 × GPBD 4 and three introgression line populations from ICGS 76 × ISATGR 278-18, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5 (Varshakumari, 2013; Sukruth *et al.*, 2014).

Further, a strong validation of the QTL and the linked markers might also come from Near Isogenic Lines (NILs), which share common genetic background, but differ for only a small genomic region (Paterson *et al.*, 1990; Kaeppler *et al.*, 1993). NILs are generally developed by backcross breeding (Young *et al.*, 1988; Paterson *et al.*, 1990), where the genomic region governing a trait is replaced with a donor genome. However, the development of NILs by this method requires several generations of backcrossing and selection. When many different loci are being investigated, the cost and labor associated with this procedure can become prohibitive. This is especially true for groundnut since it is a self-pollinated crop.

An alternative method of developing NILs utilizes a selfing and selection scheme (Allard, 1960; Fehr, 1987; Haley *et al.*, 1994). In this approach, the inbred lines that are not entirely homozygous are identified in advanced generations. Such lines segregating for a few loci or a trait are called as Heterogeneous Inbred Families (HIFs). The plants with contrasting phenotype for a trait are selected from a HIF, and the progenies are grown to develop NILs. HIFs offer a time and cost effective source of NILs as compared to backcross lines (Tuinstra *et al.*, 1997). Such NILs have been developed for seed weight in sorghum (Tuinstra *et al.*, 1997),

root traits in Arabidopsis (Loudet *et al.*, 2005) and fusarium wilt in chick pea (Castro *et al.*, 2010).

A large number of HIFs segregating for LLS and rust resistance have been developed at UAS, Dharwad from TAG 24 × GPBD 4 and TG 26 × GPBD 4. Also, the plants differing for LLS and rust resistance within such HIFs were selected and advanced to develop stabilized lines. These lines can be evaluated and characterized to identify the NILs for LLS and rust resistance. Further, such NILs identified from a large number of HIFs can be used to validate QTL and markers linked to LLS and rust resistance. In addition, a new RIL mapping population of VL 1 × 110, developed at UAS, Dharwad can also serve as the genomic resource for marker validation. Once validated, the QTL and markers can be deployed for marker assisted selection (MAS) and marker assisted backcrossing (MABC) to improve LLS and rust resistance in an otherwise susceptible genotype of groundnut. This study mainly aimed at validation of QTL and markers linked to LLS and rust resistance, and marker-assisted backcrossing of JL 24, a susceptible variety to improve resistance for LLS and rust, with the following objectives

1. To validate QTL/markers linked to LLS and rust resistance in groundnut by
    a. identifying and characterizing the NILs from HIFs
    b. analyzing the marker-trait co-segregation among the NILs
    c. single marker analysis using the RILs of VL 1 × 110
2. To develop backcross populations in JL 24 for improving LLS and rust resistance

# 2. REVIEW OF LITERATURE

Groundnut (*Arachis hypogaea* L.) is an important food crop worldwide with majority of the produce crushed for oil. Domesticated groundnut suffers from various diseases and insect pests. Late leaf spot and rust are widespread, and cause intense economic losses. Therefore, groundnut breeding programs are focusing on improving disease resistance. Recently, conventional breeding methods are complemented with the genomic resources such as molecular markers, QTL and Marker Assisted Selection (MAS) for enhancing the efficiency of selection and variety development. Literature pertaining to major diseases of groundnut, breeding strategies, molecular markers and MAS in groundnut is reviewed in this chapter.

## 2.1 Foliar diseases of groundnut

Foliar diseases are the major constraints to groundnut production. Late Leaf Spot (LLS), Early Leaf Spot (ELS) and rust are the most widely distributed and economically important foliar diseases of groundnut causing severe yield loses (McDonald *et al.*, 1985; Kokalis-Burelle, 1997). They commonly occur wherever groundnut is grown, but their incidence and severity vary with location and season. Individually each disease is capable of causing substantial yield loss to a tune of more than 50% when fungicides are not applied. Co-occurrence of LLS and rust together can cause up to 70 per cent yield loss in India (Subrahmanyam *et al.*, 1984; Subrahmanyam *et al.*, 1985; Waliyar, 1990; Shokes and Culbreath, 1997). These diseases also have an adverse effect on the recovery of pod, quality of seeds and haulm.

### 2.1.1 Rust

Rust caused by *Puccinia arachidis* Speg., is one of the most destructive fungal diseases in almost all groundnut growing areas of the world. It was first noted by Spegazzini in 1884. Rust occurs in most of the groundnut growing Indian states and more intensively in South Indian states as the growing condition favors the development and spread of the disease (Subrahmanyam and McDonald, 1982). Yield loss caused by rust can range from 50-80 per cent (Sandhikar *et al.*, 1989).

Rust disease is characterised by orange-coloured raised pustules on the lower surface of the leaflets, which rupture to release masses of reddish-brown uredospores. In susceptible cultivars, the pustules may be surrounded by colonies of secondary pustules. When disease is severe, the pustules may also appear on upper surface of the leaflets opposite to those on lower surface and appear like a yellowish mosaic spots. Lesions can be formed on all the aerial parts except flowers, while those on stem are usually elongated. Infected leaves turn necrotic and dry up but tend to remain attached to the plant. Generally, rust coincides with LLS, and the symptoms of rust are masked and cannot be distinguished due to defoliation (Subrahmanyam *et al*., 1995).

### 2.1.2 Late leaf spot

Late Leaf Spot (LLS) caused by *Cercospora personatum* (Berk and Curt) is distributed throughout the world. Recently, the pathogen was renamed as *Phaeoisariopsis personata* (Berk and curt) V. Arx. It is more predominant compared to early leaf spot because of its fast-spreading nature. It was first described in USA during 1875. The pathogen perpetuates from season to season only on infected plant debris and volunteer groundnut plants, building up an inoculum reservoir for the following season (Subrahmanyam *et al*., 1995).

Late leaf spot is characterised with nearly circular dark spots. The spots are more prominent on the abaxial (lower) surface of the leaf than adaxial (upper) surface. The sporulation is confined to the lower leaf surface where the spot turns to black lesion with slightly rough appearance. Late leaf spot can be easily distinguished from early leaf spots, which are lighter in colour and are characteristically surrounded by a yellowish chlorotic halo. When the disease attack is severe, the affected leaflets turn to chlorotic, then necrotic and the lesions often coalesce resulting in premature senescence and shedding of the leaflets. Further, the disease also spreads to petioles, stem, stipules and pegs. LLS usually coincide with rust and cause complete defoliation (Subrahmanyam *et al.*, 1995).

## 2.2 Components of resistance to rust and late leaf spot

### 2.2.1 Rust

The characterized sources of resistance to rust in *A. hypogaea* exhibit reduced rate of disease development due to longer incubation and latent periods, fewer pustules per leaf, smaller pustule diameter and lower sporulation index. In general, infection frequency, pustule diameter, per cent ruptured pustules and leaf area damage are correlated with each other and with mean field rust score. The incubation period is negatively correlated with other components.

Rust resistance components appear to work additively (Subrahmanyam *et al.*, 1983; Reddy and Khare, 1988; Mehan *et al.*, 1994; Dwivedi *et al.*, 2002). The wild *Arachis* species and their interspecific derivatives possess various components of rust resistance. In particular, uredosori on them are observed to be small (containing very few uredospores), slightly depressed, and do not rupture to release their uredospores (Subrahmanyam *et al.*, 1983). Subrahmanyam *et al.* (1983) attempted to use species that are resistant and immune to *P. arachidis* as sources of resistance to rust and hypothesised that the mechanism of resistance in such species is different from those in *A. hypogaea* which provides a possibility of combining rust resistance of wild and cultivated species to give more effective and stable resistance in the cultivated groundnut.

### 2.2.2 Late leaf spot

Most popular and widely cultivated early maturing Spanish bunch types are highly susceptible to LLS. Components of resistance to LLS in *A. hypogaea* are due to longer latent period, lesser lesions per leaf, smaller lesion diameter, reduced sporulation, lower sporulation index, lesser leaf area damage and marginal defoliation (Nevill, 1981). Sporulation, lesion size, and latent period are predominant for LLS and are highly correlated with each other and with per cent leaf necrotic area (Chiteka *et al.*, 1988). Glasshouse studies on lesion diameter, defoliation, and sporulation were shown to be correlated with field disease score (Subrahmanyam *et al.*, 1982). Thus resistance to LLS is partially due to longer incubation and latent periods and resistant genotypes show reduced infection than susceptible genotypes (Nevill, 1981; Green and Wynne, 1986; Chiteka *et al.*, 1988; Anderson *et al.*, 1990;

Waliyar *et al.*, 1993; Dwivedi *et al.*, 2002). However, resistance to LLS could be associated with late maturity and undesirable pod and seed characteristics (Subrahmanyam and McDonald, 1983; Wynne *et al.*, 1991).

## 2.3 Resistance breeding

Control of LLS and rust through the application of plant protection measures has been suggested. But this method not only increases the cost of cultivation but also leads to environmental and health hazards. Use of disease resistant cultivars is one of the best means of reducing crop losses from these diseases. In this direction, sources of resistance have been identified from cultivated types, wild species and their derivatives (Chiteka *et al.*, 1988; Anderson *et al.*, 1993; Stalker and Simpson, 1995). Valencia landraces and wild species of groundnut possess high level of resistance to foliar diseases, but the resistance is generally linked to low productivity, late maturity, poor adaptability and undesirable pod features (Wynne *et al.*, 1991; Singh *et al.*, 1997). Germplasm originating from secondary centers of diversity were resistant to foliar diseases with desirable agronomic backgrounds, but their productivity levels were low (Singh *et al.*, 1997). In an attempt to introduce variability from wild diploid species into cultivated tetraploids, several interspecific derivatives have been developed. They possessed high levels of resistance to foliar diseases but the existence of strong association between undesirable traits like late maturity and inferior pod and seed characteristics with resistance prevented their commercial release (Nigam *et al.*, 1992; Stalker and Beute, 1993; Reddy *et al.*, 1996; Moss *et al.*, 1997).

Several efforts have also been made to study the genetics of LLS and rust resistance. Resistance to LLS is controlled by a combination of both nuclear and maternal gene effects. In both these cases, additive and dominance effects controlled majority of the variation (Pasupuleti *et al.*, 2013). Motagi *et al.* (2000) reported duplicate recessive genes controlling resistance to LLS. Both two-gene (Tiwari *et al.*, 1984) and five-locus recessive genetic models (Nevill, 1982) have been reported for resistance to LLS. Five recessive genes for resistance have been reported in crosses involving cultivated groundnut and wild *Arachis* species (Sharief *et al.*, 1978). Other studies reported predominantly additive genetic variance for

most of the components of resistance to LLS (Kornegay *et al.*, 1980; Hamid *et al.*, 1981; Anderson *et al.*, 1986; Jogloy *et al.*, 1987).

Resistance to rust in *A. hypogaea* is conferred either by a few recessive genes (Kalekar *et al.*, 1984; Knauft, 1987; Paramasivam *et al.*, 1990) or predominantly controlled by additive, dominance, additive × additive and additive × dominance genetic effects (Reddy *et al.*, 1987; Varman *et al.*, 1991). In addition, partial dominance is reported in some diploid species (Singh *et al.*, 1984). While, Motagi *et al.* (2000) reported that resistance to rust is conferred by duplicate complementary genes (9:7).

To breed for high productivity and disease resistance, standard breeding methods common for self-pollinated crops are used in groundnut. But incorporation of traits with quantitative inheritance has been difficult. Several attempts have been made to develop high-yielding foliar disease resistant genotypes through hybridization, but the lines showed only moderate level of resistance while retaining certain undesirable characteristics (Wynne *et al.*, 1991).

## 2.4 Backcross breeding

A cross between a hybrid ($F_1$ or a segregating generation) and one of the parents is known as backcross. In the backcross method, the hybrid and the progenies in the subsequent generations are repeatedly backcrossed to one of the parents. As a result, the genotype of backcross progeny becomes increasingly similar to that of the parent to which it is backcrossed. At the end of 6-8 backcrosses, the progeny would be almost identical with the parent used for backcrossing. The objective of the backcross method is to improve one or two specific defects of a high yielding variety, which is well adapted to the area and has other desirable characteristics. The characters lacking in this variety are transferred to it from a donor parent without changing its genotype, except for the genes being transferred. Thus the end result of a backcross programme is a well adapted variety with one or two improved characters.

For successful development of a new variety through backcross method, the following requirements must be fulfilled. A suitable recurrent parent must be available, which lacks in one or two characteristics. A suitable donor parent must be

available. The donor parent should have the character or characters to be transferred in a highly intense form. The character to be transferred must have high heritability. Preferably, it should be determined by one or few genes. A sufficient number of backcross should be made so that the genotype of the recurrent parent is recovered in full. Ordinarily, 6-7 backcrosses are sufficient for the purpose. The backcross method has been commonly used for the transfer of disease resistance from one variety to another. But it is also suitable for the transfer of other characteristics, even quantitative characters, and is applied to both self- and cross-pollinated crops. Backcross method may also be modified to allow the recovery of transgressive segregants.

The genetic consequences of repeated backcrossing need to be considered before the programme is undertaken. First effect of repeated backcrossing is a rapid increase in homozygosity and the frequency of homozygotes. Repeated backcrossing leads to increase in homozygosity at the same rate as selfing. The frequency of the desirable homozygotes for the allele from the recurrent parent *i.e.*, the homozygotes of recurrent parent type is more in case of backcrossing than in the case of selfing. Thus it is easier to select for a desirable genotype from a backcross progeny than from a selfed progeny. When backcrossing is repeated, the frequency of desirable homozygote increases rapidly; in the sixth backcross progeny, more than 98 per cent of the plants have the alleles from the recurrent parent. This is expected when no selection is done in backcross progenies. But when the selection is done for the plant type of the recurrent parent, a faster rate of return of the backcross progenies to the genotype of the recurrent parent is realized. The gene under transfer must be maintained by selection in the backcross generations.

Backcrossing is one of the important breeding schemes used in groundnut for various applications. In order to broaden the genetic base, backcrossing has been used in groundnut. A few wild *Arachis* species are resistant to rust and/or late leaf spot. But direct utilization of cross-compatible wild relatives (diploids), to broaden the genetic base is not a straight-forward process due the genetic barrier between wild and cultivated groundnut. Also, the wild species are not agronomically well adapted. Therefore, multiple cycles of backcrossing are usually required to eliminate undesirable wild genes. Interspecific derivatives were developed by crossing diploid

*Arachis* species with the cultivated groundnut to get a triploid, which was then backcrossed to cultivated groundnut to get a stable tetraploid. A few interspecific derivatives resistant to LLS and rust have been developed (Table 1).

Backcrossing has been used for transferring root-knot nematode resistance from *A. cardenasii* to *A. hypogaea* to develop the first root-knot nematode-resistant groundnut cultivar, COAN (Simpson and Starr, 2001). Backcrossing was also used to develop genotypes with high oleic acid and low linoleic acid content (Moore and Knauft, 1989; Knauft *et al*., 1993). However, a common problem with most of the backcross programmes has been the linkage drag with undesirable traits. Selection of the genotypes based on the marker data not only helps in efficient selection but also helps in avoiding any linkage drag (Hospital, 2001). Thus genomics-assisted breeding offers a clear advantage over conventional breeding.

Table 1: Groundnut genotypes reported to be resistant/tolerant to late leaf spot and rust

| Disease | Genotypes |
|---|---|
| Rust | ICGV 86590, R 8808, ICGV 99003, ICGV 99012; VG 9514; GFDS 272; NCAc 343; DTG 57; DTG 60; DTG 58; DTG 27; TDG 56; TFDRG 5 |
| Late leaf spot (LLS) | Mutant (28-2), Dh 8, ICGV 99006, ICGV 99013, ICGV 99004, ICGV 99003, ICGV 99001; GG 7 (J-38); Kadiri 5; Kadiri 6 (K 1240); Greeshma; VLMoongphali-1 |
| Both LLS and rust | GPBD 4, B 37c, ICGV 87165, ICGV 99005, ICGV 86699; VRI (Gn) 5; Co (Gn) 4 (TNAU 269); TG 37A; Phule Unap (JL 286); Kadiri Haritandhra (K 1319); Kadiri 9; VRI (Gn) 6 (VG 9816); VRI (Gn) 7; ICGV 00348 |

Sources : Motagi *et al*., 2014; Gowda *et al*., 2002; Dwivedi *et al*., 2002 and Mondal *et al*., 2007

## 2.5 Genomics-assisted breeding

Use of molecular markers that are tightly linked to quantitative traits would enhance the efficiency of selection in backcross breeding. The quantitative trait loci (QTL) of interest can be selected using the markers in the backcross generations. When such an efficient selection is exercised for the plant type of the recurrent parent, a faster rate of return of the backcross progenies to the genotype of the recurrent parent can be realized. Moreover, such plants which are in excess frequency can be identified and confirmed by foreground and background selection using the molecular markers. Also, recombination selection allows identification of the plant (recombinant plants) heterozygous for QTL or gene under transfer while homozygous for the recipient allele at one marker locus distally flanking the QTL. This selection is useful in overcoming the linkage drag (Collard and Mackill, 2008). A backcross breeding assisted with foreground, background and recombination selection is typically known as marker assisted backcrossing (MABC).

Implementation of MABC requires the availability of genomic resources like linkage map, QTL and linked markers. In groundnut, a considerable progress has been made to establish enormous genomic resources. Recent years have witnessed accelerated development of molecular markers, genetic and physical maps, generation of expressed sequenced tags (ESTs), development of mutant resources, and functional genomics platforms that facilitate the identification of QTLs and discovery of genes associated with tolerance/resistance to abiotic and biotic stresses and agronomic traits. These genomic resources would accelerate molecular breeding for several traits for development of superior genotypes.

## 2.6 Genomic resources in groundnut

### 2.6.1 Molecular markers

A range of molecular markers such as restriction fragment length polymorphism (Halward *et al.*, 1993), random amplified polymorphic DNA (RAPD) (Halward *et al.*, 1991), amplified fragment length polymorphism (Herselman, 2003), and simple sequence repeat (SSR) markers (Hilu and Stalker, 1995; Kochert *et al.*, 1996; Subramanian *et al.*, 2000; Dwivedi *et al.*, 2001; He and Prakash, 2001; Bravo *et al.*, 2006) have been used in groundnut. Recently, Diversity Array Technology

(DArT) marker platform has also been developed for groundnut (Kilian, 2008). Currently, the advent of next-generation sequencing and faster genotyping technologies have enabled the detection of single nucleotide polymorphisms (SNPs), which have emerged as the marker of choice in crop breeding (Varshney *et al*., 2009a). SNPs have been detected from diploids (Alves *et al*., 2008; Nagy *et al*., 2012) as well as cultivated tetraploids (Chen *et al*., 2013). Zhou *et al*. (2014) reported the discovery of 53,257 SNPs and the construction of SNP-based linkage map.

With the observation that transposons represent a major component of interspersed repetitive sequences (genome-wide repeats) and their pattern of insertion varies among germplasm or individuals, a considerable effort has been made to develop and test transposable element (TE)-specific markers in groundnut. *Arachis hypogaea* miniature inverted repeat transposable element (*AhMITE1*)-specific marker was found to be associated with LLS resistance (Gowda *et al*., 2010). Subsequently, Shirasawa *et al*. (2012a) identified 504 sites within groundnut genome that had *MITE* insertion. Based on the flanking sequences at 504 sites, primers were designed in an attempt to convert them as markers. PCR analyses detected 13.4% and 30.6% among Virginia lines and between Virginia and Spanish types, respectively. Compared to the polymorphism obtained with SSR markers, MITEs displayed very high level of polymorphism. Further, *in silico* polymorphism analysis could increase the efficiency of development of transposon markers (535) by three fold (Shirasawa *et al*., 2012b). When PCR analysis was performed using these 535 primer pairs, very high level of polymorphism was noticed.

### 2.6.2 Linkage mapping

In groundnut, molecular genetic studies initially progressed using diploids rather than tetraploid cultivated types due to the greater simplicity of diploids as genetic models (Halward *et al*., 1993). Genetic maps for diploid groundnut have been reported by Moretzsohn *et al*. (2005) (1,230.89 cM, AA genome) and Moretzsohn *et al*. (2009) (1,294.0 cM, BB genome). Considering the importance of foliar diseases, several mapping populations segregating for LLS and rust resistance were developed using GPBD 4 as one of the parents at UAS, Dharwad (Bhat *et al*., 2012). Two hundred and sixty eight RILs of TAG 24 × GPBD 4 were used for map

(462.24 cM; 56 loci mapped on 14 LGs) construction using 59 SSR markers (out of total 67) (Khedikar *et al.*, 2010). Similarly, a map (657.90 cM; 45 loci mapped on 8 LGs) was constructed using the RILs of TG 26 × GPBD 4 (Sarvamangala *et al.*, 2011). Recently, improved genetic maps for the same populations, TAG 24 × GPBD 4 (1922.4 cM; 188 loci mapped on 20 LGs) and TG 26 × GPBD 4 (1963; 181 loci mapped on 21 LGs) were constructed (Sujay *et al.*, 2012). Further, using 143 markers common to the two maps, a consensus map with 225 SSR loci and total map distance of 1,152.9 cM was developed (Sujay *et al.*, 2012).

LLS and rust resistance-linked QTL have been identified using the RILs of TAG 24 × GPBD 4 and TG 26 × GPBD 4 (Sujay *et al.*, 2012). The genomic region on linkage group AhXV carried three QTL, GM2009-GM1536, GM1536-GM2301/GM2009 and IPAHM103-GM1954, contributing for both LLS and rust resistance. The highest phenotypic variance explained (PVE) across the seasons ranged from 62.35% to 82.96% for rust resistance and 17.37% to 67.98% for LLS resistance among the three QTL. Another region on AhXII flanked by GM1573-GM1009-pPGPseq8D09D exhibited 62.34% PVE for LLS resistance. The RILs of VG 95149 × TAG 24 showed strong linkage of SSR marker GO340445 with rust resistance (Mondal *et al.*, 2012). The marker was closely linked (11.9 cM) to IPAHM103.

A considerable progress has been made to involve wild germplasm in the recent genomics approaches, which not only expedite QTL mapping, fine mapping and gene discovery, but also help variety development since they involve the simultaneous transfer of QTLs into elite breeding lines. In general, when wild relatives are used, inbreeding after crossing results in sterility, thus making it difficult to generate a large, random array of segregants for mapping. Advanced backcross QTL (AB-QTL) populations help overcome this problem. Wild species (donor) are crossed to an elite variety (recurrent parent), and the $F_1$s are backcrossed. An array of $BC_2$ or $BC_3$ lines, each containing a small number of random introgressions from the donor wild species in an elite varietal background is used as the AB-QTL population (Tanksley and Nelson, 1996). Recently, such a mapping population was developed from ICGS 76 (LLS susceptible) and a LLS resistant synthetic allotetraploid, ISATGR 278-18 (*A. duranensis* × *A. batizocoi*). QTL analysis in this

population identified (Varshakumari, 2013) the genomic regions previously mapped using the RILs of TAG 24 × GPBD 4 and TG 26 × GPBD 4.

### 2.6.3 Association mapping

Association mapping based on linkage disequilibrium is another method of identifying marker-trait association. Seed quality traits (including oil content, fatty acid composition, flavonoids, and resveratrol) were studied using US peanut mini-core panel (Wang *et al.*, 2011). SNP markers associated with the quality traits were identified. Similarly, the mini-core collection in China comprising 298 accessions was genotyped using 109 simple sequence repeat (SSR) markers. Markers associated with 15 agronomic traits were identified in the panel (Jiang *et al.*, 2014). A comprehensive analysis of marker-trait association (MTA) on LLS and rust resistance was done using a multi-location and multi-season data collected on a 'reference set' of groundnut genotypes. MTAs were identified for early leaf spot, late leaf spot and rust resistance (Pandey *et al.*, 2014).

### 2.6.4 QTL validation

Some 'significant' QTLs may be false positives and QTLs responsible for significant variation within and between populations can be missed if the tested genotypes are fixed by chance for alleles with similar effects. Therefore, QTLs should be confirmed by repeated experiments using the same and different strains or genotypes before they are considered for breeding programmes. In general, the QTL/markers are validated by testing them in different genetic background. In general, stabilized populations, cultivated genotypes, popular cultivars and NILs are used as genetic material for validating the QTLs/markers.

Validation of the LLS and rust resistance QTL and their linked markers will be of great importance in molecular breeding to improve LLS and rust resistance in groundnut. The major QTL governing resistance to LLS and rust have been validated using a set of 46 resistant and susceptible germplasm lines with different genetic background including released varieties, hybrid derivatives from North Carolina Accessions, interspecific derivatives, mutant lines, cultivars from South American landraces and advanced breeding lines (Khedikar *et al.*, 2010; Sujay *et al.*, 2012). The makers linked to LLS and rust resistance were also validated using the

RILs of a new cross, TG 19 × GPBD 4 and three intogression line populations from ICGS 76 × ISATGR 278-18, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5. The type of allele at three LLS resistance-linked markers (GM1009, GM1573, pPGPseq8D09) six LLS and rust resistance-linked markers (GM1536, GM1954, GM2009, GM2301, GM2079 and IPAHM103) and one rust resistance-linked marker (GO340445) loci were checked for the co-segregation with the phenotype. The resistant genotypes had the resistant allele at all marker loci. They were validated statistically by single marker analysis, Kruskal-Wallis test and locus-by-locus AMOVA (Sukruth *et al.*, 2014). Similarly, 16 SSRs markers linked to LLS and rust resistance were validated using 95 diverse genotypes, majority of these markers were ascribed to LG_03 followed by LG_04 on consensus genetic map (Gajjar *et al.,* 2014).

The genomic resources developed for LLS and rust resistance have been employed for developing backcross lines in groundnut (Varshney *et al.*, 2014). Genomic region on linkage group AhXV governing rust resistance was transferred from GPBD 4 to three rust susceptible varieties, ICGV 91114, JL 24 and TAG 24 through MABC using IPAHM103, GM2079, GM1536 and GM2301. Two to three backcrosses and selfing could yield 200 backcross lines from all the three crosses. Field evaluation of 81 lines confirmed their improved resistance to rust. These lines had significantly increased pod yields (56–96%) in infested environments compared to the susceptible parents (Varshney *et al.*, 2014). Hence, improving groundnut for LLS and rust resistance through molecular breeding by making use of available genomic resources looks promising as similar efforts have met with success in improving various traits in other crops.

# 3. MATERIAL AND METHODS

The present study was focused on the two important aspects of molecular breeding in groundnut for improving foliar disease resistance. The first aspect included validation of LLS and rust resistance-linked markers/QTL in Near Isogenic Lines (NILs) derived from Heterogeneous Inbreed Families (HIFs) of TAG 24 × GPBD 4 and TG 26 × GPBD 4, and in recombinant inbred lines of VL 1 × 110. The second aspect was to take up marker assisted backcrossing (MABC) in JL 24 for LLS and rust resistance. The material and methods used in the development of NILs, marker validation, development and evaluation of backcross populations, foreground and background genome selection are described in this chapter.

## 3.1 Marker validation using NILs

The markers considered for validation included IPAHM103, GM1536, GM2301, GM2009 linked to both LLS and rust resistance, and GM1009, GM1573 and pPGPseq8D09 linked to only LLS resistance (Khedikar *et al.*, 2010; Sujay *et al.*, 2012; Varshakumari, 2013). NILs were employed for testing the marker validation.

### 3.1.1 Development of NILs

The crosses, TAG 24 × GPBD 4 and TG 26 × GPBD 4 involving the following parents were employed for developing the NILs. The salient features and description of the parents are as follows.

- TAG 24: A Spanish bunch type variety derived from TGE 2 × TGE 1 (Patil *et al.*, 1995). This is a popular high yielding cultivar which matures early (100-105 days) with high harvest index, better partitioning coefficient and field tolerance to bud necrosis disease. It contains 29 per cent protein, 45 per cent oil and low O/L ratio.

- TG 26: An improved Spanish bunch variety, derived from BARCG 1 × TG 23 (Kale *et al.*, 1997). It is an early maturing (95-105 days), semi dwarf, erect variety with high pod growth rate, high harvest index, greater partitioning efficiency, high linoleic acid content, tolerance to bud necrosis and rust but susceptibility to LLS (Kale *et al.*, 1997; Badigannavar *et al.*, 2002).

- GPBD 4: An improved Spanish bunch groundnut variety developed at UAS, Dharwad (Gowda *et al*., 2002) from KRG 1 × ICGV 86855. It has a desirable combination of early maturity, high yield, high pod growth rate, desirable pod and kernel features, high oil and protein content, better Oleic/Linoleic (O/L) ratio, resistance to late leaf spot and rust. KRG 1 (selection from Argentine) is an early maturing and Spanish bunch local cultivar developed at Regional Research Station, Raichur, Karnataka. It is susceptible to foliar diseases. ICGV 86855 is a Virginia bunch interspecific derivative (*A. hypogaea* × *A. cardenasii*) developed at ICRISAT, Patancheru, India. It is resistant to foliar diseases.

The crosses, TAG 24 × GPBD 4 and TG 26 × GPBD 4 were made and the progenies were advanced at the Department of Genetics and Plant Breeding, UAS, Dharwad (Khedikar *et al*., 2010; Sujay *et al*., 2012). Lines segregating for resistance to LLS and rust were identified in $F_5$, $F_6$ and $F_7$ as heterogeneous inbred families. The plants differing for reaction to LLS and rust were selected and maintained separately from each segregating family of both the crosses. They were designated as HIF-derived plants. The plants were selfed to get the stabilized lines.

Lines recovered from 13 HIFs of TAG 24 × GPBD 4 and 16 HIFs of TG 26 × GPBD 4 segregating for response to rust constituted Set I. Whereas, the lines derived from five HIFs of TAG 24 × GPBD 4 and 16 HIFs of TG 26 × GPBD 4 segregating for response to LLS constituted Set II.

### 3.1.2 Field evaluation of HIF-derived lines

HIF-derived lines were evaluated in field at IABT Garden and Botany Garden of the Main Agricultural Research Station, UAS, Dharwad. This experimental site is located in the transitional tract of Karnataka at 15° 13′ North latitude and 75° 07′ East longitude with an altitude of 678 m above mean sea level. The soil type of the experimental blocks was vertisol with pH ranging from 7.0 to 7.5. HIF-derived lines along with their respective parents were evaluated in a randomized block design with two replications.

3.1.2.1 Screening for rust and late leaf spot resistance

HIF-derived lines were evaluated for their reaction to LLS and rust during for four seasons (rainy seasons of 2010, 2011 and 2012, and post-rainy season of 2010). During these evaluations, LLS and rust inocula were maintained on TMV 2 (national check variety susceptible to LLS and rust) and mutant 28-2 (resistant to LLS but susceptible to rust) plants grown in growth chamber. The artificial epiphytotic condition for LLS and rust was created in the field using 'Spreader Row Technique' (Subrahmanyam *et al*., 1995) in which the disease spreader plants (TMV 2 and mutant 28-2) were planted at every $10^{th}$ row in the experimental plot. The infected leaves from susceptible plants grown in the growth chamber were collected and soaked in water for 30 min. LLS conidia and rust urediniospores were released by rubbing the infected leaves in the water. The inoculum containing 20,000 conidia/urediniospores per ml water was mixed with Tween 80 (0.2 ml/1,000 ml of water) as a mild surfactant. When the plants were 35 days old, they were sprayed in the evening with the inoculum using a Knapsack sprayer for a week. High humidity was maintained by irrigating the field in the night with sprinkler or furrow irrigation. Additional inoculum was provided by placing pots containing diseased plants at every 20 rows. A modified 9 point scale (1-9 score) (Subbarao *et al*., 1990) was used for scoring rust (Table 2, Fig. 1) and LLS (Table 3, Fig. 2) disease at different stages of the crop i.e., 70, 80 and 90 days after sowing (DAS).

3.1.3 Evaluation for morphological and productivity traits

HIF-derived lines were evaluated for morphological and productivity traits for two seasons (rainy seasons of 2011 and 2012). The observations on morphological and productivity traits were recorded by following the groundnut descriptor (IBPGR/ICRISAT, 1992) on randomly selected five plants per line.

3.1.3.1 Growth characters

**1)** Plant height (cm): The height of the plant was measured in centimeters (cm) from the ground level to the tip of the main stem at the time of harvest.

**2)** Number of primary branches: The number of n+1 branch on the main stem was counted at the time of harvest.

**Table 2: Modified 9-point scale used for field evaluation of rust in groundnut**

| Disease score | Description | Disease severity (%)* |
|---|---|---|
| 1 | No disease | 0 |
| 2 | Pustules sparsely distributed, largely on lower leaves | 1 - 5 |
| 3 | Many pustules on lower leaves, necrosis evident, very few pustules on middle leaves | 6 - 10 |
| 4 | Number of pustules on lower and middle leaves, severe necrosis of lower leaves | 11 – 20 |
| 5 | Severe necrosis of lower and middle leaves, pustules may be present on top leaves but less severe | 21 - 30 |
| 6 | Extensive damage to lower leaves, middle leaves, necrotic with dense distribution of pustules on top leaves | 31 - 40 |
| 7 | Severe damage of lower and middle leaves, pustules densely distributed on top leaves | 41 - 60 |
| 8 | 100 per cent damage to lower and middle leaves, pustules on top leaves | 61 – 80 |
| 9 | Almost all leaves withered, bare stems seen | 81 - 100 |

*Percentage leaf area damaged by the disease (Subbarao *et al.,* 1990)

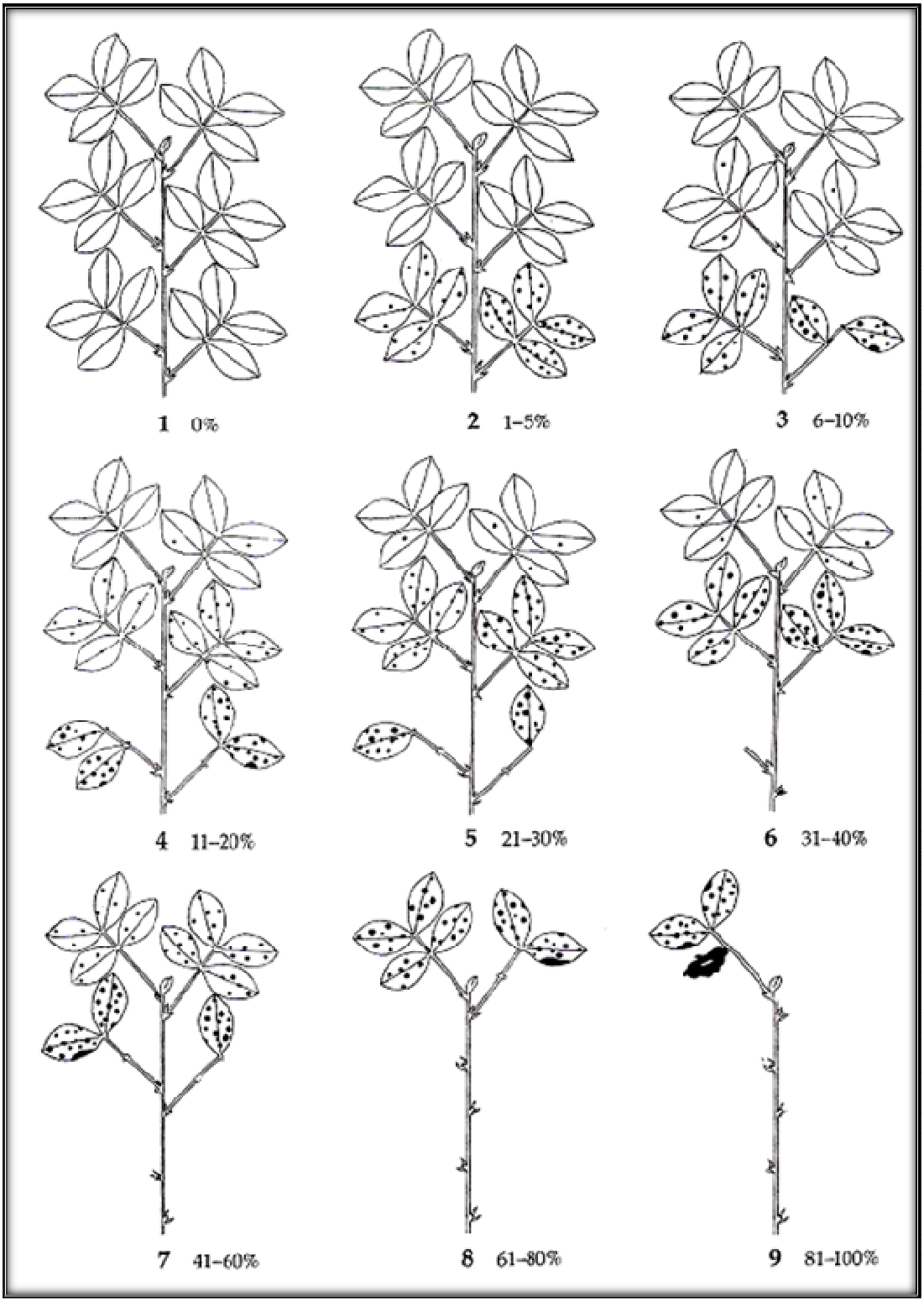


**Fig. 1: Modified 9-point scale used for field evaluation of rust in groundnut (Subbarao *et al.,* 1990)**

**Table 3: Modified 9-point scale used for field evaluation of late leaf spot in groundnut**

| Disease score | Description | Disease severity (%)* |
|---|---|---|
| 1 | No disease | 0 |
| 2 | Lesions present largely on lower leaves; no defoliation | 1 – 5 |
| 3 | Lesions present largely on lower leaves, very few on middle leaves; defoliation of some leaflets evident on lower leaves | 6 – 10 |
| 4 | Lesions present on lower and middle leaves but severe on lower leaves; defoliation of some leaflets evident on lower leaves | 11 – 20 |
| 5 | Lesions present on all lower and middle leaves; over 50% defoliation of lower leaves | 21 – 30 |
| 6 | Severe lesions on lower and middle leaves; lesions present but less severe on top leaves; extensive defoliation of lower leaves; defoliation of some defoliation on middle leaves | 31 – 40 |
| 7 | Lesions on all leaves but less severe on top leaves; defoliation of all lower and some middle leaves | 41 – 60 |
| 8 | Defoliation of all lower and middle leaves; severe lesions on top leaves; some defoliation of top leaves evident. | 61 – 80 |
| 9 | Almost leaves defoliated, leaving bare stem; some leaflets may remain, but show severe leaf spots. | 81 – 100 |

*Percentage leaf area damaged by the disease (Subbarao *et al.,* 1990)

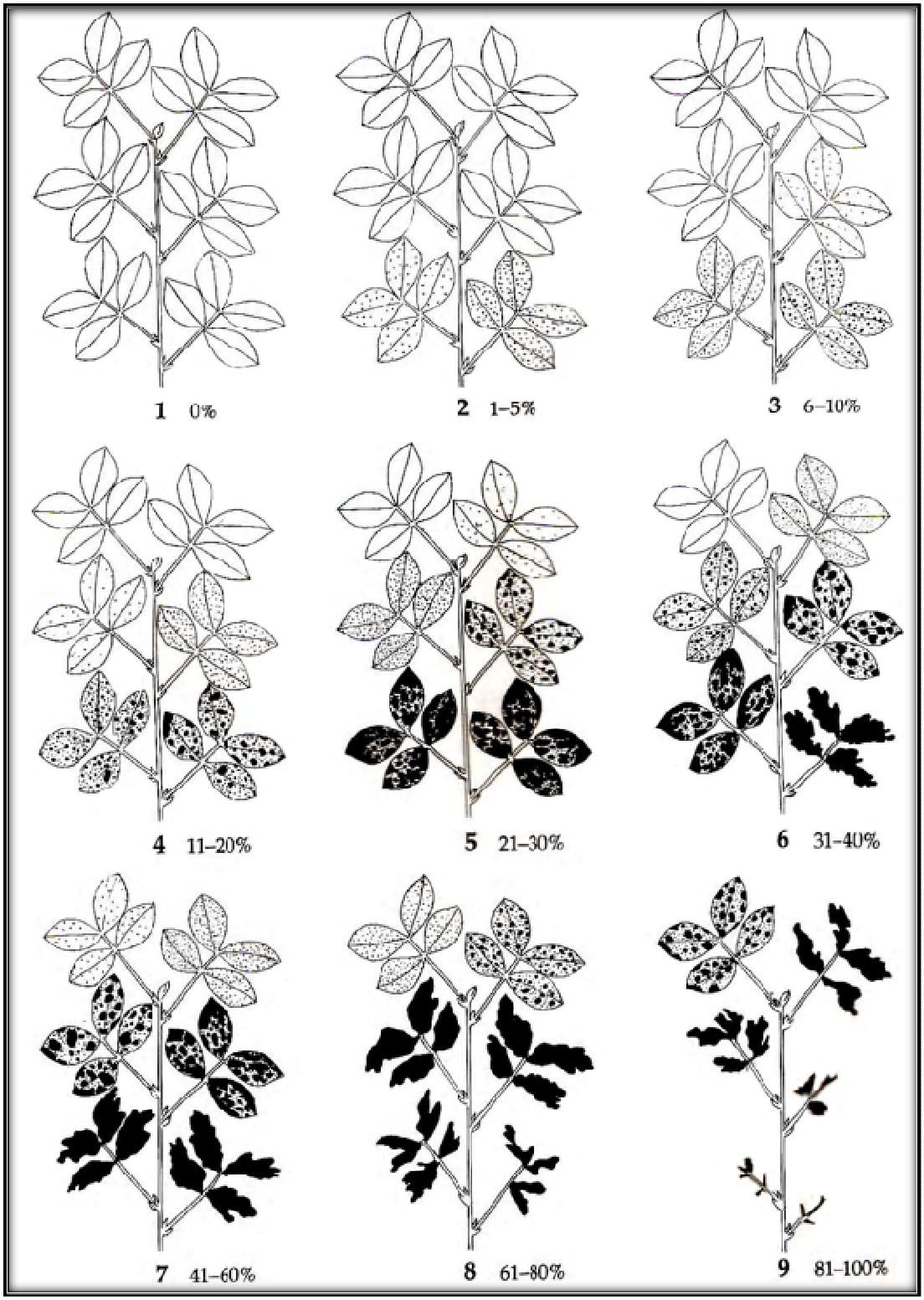


**Fig. 2: Modified 9-point scale used for field evaluation of late leaf spot in groundnut (Subbarao *et al.,* 1990)**

**3)** Leaf length (mm): The length of leaf was measured on the third leaf, apical leaflet of the main stem in centimeters (cm) when fully expanded.

**4)** Leaf width (mm): The width of leaf was measured on the third leaf, fully expanded apical leaflet on the main stem in centimeters (cm) at its widest point.

3.1.3.2 Productivity parameters

1) Pod yield per plant (PYPP): Pod yield per plant was calculated by dividing total pod yield per plot (gm) by number of plants in the plot.

2) Test weight (TW): The well dried and cleaned pods from each genotype were shelled, 100 kernels at random were counted and weight (gm) was recorded.

3) Shelling percentage (SP): Shelling percentage was calculated by weighing the kernels shelled from a unit weight of pods and expressed in percentage.

4) Sound mature kernel percentage (SMKP): The total number of kernels used in the computation of test weight included the well developed and shriveled kernels. They were separated, counted and the percentage was computed using the formula,

$$\text{Sound mature kernel percentage} = \frac{\text{Number of well developed kernels}}{\text{Total number of kernels}} \times 100$$

3.1.4 Statistical analysis for phenotypic data

The following statistical estimates were calculated using Windostat Version 8.1 and MSTAT-C statistical package.

3.1.4.1 Analysis of variance

The analysis of variance (ANOVA) for different characters was carried out using the mean phenotypic data to partition the variation due to different sources following the method given by Panse and Sukhatme (1954).

3.1.4.2 Duncan's multiple range test (DMRT)

Each treatment mean was compared with every other treatment mean using a multiple comparison test. The treatment means were arranged in descending order of magnitude, and the differences between every pair of treatment means were compared with least significant range values calculated using MSTAT-C according to Duncan (1955).

3.1.5 Genotyping of HIF-derived lines

3.1.5.1 Genomic DNA isolation

DNA was isolated from the young leaves of HIF-derived lines and their respective parents grown in the field by following the modified cetyl trimethyl ammonium bromide (CTAB) method (Cuc *et al*., 2008).

1) Young leaves were ground to a fine powder in liquid nitrogen using a mortar and pestle and transferred to a 1.5 ml micro centrifuge tube.

2) The powder was immediately added with preheated CTAB buffer (650 µl). Contents were mixed vigorously by vortexing and inverting. The tube was incubated at 65°C for 30 min with occasional inversion.

3) The samples were cooled on the bench to room temperature and were centrifuged at 13,000 rpm for 20 min to pellet the cellular debris, proteins and polysaccharides.

4) An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the extract and mixed by gentle inversion for 5 to 10 min to form a uniform milky white emulsion.

5) The mixture was centrifuged at 13,000 rpm for 20 min.

6) The aqueous phase was pipetted out gently, avoiding the interface and added with an equal volume of pre chilled isopropanol. Contents were mixed gently by inverting and incubated overnight at -20°C.

7) Content was centrifuged at 13,000 rpm for 20 min. Supernatant was discarded.

8) The pellet was washed with 70% alcohol by spinning at 9,000 rpm for 10 min. Alcohol was completely discarded to get clear pellet. Pellet was either air dried or dried by placing the tube in a hot dry bath at 37°C for 3 hr.

9) The pellet was dissolved in 200 µl of $T_{10}E_1$.

3.1.5.2 Quantification of DNA

The concentration of DNA was checked by measuring the OD for the DNA sample at 260 nm using the spectrophotometer (Varian, Cary Bio 50). Based on the OD value, the DNA quantity was worked out (Sambrook and Russell, 2001). The samples were diluted to 5 ng/µl for further use.

3.1.5.3 Analyzing genome similarity among HIF-derived lines

HIF-derived lines were analyzed for genome similarity using the marker data. Parental genotypes (TAG 24 versus GPBD 4 and TG 26 versus GPBD 4) were screened for polymorphism using 1079 (*Arachis hypogaea* Genome-SSR, AHGS) (Shirasawa *et al*., 2012b), 470 (*Arachis hypogaea* EST-SSR, AHES) (Koilkonda *et al*., 2012), 405 (*Arachis hypogaea* Transposable Element, AhTE) (Shirasawa *et al*., 2012a) and 89 SSR markers (Sujay *et al*., 2012). Subsequently, the polymorphic markers were used to genotype the HIF-derived lines of Set I and Set II using Polymerase Chain Reaction (PCR). Genotyping of HIF-derived lines was carried out in a 20 µl reaction mixture (Table 4) using a Touch-Down PCR profile (Table 5) provided by eppendorf Mastercycler® pro and BIO-RAD T100™ Thermal cyclers for AHGS, AHES and SSR markers and a standard PCR profile (Table 6) for AhTE markers.

Table 4: PCR components for AHGS, AHES, SSR and AhTE markers

| Components | Concentration (per µl) | Volume (µl) |
|---|---|---|
| Nuclease free $H_2O$ | - | 13.0 |
| Taq buffer with $Mg^{2+}$ | 10 X | 2.0 |
| dNTP's | 2 mM | 2.0 |
| Primers (F + R) | 10 pmol | (0.5 + 0.5) |
| Taq DNA polymerase | 5 U | 0.5 |
| DNA template | 5 ng | 1.5 |
| Total | | 20.0 |

Table 5: PCR profile for AHGS, AHES and SSR markers

| Steps | Temperature (°c) | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 94.0 | 5 min | 1 |
| Denaturation | 94.0 | 30 sec | |
| Annealing | 65.0* | 30 sec | 5 |
| Primer extension | 72.0 | 30 sec | |
| Denaturation | 94.0 | 30 sec | |
| Annealing | 60.0 | 30 sec | 35 |
| Primer extension | 72.0 | 30 sec | |
| Final extension | 72.0 | 10 min | 1 |
| Hold | 4.0 | - | |

* Touchdown profile: decrease in 1°C per cycle for first five cycles

Table 6: PCR profile for AhTE markers

| Steps | Temperature (°c) | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 94.0 | 5 min | 1 |
| Denaturation | 94.0 | 2 min | |
| Annealing | (45.0 – 57.0)* | 1 min | 38 |
| Primer extension | 72.0 | 2 min | |
| Final extension | 72.0 | 10 min | 1 |
| Hold | 4.0 | - | |

∗ Tm range for AhTE primers

3.1.5.4 Gel electrophoresis

The PCR amplified products of AHGS, AHES and SSR markers were separated on 3.5% agarose gel. The bands differing for the length of the microsatellites were scored as A (susceptible: TAG 24 or TG 26 type) and B (resistant: GPBD 4 type). For AhTE markers, the two products differing for the insertion of ~205 bp *AhMITE1* were separated on 1.5% agarose gel. The allele with AhMITE1 insertion was scored as A, while the allele without *AhMITE1* was scored as B.

3.1.5.5 Analyzing genome similarity and genome representation

Lines originating from each HIF were compared for the genome similarity. The number of markers that were monomorphic between the lines was counted from the total markers used for screening, and expressed in percentage.

Each line was checked for the type of allele at each background marker and scored as female parent type (TAG 24 or TG 26) or male parent type (GPBD 4) to calculate the contribution of each parent.

3.1.6 Validating the LLS and rust resistance-linked markers

LLS and rust resistance-linked markers (Table 7) were used to genotype the HIF-derived lines and their parents using PCR. Validation of IPAHM103, GM1536 and GM2301 linked to rust resistance was performed using the Set I lines. GM2009, GM1009, GM1573 and pPGPseq8D09 linked to LLS resistance were checked for their validation using the Set II lines. The PCR amplification for these markers was performed as described in 3.1.5.3. PCR products were resolved on 3.5% agarose gel and scored as mentioned in the section 3.1.5.4. Further fine-resolution of the PCR products was attempted for all the markers using Polyacrylamide Gel Electrophoresis (PAGE).

3.1.6.1 Polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide gel (4%) was prepared in a casting unit (Sequi-Gen GT) provided by (Bio-Rad Pvt. Ltd, USA). Denatured PCR products were loaded on to the gel and allowed to separate for 30 min at 75 W. The gel was scored for the banding pattern after silver staining (Goldman and Merril, 1982). The bands corresponding to each marker locus were scored as A (susceptible: TAG or TG 26 type) and B (resistant: GPBD 4 type).

3.1.6.2 Co-segregation analysis

Each line was tested for co-segregation between allele at a marker locus and disease reaction. Line showing resistance allele at linked marker locus and disease resistance (score less than 5.0) was considered positive for co-segregation. Frequency of lines showing co-segregation was compared with that of lines not

**Table 7: LLS and rust resistance- linked markers**

| Sl. No. | Primer | Primer sequence | Tm (°C) | Amplicon size | Reference |
|---|---|---|---|---|---|
| **LLS resistance-linked markers** | | | | | |
| 1 | GM1009_F | TTTCCTTCTTTCCCTTCTTCTTC | 59.6 | 414 bp | Khedikar *et al*., 2010; Sujay *et al*., 2012 |
| | GM1009_R | CGTTGTTGCCGTTAAACTGA | | | |
| 2 | GM1573_F | GAGACCGGAGACGGAGAGTAT | 51.7 | 289 bp | |
| | GM1573_R | ACGCCCATAGATTAACCCAGT | | | |
| 3 | pPGPseq8D09_F | TGAGTTTCCCCAAAAGGAGA | 51.5 | 150 bp | |
| | pPGPseq8D09_R | CAACAACAATACGGCCAACA | | | |
| **LLS and rust resistance-linked markers** | | | | | |
| 1 | GM2009_F | CAAACGCATACACCCCATAAC | 58.7 | 106 bp | Khedikar *et al*., 2010; Sujay *et al*., 2012 |
| | GM2009_R | TTTGGTTCTCGTTTGTGTTTT | | | |
| 2 | GM2301_F | GTAACCACAGCTGGCATGAAC | 60.3 | 133 bp | |
| | GM2301_R | TCTTCAAGAACCCACCAACAC | | | |
| 3 | GM2079_F | GGCCAAGGAGAAGAAGAAAGA | 60.0 | 411 bp | |
| | GM2079_R | GAAGGAGTAGTGGTGCTGCTG | | | |
| 4 | GM1536_F | AAAGCCCTGAAAAGAAAGCAG | 60.3 | 475 bp | |
| | GM1536_R | TATGCATTTGCAGGTTCTGGT | | | |
| 5 | GM1954_F | GAGGAGTGTGAGGTTCTGACG | 59.7 | 149 bp | |
| | GM1954_R | TGGTTCATTGCATTTGCATAC | | | |
| 6 | IPAHM103_F | GCATTCACCACCATAGTCCA | 59.0 | 144 bp | |
| | IPAHM103_R | TCCTCTGACTTTCCTCCATCA | | | |

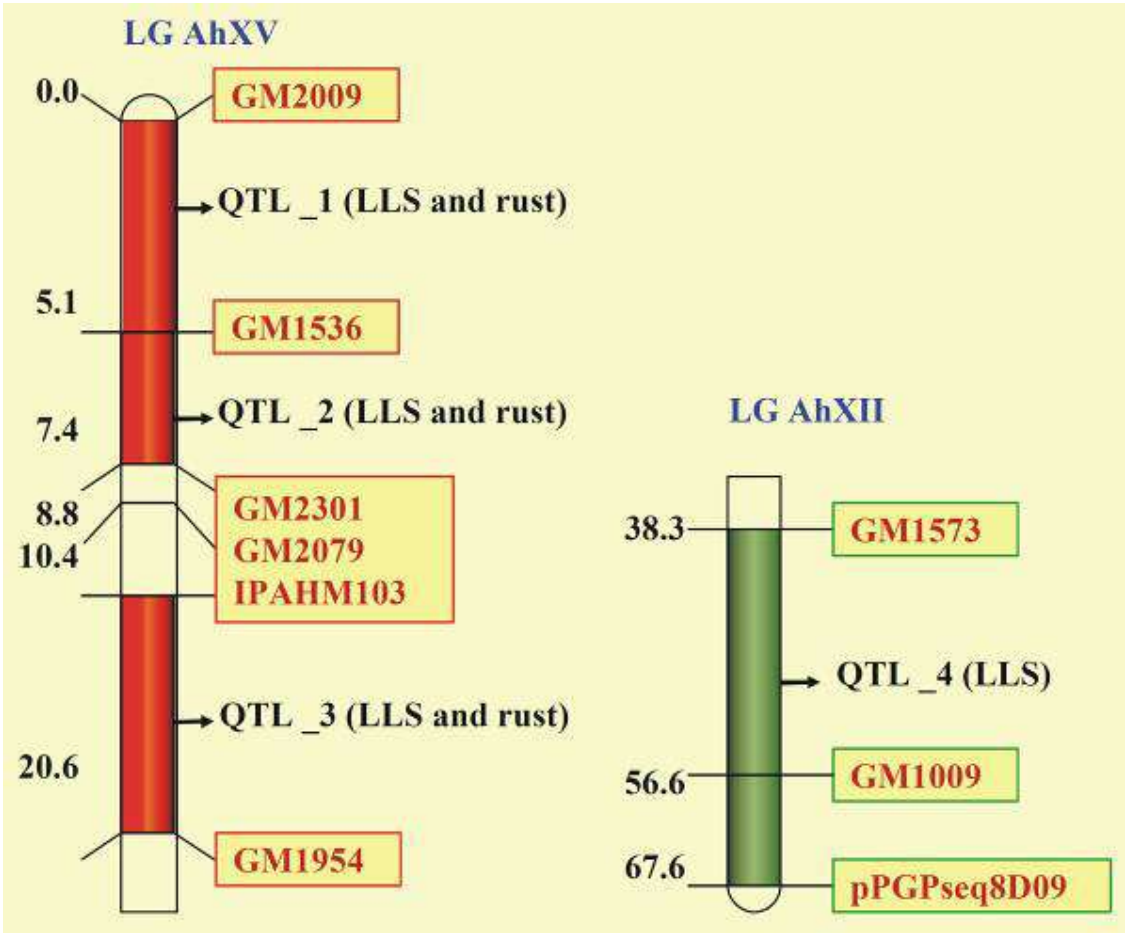


Fig. 3. Markers linked to LLS and rust resistance in groundnut (Sujay et al., 2012)

showing co-segregation in Set I and Set II separately using z test (standard normal deviate test for proportion) (Rao, 2007), where the z value was compared with the critical value of 1.96 at the 5% level of significance (irrespective degrees of freedom). High proportion of lines showing co-segregation and a significant z value was considered as a good case of marker validation.

## 3.2 Marker validation using the RILs of VL1 × 110

The mapping population consisting of 114 RILs of VL1 × 110 was used for the validation of IPAHM103, GM1954, GM1536, GM2079 and GM2301 linked to both LLS and rust resistances and GM1009, GM2009, GM1573 linked to only LLS resistance. The salient features and description of the parents used for developing the mapping population are as follows.

- VL 1: A Valencia type mutant derived from Dharwad Early Runner (Gowda *et al.*, 1996) having typical features of primitive Valencia landraces of Peru with resistance to rust, ribbed pods and coloured kernels (Gowda and Nadaf, 1992).

- 110: A Spanish bunch mutant derived by EMS mutagenesis of VL 1 having more number of primary and secondary branches, short main stem and primary branches, small leaves, high pod yield and test weight, and resistance to late leaf spot in comparison to VL 1 (Gowda *et al.*, 2010).

### 3.2.1 Genotyping of RILs

Genomic DNA was isolated from the RILs and their parents as described in 3.1.5.1. The genomic DNA was quantified as mentioned in section 3.1.5.2. Five SSR markers IPAHM103, GM1954, GM1536, GM2079 and GM2301 linked to rust resistances and GM1009, GM2009, GM1573 linked to LLS resistance were used for genotyping the parents and the RILs using PCR as described in 3.1.5.3. RILs were also genotyped with *AhMITE1*-PCR as described in section 3.1.5.3. The scoring was done for the presence (A) or absence (B) of 242 bp amplicon.

### 3.2.2 Phenotyping of RILs for LLS and rust diseases

RILs were evaluated for LLS and rust damage under epiphytotic conditions for four seasons during 2010, 2011, 2012 and 2013. The experimental details and scoring for LLS and rust diseases are mentioned in 3.1.2.1.

### 3.2.3 Statistical analysis

ANOVA for data pooled from various seasons was done as described in 3.1.4.1. Marker-trait association was worked out with Single Marker Analysis (SMA) performed using WinQTL Cartographer version 2.5 (Wang *et al*., 2007). The significance of SMA was tested by calculating F and coefficient of determination ($R^2$) statistics for each marker.

## 3.3 Marker Assisted Backcrossing (MABC) in JL 24

An effort was made to develop backcross lines in an elite variety of groundnut, JL 24 for improving LLS and rust resistance using three donors (GPBD 4, ICGV 86699 and ICGV 99005). The salient features of the parents used in MABC are

- JL 24: A high-yielding, popular, drought-tolerant and early maturing (100–110 days) variety selected from 'EC 94943', an introduction from Taiwan, at the Oilseeds Research Station, Jalgaon, Maharashtra. It was released for cultivation in India during 1979 (Patil *et al*., 1980).

- GPBD 4: Salient features are described in 3.1.1.

- ICGV 86699: A high-yielding interspecific derivative developed as (*A. batizocoi* × *A. duranensis)* × *A. hypogaea* (Reddy *et al*., 1996). It shows multiple resistance/tolerance to rust, early and late leaf spots, groundnut bud necrosis and groundnut mottle viruses, stem and pod rots (*Sclerotium rolfsii*). It is less susceptible to the tobacco caterpillar and jassids than popular Indian cultivars. It matures in 118 days during the rainy season in India with an average shelling percentage of 60% and average oil content of 48%.

- ICGV 99005: An interspecific derivative of *A. hypogaea* × [*A. batizocoi* × *A. duranensis*] showing immune or highly resistant reaction to rust and/or LLS (Subrahmanyam *et al*., 1983; Subrahmanyam *et al*., 1985). It has decumbent growth habit, alternate branching pattern and medium-sized elliptic green leaves. Pods carry 1-3 seeds. Pods show slight to moderate pod beak, moderate pod constriction and moderate to slight pod reticulation. The seeds are elongated in shape.

### 3.3.1 Crossing and selection scheme in MABC

The seeds of the parents were obtained from the Department of Genetics and Plant Breeding, UAS, Dharwad, and grown in pots. JL 24 (female parent) was crossed with GPBD 4, ICGV 86699 and ICGV 99005 (male parents) during the rainy season of 2010. Ten plants of JL 24 were used in each cross (JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005) to produce $F_1$ seeds. Crossing was performed by hand emasculating the unopened buds on previous day of pollination. Anthers were removed from the flowers of the female plant (JL 24) by holding flower carefully in one hand, and removing the anthers using the forceps with the other hand, ensuring minimum damage to the stigma of the flower. Pollination was done by applying the pollens on the receptive stigma of the female plant in the early morning (before 8.30 AM). Crossing was carried out for 15-20 days after the appearance first flower. Thereafter the new flowers were removed from the plants to avoid selfing.

The pods set on JL 24 were harvested and the seeds were sown in the field. Hybrid ($F_1$) plants were identified by screening with LLS and rust resistance-linked markers. JL 24 (recurrent and female parent) plants were crossed with respective $F_1$s (male parents) to develop the first backcross hybrid ($BC_1F_1$). Likewise, backcrossing was repeated twice to get $BC_3F_1$s.

### 3.3.2 Selfed generations of backcrosses

The backcrosses of JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005 were also selfed. The first backcross ($BC_1F_1$) progenies were advanced to $BC_1F_4$, second backcross ($BC_2F_1$) progenies were advanced to $BC_2F_3$ and the third backcross ($BC_3F_1$) progenies were advanced to $BC_3F_3$ for identifying the genotypes superior to JL 24 for LLS and rust resistance.

3.3.3 Field evaluation of backcross populations

Various backcross and selfed generations of JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005 were evaluated for LLS and rust under disease epiphytotic conditions by following the procedures as mentioned in the section 3.1.2.1. Observations on growth characters and productivity parameters were recorded as mentioned in the section 3.1.3.1 and 3.1.3.2, respectively. In addition, the following traits were also recorded.

1) Height of longest primary branches (HLPB): The height of longest primary branches was measured in centimeters (cm) from the main stem to the tip of the branch at the time of harvest.

2) Branching pattern: It was determined on cotyledonary lateral branch as ‘alternate’ if a pair of vegetative branches was followed by a reproductive branch or as ‘sequential’ if there are continuous runs of reproductive branches.

3) Growth habit: Based on the carriage of the plant and position of primary branches in relation to the main axis, the genotypes were classified as ‘spreading’, ‘semi-spreading’ or ‘erect’.

4) Pod length (PL): The length of five dried and cleaned pods was measured and recorded in centimeters (cm) using vernier callipers.

5) Pod width (PW): The width of five dried and cleaned pods was measured and recorded in centimeters (cm) using vernier callipers.

6) Pod constriction (PC): It was recorded as none, slight, moderate, deep or very deep based on the intensity of constriction on the mature pods.

7) Pod beak (PB): Based on the intensity of pod beak, the mature pods were classified as “absent, slight, moderate, prominent or very prominent”.

8) Pod reticulation (PR): It was recorded as smooth, slight moderate, prominent or very prominent based on the extent of veination on mature pods.

9) Seed colour: It was classified as off white, salmon, salmon with white flecks, rose, red with salmon flecks, purple, red, red with white flecks, tan, dark red and salmon with light purple fleck and light purple with salmon flecks based.

3.3.3.1 Estimation of genetic variability components

Phenotypic and genotypic variances were calculated as suggested by Singh and Chaudhary (1979).

$$\text{Genotypic variance } (\sigma^2 g) = \frac{\text{MSS (genotypes)} - \text{MSS (error)}}{\text{Number of replications}} = \frac{M_2 - M_3}{r}$$

$$\text{Phenotypic variance } (\sigma^2 p) = \sigma^2 g + \text{MSS (error)} = \frac{M_2 - M_3}{r} + M_3$$

Where,

$M_2$ = Mean sum of squares due to treatments

$M_3$ = Mean sum of squares due to error

r = Number of replications

3.3.3.2 Coefficient of variation

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

$$GCV = \frac{\sigma g}{\overline{X}} \times 100$$

$$PCV = \frac{\sigma p}{\overline{X}} \times 100$$

Where,

$\sigma g$: Genotypic standard deviation

$\sigma p$: Phenotypic standard deviation

$\overline{X}$: General mean of the character

GCV and PCV values were categorized as low (0-10%), moderate (10-20%) and high (>20%) as indicated by Sivasubramanian and Menon (1973).

3.3.3.3 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.

$$\text{Heritability } (h^2) = \frac{\sigma^2 g}{\sigma^2 p} \text{ X } 100$$

Where,

$\sigma^2_g$: genotypic variance

$\sigma^2_p$: phenotypic variance

The heritability in broad sense were categorized as low (0-30%), moderate (30-60%) and high (>60%) as indicated by Robinson *et al*. (1949).

3.3.3.4 Genetic advance (GA)

Genetic advance was estimated by using the formula given by 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% intensity of selection (Lush, 1940)

$\sigma_p$: Phenotypic standard deviation.

3.3.3.5 Genetic advance as per cent of mean (GAM)

$$GAM = \frac{GA}{\overline{X}} \text{ X } 100$$

Where,

GA: Genetic advance

$\overline{X}$: General mean of the character.

Genetic advance as per cent mean were categorized as low (0-10%), moderate (10-20%) and high (>20%) as indicated by Johnson *et al*. (1955).

3.3.3.6 Correlation analysis

Phenotypic correlation was computed to determine the degree of association among the characters (Diseases, quality and productivity traits) by using the formula given by Weber and Moorthy (1952).

$$r_{xy}(p) = \frac{Cov_{xy}(p)}{\sqrt{V_x(p) \times V_y(p)}}$$

Where,

$Cov_{xy}(p)$: Phenotypic covariance between characters x and y

$V_x(p)$: Phenotypic variance of character x

$V_y(p)$: Phenotypic variance of character y

$r_{xy}(p)$: Phenotypic correlation coefficient between characters x and y

Phenotypic correlation coefficients were compared against Table value at (n-2) degrees of freedom at the probability levels of 0.05 and 0.01 to test their significance (Fisher and Yates, 1963).

3.3.4 Genotyping of backcrosses and their selfed generations

Genomic DNA from $F_1$, $BC_1F_1$, $BC_2F_1$, $BC_3F_1$, $BC_3F_2$ and $BC_3F_3$ were isolated by following the standard protocol (Cuc *et al*., 2008). Genomic DNA was quantified as mentioned in the section 3.1.5.2.

3.3.4.1 Foreground selection

Both LLS and rust (IPAHM103 and GM2301) and only LLS (pPGPseq8D09) resistance-linked markers were used for the foreground selection of the target genomic region. These markers were checked for parental polymorphism using the PCR as described in 3.1.5.3.

3.3.4.2 Background selection

Two hundred ninety four AhTE markers (Appendix I) were used for background selection. Parental polymorphic survey was carried out using the PCR as detailed in 3.1.5.3.

# 4. EXPERIMENTAL RESULTS

Validation of LLS and rust resistance-linked QTL/markers is a pre-requisite for marker assisted backcrossing (MABC) in groundnut. For this, Near Isogenic Lines (NILs) isolated from Heterogeneous Inbred Families (HIFs) and a new mapping population (VL 1 × 110) were employed. Using these markers, a marker assisted backcrossing was undertaken to transfer LLS and rust resistance to JL 24, a popular and well adapted variety which is susceptible to LLS and rust.

## 4.1 Phenotypic and genotypic characterization of NILs

Previously, families of the advanced generations ($F_5$, $F_6$ and $F_7$) developed from the two crosses TAG 24 × GPBD 4 and TG 26 × GPBD 4 were evaluated for rust resistance under epiphytotic condition. Majority of such families bred true, while a few segregated for rust resistance. Advanced families segregating for rust reaction were identified as heterogeneous inbred families (HIFs). Thirteen and sixteen HIFs were identified in TAG 24 × GPBD 4 and TG 26 × GPBD 4, respectively. Plants that were either rust resistant (less than score 5) or rust susceptible (more than score 5) were selected from each HIF of both the crosses. In total, 13 rust resistant and 16 rust susceptible plants were identified from the 13 HIFs of TAG 24 × GPBD 4. Similarly, 17 rust resistant and 18 rust susceptible plants were identified from 16 HIFs of TG 26 × GPBD 4. There was at least one rust resistant and one rust susceptible line isolated from each HIF of both the crosses. True breeding lines were developed from these plants and such lines with contrasting responses to rust were grouped under Set I (Table 8). Thus Set I consisted of 30 rust resistant and 34 rust susceptible lines.

Similarly, based on LLS reaction, plants with score less than 5 were considered as LLS resistant and those with score more than 5 were regarded as susceptible. In total, five LLS resistant and six LLS susceptible plants were selected from five HIFs of TAG 24 × GPBD 4. Likewise, 16 LLS resistant and 19 LLS susceptible plants were recovered from 16 HIFs of TG 26 × GPBD 4. True breeding progenies of these plants were grouped under Set II (Table 9). At least one LLS resistant and one LLS susceptible lines isolated from each HIF of both the crosses were included in Set II. Thus Set II contained 21 LLS resistant and 25 LLS susceptible lines. These sets were obtained from the Department of Genetics and Plant Breeding, UAS, Dharwad.

**Table 8: Heterogeneous inbred families and their lines differing for rust response in Set I**

| TAG 24 × GPBD 4 | | | TG 26 × GPBD 4 | | |
|---|---|---|---|---|---|
| **Sl. No.** | **HIF-derived lines** | | **Sl. No.** | **HIF-derived lines** | |
| 1 | 9 | 9-1, 9-2, 9-3 | 1 | 1 | 1-1, 1-2 |
| 2 | 14 | 14-1, 14-2 | 2 | 7 | 7-1, 7-2 |
| 3 | 46 | 46-1, 46-2, 46-3 | 3 | 46 | 46-1, 46-2, 46-3 |
| 4 | 47 | 47-1, 47-2 | 4 | 53 | 53-1, 53-2 |
| 5 | 50 | 50-1, 50-2 | 5 | 58 | 58-1, 58-2 |
| 6 | 60 | 60-1, 60-2, 60-3 | 6 | 102 | 102-1, 102-2 |
| 7 | 64 | 64-1, 64-2 | 7 | 103 | 103-1, 103-2, 103-3 |
| 8 | 77 | 77-1, 77-2 | 8 | 113 | 113-1, 113-2, 113-3 |
| 9 | 80 | 80-1, 80-2 | 9 | 124 | 124-1, 124-2 |
| 10 | 83 | 83-1, 83-2 | 10 | 129 | 129-1, 129-2 |
| 11 | 89 | 89-1, 89-2 | 11 | 131 | 131-1, 131-2 |
| 12 | 101 | 101-1, 101-2 | 12 | 138 | 138-1, 138-2 |
| 13 | 116 | 116-1, 116-2 | 13 | 156 | 156-1, 156-2 |
| | | | 14 | 162 | 162-1, 162-2 |
| | | | 15 | 167 | 167-1, 167-2 |
| | | | 16 | 175 | 175-1, 175-2 |

**Table 9: Heterogeneous inbred families and their lines differing for LLS response in Set II**

| TAG 24 × GPBD 4 | | | TG 26 × GPBD 4 | | |
|---|---|---|---|---|---|
| **Sl. No.** | **HIF-derived lines** | | **Sl. No.** | **HIF-derived lines** | |
| 1 | 14 | 14-1, 14-2 | 1 | 1 | 1-1, 1-2 |
| 2 | 77 | 77-1, 77-2 | 2 | 7 | 7-1, 7-2 |
| 3 | 132 | 132-1, 132-2 | 3 | 46 | 46-1, 46-2, 46-3 |
| 4 | 139 | 139-1, 139-2, 139-3 | 4 | 58 | 58-1, 58-2 |
| 5 | 12 | 12-1, 12-2 | 5 | 62 | 62-1, 62-2 |
| | | | 6 | 72 | 72-1, 72-2 |
| | | | 7 | 79 | 79-1, 79-2, 79-3 |
| | | | 8 | 103 | 103-1, 103-2, 103-3 |
| | | | 9 | 107 | 107-1, 107-2, 107-3 |
| | | | 10 | 111 | 111-1, 111-2, 111-3 |
| | | | 11 | 127 | 127-1, 127-2 |
| | | | 12 | 129 | 129-1, 129-2 |
| | | | 13 | 138 | 138-1, 138-2 |
| | | | 14 | 150 | 150-1, 150-2 |
| | | | 15 | 170 | 170-1, 170-2 |
| | | | 16 | 175 | 175-1, 175-2 |

In the present study, the lines belonging to Set I and Set II were evaluated separately for their response to rust and LLS under disease epiphytotic condition during the rainy seasons of 2011 and 2012 in IABT Garden E115, Main Agricultural Research Station, UAS, Dharwad. In addition to the data collected during the rainy seasons of 2011 and 2012, the data available in the Department for the rainy seasons of 2008, 2009 and 2010, and the post-rainy season of 2010 were pooled and considered for analysis of variation (ANOVA).

### 4.1.1 Evaluation for rust response

Analysis of variance for the data pooled across the seasons for rust response is given in the Table 10. A significant variation was observed between the seasons and between the genotypes of Set I. It was noticed that the lines originating from the same HIF differed significantly for rust resistance. For example, 14-1 and 14-2 were the two lines derived from the HIF 14 from TAG 24 × GPBD 4. Line 14-1 was rust resistant with a mean disease score of 4.67, while 14-2 was rust susceptible with a score of 6.50 at 90 DAS (Table 11). Likewise, 53-1 and 53-2 were the lines derived from HIF 53 of TG 26 × GPBD 4. 53-1 was rust resistant with a mean disease score of 4.42, while 53-2 was rust susceptible with a score of 6.67 at 90 DAS (Table 12). Such lines differing for rust resistance were observed from all the 13 HIFs of TAG 24 × GPBD 4 and 16 HIFs of TG 26 × GPBD 4.

### 4.1.2 Evaluation for LLS response

ANOVA for the pooled data across the seasons for response to LLS is given in the Table 13. The lines in Set II differed significantly for LLS response at 80 and 90 DAS, but not at 70 DAS. The interaction effect of genotypes and seasons was not significant for LLS response at 70 DAS but, was significant at 80 and 90 DAS among the lines of both the crosses. Line originating from the same HIF differed significantly for response to LLS. For example, line 77-1 and 77-2 originated from the HIF 77 of TAG 24 × GPBD 4. Line 77-1 was LLS resistant with a mean disease score of 4.83, whereas 77-2 was LLS susceptible with a score of 7.08 at 90 DAS (Table 14). Similarly, line 7-1 and 7-2 were selected from HIF 7 of TG 26 × GPBD 4. Line 7-2 was LLS resistant with a mean disease score of 4.25, while 7-1 was LLS susceptible with a

**Table 10: Pooled ANOVA for response to rust among the lines of Set I**

| Crosses | df | Mean sum of squares | | | Critical difference (CD) | | SEm± | CV (%) |
|---|---|---|---|---|---|---|---|---|
| | | Replication | Treatment | Error | 5% | 1% | | |
| **TAG 24 × GPBD 4** | | | | | | | | |
| R_70 | 30 | 0.02 | 0.09 | 0.02 | 0.26 | 0.35 | 0.09 | 5.03 |
| R_80 | 30 | 0.16 | 0.74** | 0.05 | 0.45 | 0.61 | 0.16 | 5.26 |
| R_90 | 30 | 0.00 | 2.40** | 0.13 | 0.72 | 0.97 | 0.25 | 6.36 |
| **TG 26 × GPBD 4** | | | | | | | | |
| R_70 | 36 | 0.02 | 0.10** | 0.02 | 0.28 | 0.37 | 0.10 | 5.47 |
| R_80 | 36 | 0.00 | 0.58** | 0.06 | 0.50 | 0.67 | 0.17 | 5.97 |
| R_90 | 36 | 0.00 | 2.55** | 0.15 | 0.78 | 1.05 | 0.27 | 7.06 |

*, **: Significance at 5% and 1%, respectively

**df:** degrees of freedom; **CV:** Coefficient of variation; **SEm±:** Standard error of mean; **R_70:** Rust score at 70 days after sowing (DAS); **R_80:** Rust score at 80 DAS; **R_90:** Rust score at 90 DAS

**Table 11: Performance of HIF-derived lines from TAG 24 × GPBD 4 belonging to Set I for response to rust, productivity traits and agronomic traits**

| Lines | R_90 | PYPP | TW | SP | SMKP | NPB | PH | LL | LW |
|---|---|---|---|---|---|---|---|---|---|
| 9-1 | 6.58 $^{a-c}$ | 14.46 $^{a-e}$ | 49.75 $^{cd}$ | 61.88 $^{ij}$ | 87.30 $^{kl}$ | 8.13 $^{a}$ | 33.35 $^{a}$ | 5.14 $^{ab}$ | 2.60 $^{a}$ |
| 9-2 | 4.50 $^{e-g}$ | 16.06 $^{a}$ | 47.88 $^{c-g}$ | 68.13 $^{gh}$ | 93.93 $^{e-h}$ | 6.75 $^{a-d}$ | 29.98 $^{ab}$ | 5.24 $^{ab}$ | 2.31 $^{a}$ |
| 9-3 | 6.67 $^{a-c}$ | 15.00 $^{a-e}$ | 49.50 $^{cd}$ | 74.75 $^{b-d}$ | 100.00 $^{a}$ | 6.63 $^{a-d}$ | 26.73 $^{b-f}$ | 4.79 $^{ab}$ | 2.56 $^{a}$ |
| 14-1 | 4.67 $^{e-g}$ | 15.28 $^{a-d}$ | 44.00 $^{f-h}$ | 74.00 $^{b-e}$ | 92.54 $^{g-i}$ | 6.38 $^{a-d}$ | 27.79 $^{b-d}$ | 4.49 $^{ab}$ | 2.25 $^{a}$ |
| 14-2 | 6.50 $^{a-d}$ | 13.68 $^{a-e}$ | 41.00 $^{h-j}$ | 73.63 $^{b-e}$ | 100.00 $^{a}$ | 6.50 $^{a-d}$ | 23.85 $^{d-h}$ | 4.61 $^{ab}$ | 2.53 $^{a}$ |
| 46-1 | 7.00 $^{g}$ | 14.71 $^{a-e}$ | 45.00 $^{e-h}$ | 72.88 $^{b-g}$ | 95.52 $^{c-g}$ | 6.94 $^{a-d}$ | 23.43 $^{e-h}$ | 4.55 $^{ab}$ | 2.55 $^{a}$ |
| 46-2 | 5.25 $^{e-g}$ | 12.61 $^{de}$ | 42.25 $^{hi}$ | 74.38 $^{b-d}$ | 89.15 $^{jk}$ | 6.13 $^{a-d}$ | 27.31 $^{b-e}$ | 4.58 $^{ab}$ | 2.78 $^{a}$ |
| 46-3 | 4.50 $^{a}$ | 14.09 $^{a-e}$ | 42.25 $^{hi}$ | 75.13 $^{b-d}$ | 95.54 $^{c-g}$ | 4.75 $^{d}$ | 25.13 $^{c-h}$ | 5.31 $^{ab}$ | 2.59 $^{a}$ |
| 47-1 | 4.92 $^{e-g}$ | 13.56 $^{a-e}$ | 43.00 $^{hi}$ | 69.25 $^{e-h}$ | 92.23 $^{hi}$ | 6.25 $^{a-d}$ | 25.10 $^{c-h}$ | 5.19 $^{ab}$ | 2.35 $^{a}$ |
| 47-2 | 5.83 $^{b-e}$ | 13.23 $^{b-e}$ | 37.25 $^{j}$ | 77.13 $^{b}$ | 88.37 $^{j-l}$ | 6.13 $^{a-d}$ | 27.89 $^{b-d}$ | 4.86 $^{ab}$ | 2.38 $^{a}$ |
| 50-1 | 5.83 $^{e-g}$ | 12.87 $^{c-e}$ | 41.25 $^{h-j}$ | 76.25 $^{bc}$ | 93.86 $^{e-h}$ | 7.75 $^{ab}$ | 24.43 $^{c-h}$ | 4.49 $^{ab}$ | 2.51 $^{a}$ |
| 50-2 | 4.67 $^{c-f}$ | 13.72 $^{a-e}$ | 49.75 $^{cd}$ | 64.75 $^{hi}$ | 96.48 $^{b-e}$ | 7.25 $^{a-c}$ | 27.38 $^{b-e}$ | 4.61 $^{ab}$ | 2.59 $^{a}$ |
| 60-1 | 7.08 $^{d-g}$ | 12.51 $^{e}$ | 42.13 $^{hi}$ | 68.50 $^{f-h}$ | 100.00 $^{a}$ | 5.38 $^{b-d}$ | 18.80 $^{j}$ | 4.01 $^{b}$ | 2.48 $^{a}$ |
| 60-2 | 7.00 $^{b-e}$ | 12.88 $^{c-e}$ | 38.75 $^{ij}$ | 73.50 $^{a}$ | 95.73 $^{c-f}$ | 5.75 $^{a-d}$ | 32.33 $^{a}$ | 5.65 $^{a}$ | 3.09 $^{a}$ |
| 60-3 | 4.42 $^{a-c}$ | 15.32 $^{a-d}$ | 45.50 $^{d-h}$ | 72.50 $^{b-g}$ | 98.02 $^{a-c}$ | 6.88 $^{a-d}$ | 25.76 $^{c-g}$ | 5.38 $^{ab}$ | 2.54 $^{a}$ |
| 64-1 | 6.17 $^{g}$ | 14.11 $^{a-e}$ | 44.25 $^{f-h}$ | 70.25 $^{d-g}$ | 95.45 $^{c-g}$ | 6.75 $^{a-d}$ | 25.74c $^{d-g}$ | 5.18 $^{ab}$ | 2.79 $^{a}$ |
| 64-2 | 4.33 $^{a-c}$ | 13.85 $^{a-e}$ | 44.50 $^{f-h}$ | 71.25 $^{c-g}$ | 100.00 $^{a}$ | 7.13 $^{a-d}$ | 24.28 $^{c-h}$ | 4.75 $^{ab}$ | 2.31 $^{a}$ |
| 77-1 | 7.08 $^{ab}$ | 13.88 $^{a-e}$ | 49.63 $^{cd}$ | 73.38 $^{b-e}$ | 97.86 $^{a-d}$ | 6.63 $^{a-d}$ | 27.36 $^{b-e}$ | 4.70 $^{ab}$ | 2.40 $^{a}$ |
| 77-2 | 4.83 $^{d-g}$ | 14.50 $^{a-e}$ | 48.50 $^{c-f}$ | 74.75 $^{b-d}$ | 92.55 $^{g-i}$ | 6.00 $^{a-d}$ | 21.14 $^{h-j}$ | 4.93 $^{ab}$ | 2.58 $^{a}$ |
| 80-1 | 4.92 $^{g}$ | 15.47 $^{a-c}$ | 55.50 $^{b}$ | 73.13 $^{b-f}$ | 93.34 $^{f-i}$ | 7.00 $^{a-d}$ | 28.08 $^{bc}$ | 5.33 $^{ab}$ | 2.54 $^{a}$ |
| 80-2 | 5.83 $^{b-e}$ | 15.41 $^{a-c}$ | 51.00 $^{c}$ | 63.00 $^{ij}$ | 94.87 $^{d-h}$ | 5.88 $^{a-d}$ | 22.75 $^{f-j}$ | 4.73 $^{ab}$ | 2.45 $^{a}$ |
| 83-1 | 4.67 $^{e-g}$ | 13.93 $^{a-e}$ | 49.38 $^{c-e}$ | 72.38 $^{b-g}$ | 96.30 $^{b-f}$ | 7.38 $^{a-c}$ | 23.55 $^{e-h}$ | 5.54 $^{ab}$ | 2.79 $^{a}$ |
| 83-2 | 7.08 $^{a}$ | 15.72 $^{ab}$ | 43.75 $^{gh}$ | 76.13 $^{bc}$ | 96.81 $^{b-e}$ | 7.25 $^{a-c}$ | 25.23 $^{c-h}$ | 4.56 $^{ab}$ | 2.39 $^{a}$ |
| 89-1 | 4.50 $^{ab}$ | 14.14 $^{a-e}$ | 44.25 $^{f-h}$ | 73.75 $^{b-e}$ | 99.33 $^{ab}$ | 5.50 $^{b-d}$ | 19.26 $^{ij}$ | 4.30 $^{ab}$ | 2.59 $^{a}$ |
| 89-2 | 6.50 $^{g}$ | 15.25 $^{a-e}$ | 49.75 $^{cd}$ | 75.75 $^{bc}$ | 87.71 $^{kl}$ | 7.50 $^{a-c}$ | 22.29 $^{g-j}$ | 5.43 $^{ab}$ | 2.83 $^{a}$ |
| 101-1 | 4.58 $^{g}$ | 14.22 $^{a-e}$ | 40.88 $^{hij}$ | 73.75 $^{b-e}$ | 96.34 $^{b-f}$ | 6.13 $^{a-d}$ | 22.24 $^{g-j}$ | 4.69 $^{ab}$ | 2.88 $^{a}$ |
| 101-2 | 6.50 $^{a-c}$ | 15.80 $^{ab}$ | 40.38 $^{a}$ | 73.88 $^{b-e}$ | 100.00 $^{a}$ | 6.88 $^{a-d}$ | 23.05 $^{f-i}$ | 4.85 $^{ab}$ | 2.58 $^{a}$ |
| 116-1 | 4.58 $^{fg}$ | 16.11 $^{a}$ | 41.50 $^{h-j}$ | 75.25 $^{bc}$ | 90.81 $^{ij}$ | 7.75 $^{ab}$ | 25.56 $^{c-g}$ | 4.50 $^{ab}$ | 2.59 $^{a}$ |
| 116-2 | 6.58 $^{a-c}$ | 14.95 $^{a-e}$ | 44.50 $^{f-h}$ | 73.88 $^{b-e}$ | 98.51 $^{a-c}$ | 7.13 $^{a-d}$ | 22.08 $^{g-j}$ | 5.44 $^{ab}$ | 2.66 $^{a}$ |
| TAG 24 | 7.17 | 13.84 | 42.00 | 60.00 | 92.21 | 5.25 | 21.24 | 4.34 | 2.33 |
| GPBD 4 | 3.00 | 13.91 | 41.25 | 75.13 | 85.75 | 6.13 | 27.58 | 5.14 | 2.54 |
| SEm± | 0.25 | 1.26 | 3.78 | 4.16 | 1.68 | 0.96 | 2.93 | 0.40 | 0.23 |

**R_90:** Rust score at 90 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

**Table 12: Performance of HIF-derived lines from TG 26 × GPBD 4 belonging to Set I for response to rust, productivity traits and agronomic traits**

| Lines | R_90 | PYPP | TW | SP | SMKP | NPB | PH | LL | LW |
|---|---|---|---|---|---|---|---|---|---|
| 1-1 | 4.50 k-m | 15.88 a-d | 47.50 b-d | 74.25 ab | 96.06 a-d | 7.88 a-d | 31.19 b-f | 4.84 a-d | 2.64 a |
| 1-2 | 7.40 a-c | 14.49 c-f | 43.50 d-h | 74.00 ab | 96.97 ab | 7.75 a-e | 27.28 f-o | 5.09 a-d | 2.61 a |
| 7-1 | 6.08 d-h | 14.61 c-f | 54.25 a | 68.25 f-i | 98.30 a | 8.25 a-c | 24.99 l-p | 5.00 a-d | 2.73 a |
| 7-2 | 4.25 m | 14.89 b-e | 40.75 g-k | 74.50 ab | 95.48 a-d | 8.75 a | 36.50 a | 5.70 ab | 2.74 a |
| 46-1 | 6.42 b-g | 16.54 ab | 55.00 a | 73.50 a-d | 95.59 a-d | 6.13 c-g | 29.45 c-i | 5.69 ab | 2.71 a |
| 46-2 | 7.92 a-e | 15.31 a-e | 38.25 j-l | 71.25 a-g | 96.85 ab | 5.13 g | 19.30 q | 4.63 a-d | 2.46 a |
| 46-3 | 4.83 i-m | 14.99 b-e | 47.50 b-d | 70.50 b-h | 97.06 ab | 8.75 a | 27.30 f-o | 4.90 a-d | 2.36 a |
| 53-1 | 4.42 lm | 16.18 a-c | 49.75 b | 73.88 a-c | 95.14 a-e | 6.50 a-g | 29.95 c-j | 5.53 a-d | 2.43 a |
| 53-2 | 6.67 b-f | 16.00 a-d | 49.38 b | 75.13 a | 96.48 a-c | 7.63 a-e | 31.66 b-d | 5.21 a-d | 2.60 a |
| 58-1 | 4.42 lm | 15.35 a-e | 49.50 b | 69.38 c-i | 95.34 a-e | 6.88 a-g | 29.23 d-g | 5.13 a-d | 2.53 a |
| 58-2 | 6.00 e-i | 16.04 a-d | 49.50 b | 73.50 a-d | 93.07 b-f | 8.00 a-d | 27.23 g-o | 4.91 a-d | 2.56 a |
| 102-1 | 4.83 i-m | 16.76 a | 46.00 b-f | 65.75 i | 97.61 a | 7.00 a-g | 23.75 op | 5.26 a-d | 2.51 a |
| 102-2 | 6.17 d-g | 16.08 a-d | 46.00 b-f | 73.75 a-d | 90.43 f-i | 5.25 fg | 22.48 pq | 4.85 a-d | 2.41 a |
| 103-1 | 6.00 e-i | 15.83 a-d | 42.25 f-j | 66.63 hi | 95.77 a-d | 6.50 a-g | 27.04 g-o | 4.86 a-d | 2.38 a |
| 103-2 | 4.67 j-m | 15.26 a-e | 43.00 e-h | 73.50 a-d | 95.00 a-e | 6.75 a-g | 28.43 c-m | 5.33 a-d | 2.80 a |
| 103-3 | 4.92 h-m | 14.02 e-g | 43.00 e-h | 69.25 d-i | 95.30 a-e | 6.38 b-g | 25.19 k-p | 4.93 a-d | 2.84 a |
| 113-1 | 4.67 j-m | 14.79 b-e | 38.75 i-l | 71.75 a-g | 93.14 b-f | 6.75 a-g | 24.54 m-p | 4.54 b-d | 2.40 a |
| 113-2 | 5.25 g-m | 15.00 b-e | 42.50 f-i | 72.00 a-f | 90.48 f-i | 6.88 a-g | 28.01 d-m | 5.26 a-d | 2.96 a |
| 113-3 | 5.67 a-e | 15.25 a-e | 41.75 g-j | 70.63 a-h | 94.79 a-e | 6.00 c-g | 25.90 i-p | 4.96 a-d | 2.75 a |
| 124-1 | 4.67 j-m | 12.59 g | 40.13 h-k | 70.13 b-i | 92.10 d-h | 5.50 e-g | 24.08 n-p | 4.91 a-d | 2.40 a |
| 124-2 | 5.42 g-m | 14.57 c-f | 48.25 bc | 68.38 e-i | 87.79 i | 7.50 a-f | 28.33 d-m | 4.84 a-d | 2.58 a |
| 129-1 | 7.25 h-m | 15.90 a-d | 43.00 e-h | 59.75 j | 95.20 a-e | 6.13 c-g | 29.16 c-k | 5.36 a-d | 2.49 a |
| 129-2 | 4.58 j-m | 15.30 a-e | 49.25 b | 67.88 f-i | 94.86 a-e | 6.25 b-g | 31.33 b-e | 5.21 a-d | 2.40 a |
| 131-1 | 5.42 g-m | 12.54 g | 42.25 f-j | 70.88 a-h | 95.40 a-e | 6.13 c-g | 27.28 | 5.29 a-d | 2.48 a |
| 131-2 | 4.92 h-m | 13.87 fg | 44.75 c-g | 73.88 a-c | 92.81 c-g | 7.13 a-g | 32.31 | 5.56 a-c | 2.71 a |
| 138-1 | 7.58 ab | 12.41 g | 35.50 l | 66.00 i | 97.08 ab | 6.63 a-g | 31.74 | 5.18 a-d | 2.73 a |
| 138-2 | 4.83 i-m | 13.08 fg | 42.75 f-i | 65.88 i | 96.84 ab | 8.50 ab | 28.56 | 5.15 a-d | 2.44 a |
| 156-1 | 4.50 k-m | 15.09 a-e | 42.75 f-i | 69.00 e-i | 88.42 hi | 5.25 fg | 27.45 | 4.64 b-d | 2.33 a |
| 156-2 | 5.75 f-k | 14.33 d-f | 37.00 kl | 73.63 a-d | 91.47 e-i | 5.88 d-g | 26.16 | 4.29 cd | 2.35 a |
| 162-1 | 5.83 f-j | 16.10 a-c | 47.00 b-e | 61.38 j | 93.07 b-f | 7.13 a-g | 34.03 | 6.10 a | 2.65 a |
| 162-2 | 4.75 i-m | 14.78 c-e | 40.25 h-k | 66.75 hi | 95.74 a-d | 7.00 a-g | 27.85 | 4.95 a-d | 2.31 a |
| 167-1 | 6.33 c-g | 14.89 b-e | 35.75 l | 67.50 g-i | 96.22 a-c | 7.00 a-g | 24.65 | 4.46 b-d | 2.46 a |
| 167-2 | 4.50 k-m | 14.47 c-f | 44.75 c-g | 67.38 g-i | 89.19 g-i | 6.25 b-g | 25.29 | 5.10 a-d | 2.51 a |
| 175-1 | 6.50 b-g | 15.80 a-d | 36.00 l | 72.25 a-f | 93.24 b-f | 7.88 a-d | 27.15 | 4.15 d | 2.46 a |
| 175-2 | 4.42 lm | 15.96 a-d | 43.25 e-h | 72.75 a-e | 95.59 a-d | 6.50 a-g | 26.40 | 4.88 a-d | 2.31 a |
| TG 26 | 7.17 | 12.42 | 40.75 | 70.00 | 97.04 | 6.13 | 20.28 | 4.35 | 2.33 |
| GPBD 4 | 2.83 | 14.52 | 40.50 | 74.50 | 96.73 | 7.00 | 30.19 | 5.16 | 2.71 |
| SEm± | 0.27 | 0.51 | 2.96 | 3.41 | 2.64 | 0.89 | 2.64 | 0.32 | 0.22 |

**R_90:** Rust score at 90 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

**Table 13: Pooled ANOVA for response to LLS among the lines of Set II**

| Crosses | df | Mean sum of squares | | | Critical difference (CD) | | SEm± | CV (%) |
|---|---|---|---|---|---|---|---|---|
| | | Replication | Treatment | Error | 5% | 1% | | |
| **TAG 24 × GPBD 4** | | | | | | | | |
| LLS_70 | 12 | 0.00 | 0.12 | 0.19 | 0.27 | 0.38 | 0.09 | 5.04 |
| LLS_80 | 12 | 0.00 | 0.83** | 0.83 | 0.57 | 0.80 | 0.19 | 6.16 |
| LLS_90 | 12 | 0.07 | 3.02 | 2.43 | 0.98 | 1.38 | 0.32 | 7.82 |
| **TG 26 × GPBD 4** | | | | | | | | |
| LLS_70 | 36 | 0.14 | 0.15** | 0.04 | 0.39 | 0.52 | 0.14 | 6.86 |
| LLS_80 | 36 | 0.03 | 1.34** | 0.06 | 0.50 | 0.67 | 0.18 | 5.51 |
| LLS_90 | 36 | 0.38 | 3.27** | 0.13 | 0.74 | 1.00 | 0.26 | 6.84 |

*, **: Significance at 5% and 1%, respectively

**df:** degrees of freedom; **CV:** Coefficient of variation; **SEm±:** Standard error of mean; **LLS_70:** LLS score at 70 days after sowing (DAS); **LLS_80:** LLS score at 80 DAS; **LLS_90:** LLS score at 90 DAS

**Table 14: Performance of HIF-derived lines from TAG 24 × GPBD 4 belonging to Set II for response to LLS, productivity traits and agronomic traits**

| Lines | LLS_90 | PYPP | TW | SP | SMKP | NPB | PH | LL | LW |
|---|---|---|---|---|---|---|---|---|---|
| 14-1 | 4.67 $^{c}$ | 16.06 $^{ab}$ | 47.88 $^{ab}$ | 68.13 $^{c}$ | 93.93 $^{bc}$ | 8.00 $^{c-e}$ | 31.10 $^{b}$ | 5.95 $^{a}$ | 2.20 $^{a}$ |
| 14-2 | 6.50 $^{ab}$ | 14.46 $^{a-c}$ | 49.75 $^{a}$ | 61.88 $^{d}$ | 87.30 $^{de}$ | 8.25 $^{cd}$ | 34.55 $^{a}$ | 5.03 $^{bc}$ | 2.45 $^{a}$ |
| 77-1 | 4.83 $^{c}$ | 12.61 $^{c}$ | 42.25 $^{cd}$ | 74.38 $^{ab}$ | 89.15 $^{d}$ | 7.00 $^{def}$ | 27.08 $^{cd}$ | 4.75 $^{b-d}$ | 2.90 $^{a}$ |
| 77-2 | 7.08 $^{ab}$ | 14.09 $^{a-c}$ | 42.25 $^{cd}$ | 75.13 $^{ab}$ | 95.54 $^{b}$ | 5.00 $^{h}$ | 25.18 $^{d}$ | 4.80 $^{b-d}$ | 2.45 $^{a}$ |
| 132-1 | 4.58 $^{c}$ | 13.77 $^{bc}$ | 48.00 $^{ab}$ | 73.75 $^{ab}$ | 100.00 $^{a}$ | 7.75 $^{c-f}$ | 25.98 $^{d}$ | 5.05 $^{bc}$ | 2.33 $^{a}$ |
| 132-3 | 7.67 $^{ab}$ | 14.37 $^{a-c}$ | 44.63 $^{bc}$ | 75.25 $^{ab}$ | 100.00 $^{a}$ | 7.00 $^{d-f}$ | 24.13 $^{d}$ | 4.55 $^{cd}$ | 3.00 $^{a}$ |
| I39-1 | 4.42 $^{c}$ | 14.73 $^{a-c}$ | 39.00 $^{d}$ | 76.75 $^{a}$ | 99.49 $^{a}$ | 9.00 $^{bc}$ | 30.28 $^{b}$ | 4.30 $^{cd}$ | 2.35 $^{a}$ |
| I39-2 | 6.25 $^{ab}$ | 14.10 $^{a-c}$ | 43.25 $^{b-d}$ | 72.38 $^{b}$ | 91.90 $^{c}$ | 10.75 $^{a}$ | 30.53 $^{b}$ | 4.48 $^{cd}$ | 2.45 $^{a}$ |
| I39-3 | 5.67 $^{c}$ | 16.37 $^{a}$ | 47.75 $^{ab}$ | 73.25 $^{ab}$ | 95.96 $^{b}$ | 9.75 $^{ab}$ | 24.55 $^{d}$ | 4.45 $^{cd}$ | 2.38 $^{a}$ |
| 12□1 | 4.50 $^{c}$ | 13.65 $^{c}$ | 51.50 $^{a}$ | 75.50 $^{ab}$ | 91.48 $^{c}$ | 6.50 $^{fg}$ | 26.13 $^{d}$ | 4.70 $^{b-d}$ | 2.58 $^{a}$ |
| 12□2 | 6.92 $^{ab}$ | 12.53 $^{c}$ | 51.75 $^{a}$ | 74.13 $^{ab}$ | 95.35 $^{b}$ | 9.00 $^{bc}$ | 30.25 $^{b}$ | 4.50 $^{cd}$ | 2.33 $^{a}$ |
| TAG 24 | 7.83 | 13.84 | 42.00 | 60.00 | 92.21 | 5.50 | 17.25 | 4.05 | 2.28 |
| GPBD 4 | 3.00 | 13.91 | 41.25 | 75.13 | 85.75 | 6.75 | 29.80 | 5.43 | 2.63 |
| SEm± | 0.32 | 0.89 | 4.03 | 2.66 | 1.10 | 0.31 | 1.60 | 0.13 | 0.12 |

**LLS_90:** LLS score at 90 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

score of 6.00 at 90 DAS (Table 15). Such LLS resistant and LLS susceptible lines were observed in all the 5 HIFs of TAG 24 × GPBD 4 and 16 HIFs of TG 26 × GPBD 4.

### 4.1.3 Evaluation for agronomic and productivity traits

Lines of Set I were also evaluated for some of the agronomic and productivity traits during the rainy seasons of 2011 and 2012. They differed significantly for most of the traits except for the number of primary branches, leaf length and leaf width during both the seasons. Lines originating from the same HIF, but differing for reaction to rust, were compared for the agronomic and productivity traits in Set I. Such lines did not differ significantly for the majority of agronomic and productivity traits (Table 11 and 12). For example, 14-1and 14-2 from HIF 83 (TAG 24 × GPBD 4) and 53-1 and 53-2 from HIF 53 (TG 26 × GPBD 4) differed significantly for response to rust, but not for the majority of agronomic and productivity traits.

Similarly, the lines of Set II were also evaluated for some of the agronomic and productivity traits, and the lines from the common source (HIF) were compared. The lines from the same HIF did not differ significantly for majority of the agronomic and productivity traits (Table 14 and 15). For example, 77-1and 77-2 from HIF 77 (TAG 24 × GPBD 4) and 7-1 and 7-2 from HIF 7 (TG 26 × GPBD 4) showed significant differences for response to LLS, but not for the majority of agronomic and productivity traits.

### 4.1.4 Background genome similarity

Since the lines from the same HIF in Set I and Set II differed for reaction to rust and LLS, respectively but not for most of the morphological and productivity traits, an attempt was made to compare the genome similarity in terms of background markers among the lines of Set I. For this, 1079 (*Arachis hypogaea* Genome-SSR-AHGS), 470 (*Arachis hypogaea* EST-SSR-AHS), 405 (*Arachis hypogaea* Transposable Element-AhTE) and 89 SSR markers were employed for assessing the parental polymorphism between TAG 24 versus GPBD 4, and TG 26 versus GPBD 4. In total, 168 and 252 markers were polymorphic between TAG 24 versus GPBD 4 and TG 26 versus GPBD 4, respectively. Very high similarity (indicated by monomorphic markers) was observed among pairs of lines derived from each HIF. The average similarity was 67.84% and 67.18% among the lines of TAG 24 × GPBD 4 and TG 26 × GPBD 4, respectively. The

**Table 15: Performance of HIF-derived lines from TG 26 × GPBD 4 belonging to Set II for response to LLS, productivity traits and agronomic traits**

| Lines | LLS_90 | PYPP | TW | SP | SMKP | NPB | PH | LL | LW |
|---|---|---|---|---|---|---|---|---|---|
| 1-1 | 7.00 [a-c] | 15.88 [a-d] | 47.50 [bc] | 74.25 [a-c] | 96.06 [a-d] | 7.88 [a-d] | 31.19 [b-d] | 4.84 [a-d] | 2.64 [a] |
| 1-2 | 3.75 [lm] | 14.49 [a-e] | 43.50 [c-f] | 74.00 [a-d] | 96.97 [ab] | 7.75 [a-d] | 27.28 [d-i] | 5.09 [a-d] | 2.61 [a] |
| 7-1 | 6.00 [b-g] | 14.61 [a-e] | 54.25 [a] | 68.25 [e-h] | 98.30 [a] | 8.25 [a-d] | 24.99 [h-k] | 5.00 [a-d] | 2.73 [a] |
| 7-2 | 4.25 [j-l] | 14.89 [a-e] | 40.75 [e-g] | 74.50 [ab] | 95.48 [a-e] | 8.75 [ab] | 36.50 [a] | 5.70 [ab] | 2.74 [a] |
| 46-1 | 6.08 [a-g] | 16.54 [a] | 55.00 [a] | 73.50 [a-d] | 95.59 [a-e] | 6.13 [c-e] | 29.45 [c-f] | 5.69 [ab] | 2.71 [a] |
| 46-2 | 4.00 [lm] | 15.31 [a-e] | 38.25 [g-j] | 71.25 [a-f] | 96.85 [a-c] | 5.13 [e] | 19.30 [lm] | 4.63 [a-d] | 2.46 [a] |
| 46-3 | 7.25 [a] | 14.99 [a-e] | 47.50 [bc] | 70.50 [b-h] | 97.06 [ab] | 8.75 [ab] | 27.30 [d-i] | 4.90 [a-d] | 2.36 [a] |
| 58-1 | 4.25 [j-l] | 15.35 [a-e] | 49.50 [b] | 69.38 [c-h] | 95.34 [a-e] | 6.88 [a-e] | 29.23 [c-g] | 5.13 [a-d] | 2.53 [a] |
| 58-2 | 6.42 [a-f] | 16.04 [a-c] | 49.50 [b] | 73.50 [a-d] | 93.07 [d-g] | 8.00 [a-d] | 27.23 [d-i] | 4.91 [a-d] | 2.56 [a] |
| 62-1 | 4.67 [h-l] | 14.06 [b-f] | 43.75 [c-f] | 74.88 [ab] | 96.83 [a-c] | 7.25 [a-e] | 26.71 [e-i] | 4.79 [a-d] | 2.46 [a] |
| 62-2 | 6.67 [a-e] | 15.09 [a-e] | 44.50 [c-e] | 70.63 [b-h] | 94.43 [b-f] | 7.75 [a-d] | 26.31 [e-j] | 4.70 [a-d] | 2.33 [a] |
| 72-1 | 7.25 [a] | 13.93 [c-f] | 36.50 [h-j] | 73.50 [a-d] | 96.47 [a-d] | 6.63 [b-e] | 19.28 [lm] | 3.93 [d] | 2.04 [a] |
| 72-2 | 4.17 [kl] | 13.98 [b-f] | 40.25 [e-h] | 74.00 [a-d] | 91.40 [f-h] | 6.38 [b-e] | 31.34 [bc] | 5.19 [a-d] | 2.73 [a] |
| 79-1 | 4.00 [lm] | 16.40 [a] | 38.25 [g-j] | 72.50 [a-e] | 95.87 [a-d] | 6.38 [b-e] | 28.36 [c-h] | 4.89 [a-d] | 2.46 [a] |
| 79-2 | 5.42 [f-j] | 15.70 [a-e] | 42.00 [d-g] | 74.00 [a-d] | 96.84 [a-c] | 6.50 [b-e] | 25.68 [f-k] | 4.73 [a-d] | 2.26 [a] |
| 79-3 | 6.92 [a-d] | 16.05 [ab] | 48.50 [b] | 71.00 [b-g] | 95.82 [a-e] | 6.75 [b-e] | 24.18 [i-k] | 5.00 [a-d] | 2.53 [a] |
| 103-1 | 5.75 [d-h] | 15.83 [a-d] | 42.25 [d-g] | 66.63 [f-h] | 95.77 [a-e] | 6.50 [b-e] | 27.04 [e-i] | 4.86 [a-d] | 2.38 [a] |
| 103-2 | 4.42 [i-l] | 15.50 [a-e] | 43.00 [d-f] | 73.50 [a-d] | 95.00 [a-e] | 6.75 [a-e] | 28.43 [c-h] | 5.33 [a-d] | 2.80 [a] |
| 103-3 | 5.42 [f-j] | 14.59 [a-e] | 43.00 [d-f] | 69.25 [d-h] | 95.30 [a-e] | 6.38 [b-e] | 25.19 [g-k] | 4.93 [a-d] | 2.84 [a] |
| 107-1 | 5.50 [e-i] | 13.98 [d-f] | 41.75 [e-g] | 68.25 [e-h] | 90.82 [gh] | 7.63 [a-d] | 22.04 [kl] | 4.38 [b-d] | 2.23 [a] |
| 107-2 | 4.33 [i-l] | 14.68 [a-e] | 38.00 [g-j] | 76.13 [a] | 92.21 [e-h] | 6.25 [c-e] | 16.58 [m] | 3.94 [cd] | 2.36 [a] |
| 111-1 | 4.58 [g-l] | 16.07 [ab] | 41.25 [e-g] | 71.13 [b-g] | 96.35 [a-d] | 6.75 [a-e] | 28.58 [c-h] | 4.93 [a-d] | 2.46 [a] |
| 111-2 | 5.92 [c-g] | 15.25 [a-e] | 41.75 [e-g] | 69.50 [c-h] | 90.98 [gh] | 9.13 [a] | 33.33 [ab] | 5.46 [a-c] | 2.68 [a] |
| 127-1 | 6.17 [a-f] | 13.60 [ef] | 34.75 [j] | 71.00 [b-g] | 96.78 [a-c] | 5.88 [de] | 24.05 [i-k] | 4.46 [a-d] | 2.11 [a] |
| 127-2 | 4.08 [kl] | 15.85 [a-d] | 41.25 [e-g] | 70.88 [b-g] | 95.24 [a-e] | 7.25 [a-e] | 28.81 [c-h] | 5.11 [a-d] | 2.15 [a] |
| 129-1 | 4.00 [lm] | 15.59 [a-e] | 43.00 [e-g] | 59.75 [i] | 95.20 [a-e] | 6.13 [c-e] | 29.16 [c-g] | 5.36 [a-d] | 2.49 [a] |
| 129-2 | 7.17 [ab] | 15.09 [a-e] | 49.25 [b] | 67.88 [e-h] | 94.86 [a-e] | 6.25 [c-e] | 31.33 [bc] | 5.21 [a-d] | 2.40 [a] |
| 138-1 | 3.75 [lm] | 12.41 [f] | 39.50 [f-i] | 66.25 [gh] | 96.00 [a-d] | 6.50 [b-e] | 28.71 [c-h] | 5.04 [a-d] | 2.44 [a] |
| 138-2 | 6.92 [a-d] | 15.59 [a-e] | 42.75 [d-f] | 65.88 [h] | 96.84 [a-c] | 8.50 [a-c] | 28.56 [c-h] | 5.15 [a-d] | 2.44 [a] |
| 150-1 | 7.08 [a-c] | 16.24 [a] | 41.25 [e-g] | 65.88 [h] | 88.93 [h] | 7.50 [a-e] | 28.79 [c-h] | 5.18 [a-d] | 2.40 [a] |
| 150-2 | 3.75 [lm] | 15.31 [a-e] | 46.00 [b-d] | 73.38 [a-d] | 95.89 [a-d] | 7.75 [a-d] | 29.53 [c-f] | 5.96 [a] | 2.91 [a] |
| 170-1 | 5.75 [a-d] | 14.58 [a-e] | 34.75 [j] | 70.00 [b-h] | 93.40 [b-g] | 6.63 [b-e] | 25.01 [h-k] | 4.83 [a-d] | 2.45 [a] |
| 170-2 | 4.25 [j-l] | 13.99 [b-f] | 35.50 [ij] | 67.88 [e-h] | 96.17 [a-d] | 8.50 [a-c] | 22.53 [j-l] | 5.08 [a-d] | 2.45 [a] |
| 175-1 | 5.25 [f-k] | 15.80 [a-d] | 36.00 [ij] | 72.25 [a-e] | 93.24 [c-g] | 7.88 [a-d] | 27.15 [d-i] | 4.15 [cd] | 2.46 [a] |
| 175-2 | 4.33 [j-l] | 15.96 [a-d] | 43.25 [d-f] | 72.75 [a-e] | 95.59 [a-e] | 6.50 [b-e] | 26.40 [e-j] | 4.88 [a-d] | 2.31 [a] |
| TG 26 | 6.25 | 12.42 | 40.75 | 70.00 | 97.04 | 6.13 | 20.28 | 4.35 | 2.33 |
| GPBD 4 | 2.83 | 14.52 | 40.50 | 74.50 | 96.73 | 7.00 | 30.19 | 5.16 | 2.71 |
| SEm± | 0.26 | 0.73 | 3.12 | 4.03 | 2.21 | 1.00 | 2.75 | 0.38 | 0.20 |

**LLS_90:** LLS score at 90 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

similarity ranged from 51.7% (among the lines of HIF 77) to 86.4% (among the lines of HIF 14) in TAG 24 × GPBD 4. Similarly, it varied from 54.8% (among the lines of HIFs 1) to 83.1% (among the lines of HIF 53) in TG 26 × GPBD 4 (Table 16).

#### 4.1.4.1 Contribution of parental genomes

HIFs derived from biparental crossing and selfing represent an array of recombination events. Therefore, an effort was made to check the relative representation of the two parental genomes among the lines of Set I using the background markers. In general, the lines with TAG 24 type alleles across the loci were more common than those with GPBD 4 type alleles among the lines of TAG 24 × GPBD 4. Contribution from TAG 24 ranged from 41.1-62.7% among the lines. Similarly, those with TG 26 type alleles across the loci were more common than the lines with GPBD 4 type alleles in TG 26 × GPBD 4. Contribution of TG 26 ranged from 41.9-64.4% among the lines based on the marker data (Table 17). This wide range of allele representation is indicative of diverse recombination events among the HIF derived lines.

#### 4.1.4.2 Validation of markers linked LLS and rust resistance using NILs

The lines derived from each HIF in Set I and Set II differed significantly for the response to rust and LLS, respectively. On the other hand, they did not differ for most of the morphological and productivity traits. Also, they had very high background similarity across a large number of marker loci. Hence, these lines were regarded as Near Isogenic Lines (NILs). The NILs across the HIFs represented varying background genome in terms of parental contributions. Such NILs constitute an ideal genomic resource for validating the markers linked to LLS and rust resistance.

#### 4.1.4.3 Validation of rust resistance-linked markers

Three SSR markers IPAHM103, GM1536 and GM2301 linked to rust resistance were selected for their validation using the NILs of Set I. All the three markers showed polymorphism between the parents (TAG 24 versus GPBD 4 and TG 26 versus GPBD 4) of NILs. Twenty nine NILs from TAG 24 × GPBD 4 and 35 NILs from TG 26 × GPBD 4 were genotyped with IPAHM103, GM1536 and GM2301. The pairs of NILs differing

**Table 16: Genome similarity between the HIF-derived lines**

| TAG 24 × GPBD 4 | | | TG 26 × GPBD 4 | | |
|---|---|---|---|---|---|
| **Pair of HIF-derived lines** | **Total number of markers** | **Monomorphic markers** | **Pair of HIF-derived lines** | **Total number of markers** | **Monomorphic markers** |
| 9-1:9-2 | 162 | 127 (78.4) | 1-1:1-2 | 228 | 125 (54.8) |
| 9-2:9-3 | 162 | 128 (79.0) | 7-1:7-2 | 187 | 112 (59.9) |
| 9-1:9-3 | 161 | 133 (82.6) | 46-1:46-2 | 224 | 149 (66.5) |
| 14-1:14-2 | 132 | 114 (86.4) | 46-2:46-3 | 216 | 131 (60.7) |
| 50-1:50-2 | 162 | 99 (61.1) | 46-1:46-3 | 209 | 163 (80.0) |
| 60-1:60-2 | 151 | 90 (59.6) | 53-1:53-2 | 219 | 182 (83.1) |
| 60-2:60-3 | 145 | 95 (65.5) | | | |
| 60-1:60-3 | 150 | 88 (58.7) | | | |
| 77-1: 77-2 | 151 | 78 (51.7) | | | |
| 83-1:83-2 | 140 | 77 (55.0) | | | |
| 89-1:89-2 | 135 | 97 (71.9) | | | |
| 101-1:101-2 | 138 | 91 (65.9) | | | |
| 116-1:116-2 | 133 | 87 (65.4) | | | |

Values in the parentheses indicate the percentage similarity

**Table 17: Representation of the parental alleles among the HIF-derived lines of Set I**

| TAG 24 × GPBD 4 | | | TG 26 × GPBD 4 | | |
|---|---|---|---|---|---|
| **HIF-derived lines** | **TAG 24** | **GPBD 4** | **HIF-derived lines** | **TG 26** | **GPBD 4** |
| 9-1 | 78 (48.2) | 84 (51.9) | 1-1 | 145 (60.7) | 94 (39.3) |
| 9-2 | 87 (53.1) | 77 (47.0) | 1-2 | 111 (46.8) | 126 (53.2) |
| 9-3 | 84 (51.95 | 78 (48.2) | 7-1 | 132 (64.4) | 73 (35.6) |
| 14-1 | 82 (61.7) | 51 (38.4) | 7-2 | 96 (42.3) | 131 (57.7) |
| 14-2 | 93 (58.9) | 65 (41.1) | 46-1 | 104 (45.2) | 126 (54.8) |
| 50-1 | 74 (44.6) | 92 (55.4) | 46-2 | 154 (64.2) | 86 (35.8) |
| 50-2 | 91 (55.5) | 73 (44.5) | 46-3 | 93 (41.9) | 129 (58.1) |
| 60-1 | 71 (43.9) | 91(56.2) | 53-1 | 117 (48.8) | 123 (51.3) |
| 60-2 | 81 (52.6) | 73 (47.4) | 53-2 | 115 (51.3) | 109 (48.7) |
| 60-3 | 87 (56.9) | 66 (43.1) | | | |
| 77-1 | 91 (57.2) | 68 (42.8) | | | |
| 77-2 | 78 (49.7) | 79 (50.3) | | | |
| 83-1 | 75 (47.2) | 84 (52.8) | | | |
| 83-2 | 74 (51.0) | 71 (49.0) | | | |
| 89-1 | 96 (60.8) | 62 (39.2) | | | |
| 89-2 | 75 (52.8) | 67 (47.2) | | | |
| 101-1 | 91 (60.7) | 59 (39.3) | | | |
| 101-2 | 62 (41.1) | 89 (58.9) | | | |
| 116-1 | 71 (46.4) | 82 (53.6) | | | |
| 116-2 | 89 (62.7) | 53 (37.3) | | | |

Values in the parentheses indicate the percentage

for reaction to rust showed contrasting alleles at all the three marker loci. In general, rust resistant NILs from both the crosses showed resistant (GPBD 4 type) allele for IPAHM103, GM1536 and GM2301. For example, 14-1 and 14-2 NILs were selected from HIF 14 in TAG 24 × GPBD 4. NIL 14-1 showed resistant allele (GPBD 4 type) and 14-2 showed susceptible allele (TAG 24 type). Similarly, the pair of NILs, 7-1 and 7-2 originating from HIF 7 in TG 26 × GPBD 4 differed for the type of allele at marker loci. NIL 7-1 showed resistant allele (GPBD 4 type) and 7-2 showed susceptible allele (TG 26 type).

For marker validation, the frequency of NILs showing co-segregation between allele and disease reaction was compared statistically (z test) with the frequency of NILs not showing such a co-segregation. Of the total 29 NILs from TAG 24 × GPBD 4, 25 NILs showed perfect co-segregation between the allele and the phenotype for IPAHM103. Of them, 11 NILs showed resistant allele and resistant phenotype, and 14 NILs showed susceptible allele and susceptible phenotype. The z value worked out was 5.51, which was significant, indicating a strong association of IPAHM103 with rust resistance among the NILs of TAG 24 × GPBD 4. Of the 35 NILs in TG 26 × GPBD 4, 23 NILs showed perfect co-segregation between the allele and the phenotype. Of them, 12 NILs showed resistant allele and resistant phenotype, and 11 NILs showed susceptible allele and susceptible phenotype. A significant z value of 2.63 indicated a strong validation of IPAHM103 among the NILs of TG 26 × GPBD 4. GM1536 and GM2301 also recorded significant z value in both the crosses, indicating strong co-segregation between the allele and the phenotype (Table 18).

#### 4.1.4.4 Validation of LLS resistance-linked markers

Four SSR markers GM2009, GM1009, GM1573 and pPGPseq8D09 linked to LLS resistance were selected for their validation using the NILs of Set II. All the four markers showed polymorphism between the parents (TAG 24 versus GPBD 4 and TG 26 versus GPBD 4) of NILs. Of the eleven NILs from TAG 24 × GPBD 4, only five showed perfect co-segregation, while a majority (six) of the NILs failed to show co-segregation for GM2009. The z value was 0.43, which was non-significant. Hence, GM2009 failed to show significant association with LLS resistance among the NILs of TAG 24 × GPBD 4. Of the 24 NILs from TG 26 × GPBD 4, only five showed perfect co-segregation for GM2009, while remaining nineteen NILs failed to show any co-

segregation (non-significant z value). Therefore, GM2009 did not show significant association with LLS resistance among the NILs of TG 26 × GPBD 4.

For GM1009, of the eleven NILs from TAG 24 × GPBD 4 and 24 NILs from TG 26 × GPBD 4, only five and twelve NILs showed perfect co-segregation, respectively. But, majority of the NILs (Table 19) from both the crosses failed to show co-segregation of the phenotype with the alleles of GM1009, indicating non-significant association of GM1009 with LLS resistance. Alleles at GM1573 showed co-segregation in six out of eleven NILs from TAG 24 × GPBD 4 and 12 out of 24 NILs from TG 26 × GPBD 4 with non-significant z values in both the crosses. Alleles of pPGPseq8D09 showed co-segregation in only five out of eleven NILs from TAG 24 × GPBD 4 and 13 out of 24 NILs from TG 26× GPBD 4. The z values were non-significant in both the crosses, indicating non-significant association of pPGPseq8D09 with LLS resistance among the NILs of both the crosses. Overall, GM2009, GM1009, GM1573 and pPGPseq8D09 showed non-significant association with LLS resistance among the NILs of TAG 24 × GPBD 4 and TG 26× GPBD 4, indicating their weak validation.

## 4.2 Validation of LLS and rust resistance-linked markers using the RILs of VL 1 × 110

An attempt was also made to validate LLS resistance-linked markers using a new RIL population derived from VL 1 × 110. The RILs of VL 1 × 110 was already available at the Department of Genetics and Plant Breeding of UAS, Dharwad. The population comprised of 114 RILs. In the present study, efforts were made to phenotypically evaluate the RILs for their reaction to LLS and rust, and to genotype with LLS and rust resistance-linked markers in an attempt to validate the markers.

### 4.2.1 Phenotypic evaluation for resistance to LLS and rust

RILs were evaluated for LLS and rust resistance under disease epiphytotic conditions during the four rainy seasons of 2010, 2011, 2012 and 2013. The pooled analysis of variance was performed and the estimates of the genetic parameters for response to rust and LLS were worked out. RILs differed significantly for the response to LLS and rust at all the stages of growth. Seasons also differed significantly in

**Table 18: Co-segregation of the markers and reaction to rust among the NILs of Set I**

| Markers | Number of lines showing | | Total | z value |
|---|---|---|---|---|
| | Co-segregation | Non co-segregation | | |
| **TAG 24 × GPBD 4** | | | | |
| IPAHM103 | 25 | 04 | 29 | 5.51* |
| GM1536 | 25 | 04 | 29 | 5.51* |
| GM2301 | 25 | 04 | 29 | 5.51* |
| **TG 26 × GPBD 4** | | | | |
| IPAHM103 | 23 | 12 | 35 | 2.63* |
| GM1536 | 23 | 12 | 35 | 2.63* |
| GM2301 | 23 | 12 | 35 | 2.63* |

*: z value compared with the critical value of 1.96 at 5% level of significance

**Table 19: Co-segregation of the markers and reaction to late leaf spot among the NILs of Set II**

| Markers | Number of lines showing | | Total | z value |
|---|---|---|---|---|
| | Co-segregation | Non co-segregation | | |
| **TAG 24 × GPBD 4** | | | | |
| GM2009 | 5 | 6 | 11 | 0.43 |
| GM1009 | 5 | 6 | 11 | 0.43 |
| GM1573 | 6 | 5 | 11 | 0.43 |
| pPGPseq8D09 | 5 | 6 | 11 | 0.43 |
| **TG 26 × GPBD 4** | | | | |
| GM2009 | 5 | 19 | 24 | 4.04 |
| GM1009 | 12 | 12 | 24 | 0.00 |
| GM1573 | 12 | 12 | 24 | 0.00 |
| pPGPseq8D09 | 13 | 11 | 24 | 0.58 |

*: z value compared with the critical value of 1.96 at 5% level of significance

influencing the disease development (Table 20). VL 1 and 110 differed significantly for response to LLS and rust at all the three stages. VL 1 was susceptible to LLS and resistant to rust, while 110 was resistant to LLS and susceptible to rust. The nature and magnitude of variation was assessed among the RILs using phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV). PCV levels of >20%, 10-20% and 0-10% were considered as high, moderate and low, respectively. High PCV and GCV were observed for LLS at 80 DAS and rust at 80 and 90 DAS (Table 21).

### 4.2.2 Genotyping with LLS and rust resistance-linked markers

Five SSR markers IPAHM103, GM1954, GM1536, GM2079, GM2301 and GM2009 linked to both LLS and rust resistances were selected. GM1009 and GM1573 SSR markers linked to only LLS resistance, and a transposable element marker, *AhMITE1*-PCR associated with LLS resistance were selected for validation. All the markers were tested for polymorphism between the parents (VL1 versus 110). Four markers, IPAHM103, GM2009, GM1009 and *AhMITE1*-PCR showed polymorphism between the parents. RILs were genotyped with IPAHM103, GM1009, GM2009, and *AhMITE1*-PCR markers. The type of allele was scored as A or B for the three SSR markers. For *AhMITE1*-PCR, presence or absence of 242 bp amplicon was checked, and scored as A and B, respectively.

### 4.2.3 Single marker analysis (SMA)

Marker validation was attended using single marker analysis, where F statistic and $R^2$ were worked out using WinQTL Cartographer version 2.5 (Wang *et al*., 2007). A non-significant F was observed for all the four (IPAHM103, GM1009, GM2009 and *AhMITE1*-PCR) markers with resistance to rust at 70, 80 and 90 DAS, indicating a non-significant association of the markers with rust resistance.

GM1009, GM2009, IPAHM103 and *AhMITE1*-PCR markers showed a significant association with LLS resistance only at 70 DAS but not at 80 and 90 DAS (Table 22). The phenotypic variance explained (PVE) by the markers at 70 DAS was highest for GM1009 (5.04%) followed by *AhMITE1*-PCR (3.56%), GM2009 (1.47%) and IPAHM103 (1.33%).

**Table 20: Pooled ANOVA for response to LLS and rust at 70, 80 and 90 DAS among the RILs of VL 1 × 110**

| Sources of variation | df | R_ 70 | R_80 | R_90 | LLS_70 | LLS_80 | LLS_90 |
|---|---|---|---|---|---|---|---|
| Genotypes | 115 | 0.34** | 0.68** | 3.24** | 0.41** | 1.11** | 3.38** |
| Replications | 1 | 0.01 | 0.11 | 9.12** | 0.62* | 2.09* | 2.48* |
| Seasons | 3 | 63.83** | 340.32** | 382.77** | 79.04** | 633.01** | 184.77** |
| Interactions (G × S) | 3 | 0.65** | 0.27 | 5.57** | 1.07** | 2.90** | 3.38** |
| Error | 805 | 0.09 | 0.36 | 0.54 | 0.14 | 0.32 | 0.41 |

*, **: Significance at 5% and 1%, respectively

**R_70:** Rust score at 70 days after sowing (DAS); **R_80:** Rust score at 80 DAS; **R_90:** Rust score at 90 DAS; **LLS_70:** LLS score at 70 DAS; **LLS_80:** LLS score at 80 DAS; **LLS_90:** LLS score at 90 DAS

**Table 21: Components of genetic variation for response to LLS and rust at 70, 80 and 90 DAS among the RILs of VL 1 × 110**

| Traits | Mean | Range | | Variance | | Coefficient of variation (%) | | $h^2$ (%) | GA | GAM |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Min. | Max. | Vp | Vg | PCV | GCV | | | |
| R_70 | 2.82 | 2.00 | 3.50 | 0.23 | 0.22 | 17.05 | 16.52 | 93.90 | 0.929 | 32.97 |
| R_80 | 3.84 | 2.00 | 6.75 | 1.23 | 1.19 | 28.84 | 28.36 | 96.70 | 2.207 | 57.45 |
| R_90 | 4.94 | 3.00 | 7.38 | 1.54 | 1.45 | 25.09 | 24.39 | 94.40 | 2.410 | 48.82 |
| LLS_70 | 2.87 | 2.00 | 3.63 | 0.29 | 0.27 | 18.88 | 18.22 | 93.10 | 1.040 | 36.20 |
| LLS_80 | 4.55 | 2.00 | 6.88 | 2.18 | 2.14 | 32.52 | 32.19 | 98.00 | 2.983 | 65.64 |
| LLS_90 | 7.05 | 4.38 | 8.13 | 0.89 | 0.81 | 13.35 | 12.79 | 91.90 | 1.781 | 25.26 |

**R_70:** Rust score at 70 days after sowing (DAS); **R_80:** Rust score at 80 DAS; **R_90:** Rust score at 90 DAS; **LLS_70:** LLS score at 70 DAS; **LLS_80:** LLS score at 80 DAS; **LLS_90:** LLS score at 90 DAS; **Vp:** Phenotypic variance; **Vg:** Genotypic variance; **PCV:** Phenotypic coefficient of variation; **GCV:** Genotypic coefficient of variation; **$h^2$:** Heritability in broad sense; **GA:** Genetic advance; **GAM:** Genetic advance as percent of mean

**Table 22: Single marker analysis for LLS and rust resistance-linked/associated markers and *AhMITE1*-PCR among the RILs of VL1 × 110**

| Traits | IPAHM103 | | GM1009 | | GM2009 | | *AhMITE1*-PCR | |
|---|---|---|---|---|---|---|---|---|
| | F | $R^2$ (%) | F | $R^2$ (%) | F | $R^2$ (%) | F | $R^2$ (%) |
| R_70 | 0.718 | 0.70 | 0.532 | 0.51 | 1.182 | 1.61 | 1.987 | 2.23 |
| R_80 | 0.031 | 0.60 | 0.145 | 0.02 | 0.289 | 0.18 | 0.652 | 1.37 |
| R_90 | 0.687 | 0.18 | 0.629 | 1.97 | 0.987 | 1.55 | 1.497 | 0.75 |
| LLS_70 | 5.056* | 1.33 | 5.618* | 5.04 | 5.470* | 1.47 | 4.528* | 3.56 |
| LLS_80 | 0.972 | 1.38 | 0.850 | 2.60 | 0.913 | 0.91 | 0.613 | 1.35 |
| LLS_90 | 1.694 | 3.13 | 1.443 | 11.48 | 1.117 | 2.26 | 1.115 | 1.62 |

*, **: Significance at 5% and 1%, respectively; $R^2$: Per cent phenotypic variance explained

**R_70:** Rust score at 70 DAS; **R_80:** Rust score at 80 DAS; **R_90:** Rust score at 90 DAS; **LLS_70:** LLS score at 70 DAS; **LLS_80:** LLS score at 80 DAS; **LLS_90:** LLS score at 90 DAS

## 4.3 Marker assisted backcrossing in JL 24

JL 24, a LLS and rust susceptible variety (recurrent parent) was selected for developing backcross lines. LLS and rust resistant improved variety GPBD 4, and two LLS and rust resistant interspecific derivatives (ICGV 86699 and ICGV 99005) were used as the donor parents. For marker assisted selection, three SSR markers, IPAHM103 and GM2301 linked to LLS and rust resistance, and pPGPseq8D09linked to only LLS resistance were used for foreground selection.

### 4.3.1 Hybridization and development of $BC_1F_1$

JL 24 (female parent) was crossed independently with three donors (male parents) during the rainy season of 2011. Ten female plants were used for each cross. In total, 700, 680 and 710 flowers were emasculated and pollinated for JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively over a period of 15 days starting from the appearance of first flower at 25-30 DAS. From these flowers, 132, 78 and 75 pods, and 219, 117 and 114 seeds were harvested from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively.

These seeds were sown in the field during the post-rainy season of 2011. Genomic DNA was isolated from 100 young seedlings from each cross. IPAHM103, GM2301 and pPGPseq8D09 showed polymorphism between the parents of all the three crosses. Using these markers, 21 and 18 plants were confirmed as $F_1$ from JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. Eleven plants were confirmed as $F_1$ by phenotyping in JL 24 × GPBD 4.

These $F_1$ plants were employed as male parents for crossing with the recurrent parent, JL 24 grown in pots during the post-rainy season of 2011. In total, 750, 710 and 690 flowers were emasculated and pollinated for JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. From these flowers, 116, 98 and 105 pods, and 207, 169 and 185 seeds ($BC_1F_1$) were harvested from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively.

### 4.3.2 Development of $BC_2F_1$

$BC_1F_1$ seeds were sown in the field during the rainy season of 2012. Genomic DNA was isolated from 101, 106 and 97 young seedlings from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. Using IPAHM103, GM2301 and pPGPseq8D09 marker-specific PCR, 9, 13 and 11 plants were confirmed as $BC_1F_1$ from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively.

These $BC_1F_1$ plants were employed as male parents for crossing with the recurrent parent, JL 24 grown in pots during the rainy season of 2012. In total, 730, 640 and 720 flowers were emasculated and pollinated for JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. From these flowers, 35, 76 and 38 pods, and 52, 106 and 46 seeds ($BC_2F_1$) were harvested from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively.

### 4.3.3 Development of $BC_3F_1$

$BC_2F_1$ seeds were sown in the field during the post-rainy season of 2012. Seeds of JL 24 × GPBD 4, JL 24 × ICGV 86699 could germinate, while those of JL 24 × ICGV 99005 failed to germinate. Genomic DNA was isolated from 38 and 88 young seedlings from JL 24 × GPBD 4 and JL 24 × ICGV 86699, respectively. Using IPAHM103, GM2301 and pPGPseq8D09 marker-specific PCR, five and seven plants were confirmed as $BC_2F_1$ from JL 24 × GPBD 4, JL 24 × ICGV 86699, respectively. These $BC_2F_1$ plants were employed as male parents for crossing with the recurrent parent, JL 24 grown in pots during the post-rainy season of 2012. But in case of JL 24 × ICGV 99005, $BC_1F_2$ (positive for IPAHM103 and GM2301) were used as male parents to develop $BC_3F_1$ plants. In total, 680, 700 and 700 flowers were emasculated and pollinated for JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. From these flowers, 110, 106 and 112 pods, and 180, 175 and 170 seeds ($BC_3F_1$) were harvested from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. $BC_3F_1$ seeds were sown in the field during the rainy season of 2013. Genomic DNA was isolated from 97, 117 and 102 young seedlings from the three crosses, respectively. Using IPAHM103, GM2301 and pPGPseq8D09 marker-specific PCR, 11, 17 and 13 plants were confirmed as $BC_3F_1$ from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively (Table 23 and 24).

**Table 23: Details of crosses effected to generate backcross lines**

| Generations | Crosses | Total number of flowers crossed | Number of pods set | Number of seeds collected | Confirmed plants |
|---|---|---|---|---|---|
| **JL 24 × GPBD 4** | | | | | |
| $F_1$ | JL 24 × GPBD 4 | 700 | 132 | 219 | 8 |
| $BC_1F_1$ | JL 24 × $F_1$ | 750 | 116 | 207 | 9 |
| $BC_2F_1$ | JL 24 × $BC_1F_1$ | 730 | 35 | 52 | 5 |
| $BC_3F_1$ | JL 24 × $BC_2F_1$ | 680 | 110 | 180 | 11 |
| **JL 24 × ICGV 86699** | | | | | |
| $F_1$ | JL 24 × ICGV 86699 | 680 | 78 | 117 | 21 |
| $BC_1F_1$ | JL 24 × $F_1$ | 710 | 98 | 169 | 13 |
| $BC_2F_1$ | JL 24 × $BC_1F_1$ | 640 | 76 | 106 | 7 |
| $BC_3F_1$ | JL 24 × $BC_2F_1$ | 700 | 106 | 175 | 17 |
| **JL 24 × ICGV 99005** | | | | | |
| $F_1$ | JL 24 × ICGV 99005 | 710 | 75 | 114 | 18 |
| $BC_1F_1$ | JL 24 × $F_1$ | 690 | 105 | 185 | 11 |
| $BC_2F_1$ | JL 24 × $BC_1F_1$ | 720 | - | - | - |
| $BC_3F_1$ | JL 24 × $BC_2F_1$ | 700 | 112 | 170 | 13 |

**Table 24: Details of PCR analysis of $F_1$, $BC_1F_1$, $BC_2F_1$ and $BC_3F_1$ for foreground selection at LLS and rust resistance-linked markers (IPAHM103 and GM2301)**

| Cross | Number of plants | |
|---|---|---|
| | Screened | Confirmed |
| **$F_1$** | | |
| JL 24 × GPBD 4 | 100 | - |
| JL 24 × ICGV 86699 | 100 | 21 |
| JL 24 × ICGV 99005 | 100 | 18 |
| **$BC_1F_1$** | | |
| JL 24 × GPBD 4 | 101 | 9 |
| JL 24 × ICGV 86699 | 106 | 13 |
| JL 24 × ICGV 99005 | 97 | 11 |
| **$BC_2F_1$** | | |
| JL 24 × GPBD 4 | 38 | 5 |
| JL 24 × ICGV 86699 | 88 | 7 |
| JL 24 × ICGV 99005* | - | - |
| **$BC_3F_1$** | | |
| JL 24 × GPBD 4 | 97 | 11 |
| JL 24 × ICGV 86699 | 117 | 17 |
| JL 24 × ICGV 99005 | 102 | 13 |

* Seeds failed to germinate

### 4.3.4 Selfed generations of backcrosses

#### 4.3.4.1 First backcross

Seeds from 24, 28 and 20 $BC_1F_1$ plants from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively were harvested and sown in the field during the rainy season of 2012. These plants were evaluated under disease epiphytotic condition. At the time of harvest, JL 24 recorded a disease score of 9.0 for LLS and 8.0 for rust, while the score ranged from 5.0-9.0 for LLS and 4.0-9.0 for rust among $BC_1F_2$ plants. Single plant selection was exercised to select plants resistant to LLS or rust or both in addition to possessing pod features similar to those of JL 24. Of the 106 plants selected in JL 24 × GPBD 4, 42 plants were resistant to LLS, 36 plants were resistant to rust and 28 were resistant to both. Similarly, of the 101 plants in JL 24 × ICGV 86699, 40 plants were resistant to LLS, 35 plants were resistant to rust, and 26 were resistant to both. Whereas among the 90 plants in JL 24 × ICGV 99005, 43 plants were resistant to LLS, 30 plants were resistant to rust and 17 were resistant to both.

$BC_1F_3$ families were raised during the post-rainy season of 2012 from the seeds of 159, 114 and 71 $BC_1F_2$ plants selected from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. At the time of harvest, JL 24 recorded a disease score of 9.0 for LLS and 7.0 for rust, while the score ranged from 3.0-8.0 for LLS and rust among $BC_1F_3$ families. Segregation was observed within the families for resistance to LLS and rust. Hence single plant selection was exercised to select for the plants resistant to LLS or rust or both in addition to possessing the pod features of JL 24. A total of 106 plants were selected from 159 families of JL 24 × GPBD 4. They included 8 plants (from eight families) resistant to rust, 30 plants (from 22 families) resistant to LLS and 68 plants (from 41 families) resistant to both LLS and rust. Similarly, from 114 families in JL 24 × ICGV 86699, 82 plants were selected, of which 10 plants (from six families) were resistant to rust, 19 plants (from 12 families) were resistant to LLS and 53 plants (from 40 families) were resistant to both LLS and rust. From 71 families in JL 24 × ICGV 99005, 56 plants were selected, of which 8 plants (from four families) were resistant to rust, 23 plants (from 11 families) were resistant to LLS and 28 plants (from 13 families) were resistant to both LLS and rust.

A total of 106, 82 and 56 $BC_1F_4$ lines were raised from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively during the rainy season of 2013. The $BC_1F_4$ lines from the three crosses were evaluated for LLS and rust resistance, and a few productivity as well as agronomic traits. In general, backcross lines of all the three crosses differed significantly for all the traits except leaf length and leaf width (Table 25). A moderate to high PCV and GCV were observed for most of the traits, indicating the scope for selecting the superior lines. PCV and GCV for the traits did not differ so much between the crosses. Further, a moderate to high heritability ($h^2_{bs}$), genetic advance (GA) and genetic advance as per cent of mean (GAM) were also observed for most of the traits in all the three crosses, indicating the improvement that can be achieved with selection (Table 26).

LLS incidence resulted in significantly reduced pod yield per plant (PYPP) among the $BC_1F_4$ lines of JL 24 × GPBD 4 and JL 24 × ICGV 86699. Test weight (TW), shelling percentage (SP) and sound mature kernel percentage (SMKP) had positive correlation with PYPP in all the three crosses (Table 27).

An effort was made to select the superior lines with resistance to LLS and rust along with productivity traits. Since the rust incidence was less during the rainy season of 2013, top 20 per cent lines were selected only for LLS resistance based on normality concept (Khan *et al*., 2013). From 106 $BC_1F_4$ lines of JL 24 × GPBD 4, 20 backcross lines superior to JL 24 (female parent) for LLS resistance were selected. They had LLS scores of 3.0-4.0 while JL 24 had a mean score of 8.0. Many of such lines also possessed either marginal or significantly superior productivity traits like PYPP, TW, SP and SMKP. Line JG4_81 was significantly superior for PYPP and TW, while it was marginally superior for SP and SMKP over JL 24. Line JG4_43 was significantly superior for PYPP and SMKP, while it was marginally superior for TW and SP over JL 24 (Table 28).

Similarly, 27 lines were selected from 82 $BC_1F_4$ lines of JL 24 × ICGV 86699 based on LLS resistance. Of them, 15 lines were significantly superior for PYPP over JL 24, while four lines were marginally superior over JL 24. In addition a few of them were also superior for other productivity traits. Line J8-4_10 had significant superiority for PYPP, TW and SMKP, and marginal superiority for SP over JL 24. Line J8-4_24

**Table 25: ANOVA for agronomic traits, productivity traits and response to LLS and rust among $BC_1F_4$ populations**

| Crosses | df | Mean sum of squares | | | Critical difference (CD) | | SEm± | CV (%) |
|---|---|---|---|---|---|---|---|---|
| | | Replication | Treatment | Error | 5% | 1% | | |
| **Agronomic traits** | | | | | | | | |
| **Plant height (cm)** | | | | | | | | |
| JL 24 × GPBD 4 | 107 | 130.66 | 31.80** | 7.70 | 5.50 | 7.27 | 1.95 | 8.11 |
| JL 24 × ICGV 86699 | 83 | 324.39 | 62.02** | 4.23 | 4.09 | 5.42 | 1.45 | 5.55 |
| JL 24 × ICGV 99005 | 57 | 58.18 | 57.65** | 7.96 | 5.64 | 7.51 | 1.98 | 9.13 |
| **Number of primary branches** | | | | | | | | |
| JL 24 × GPBD 4 | 107 | 0.07 | 2.51** | 0.63 | 1.56 | 2.07 | 0.56 | 13.70 |
| JL 24 × ICGV 86699 | 83 | 0.02 | 2.14** | 0.15 | 0.77 | 1.02 | 0.27 | 6.81 |
| JL 24 × ICGV 99005 | 57 | 1.81 | 1.30** | 0.19 | 0.86 | 1.14 | 0.30 | 8.13 |
| **Height of longest primary branches (cm)** | | | | | | | | |
| JL 24 × GPBD 4 | 107 | 37.42 | 34.82** | 8.39 | 5.74 | 7.59 | 2.04 | 7.80 |
| JL 24 × ICGV 86699 | 83 | 282.23 | 68.90** | 5.81 | 4.79 | 6.35 | 1.69 | 5.84 |
| JL 24 × ICGV 99005 | 57 | 25.65 | 67.92** | 9.54 | 6.18 | 8.23 | 2.17 | 9.08 |
| **Leaf length (cm)** | | | | | | | | |
| JL 24 × GPBD 4 | 107 | 0.01 | 0.95* | 0.20 | 0.89 | 1.18 | 0.32 | 7.12 |
| JL 24 × ICGV 86699 | 83 | 0.64 | 0.84* | 0.13 | 0.72 | 0.95 | 0.23 | 6.21 |
| JL 24 × ICGV 99005 | 57 | 1.49 | 0.74 | 0.18 | 0.85 | 1.13 | 0.30 | 6.71 |
| **Leaf width (cm)** | | | | | | | | |
| JL 24 × GPBD 4 | 107 | 0.18 | 0.14** | 0.03 | 0.36 | 0.48 | 0.13 | 5.68 |
| JL 24 × ICGV 86699 | 83 | 0.00 | 0.30 | 0.03 | 0.39 | 0.51 | 0.14 | 5.98 |
| JL 24 × ICGV 99005 | 57 | 0.02 | 0.31 | 0.04 | 0.41 | 0.55 | 0.14 | 6.31 |
| **Productivity traits** | | | | | | | | |
| **Pod yield per plant (gm)** | | | | | | | | |
| JL 24 × GPBD 4 | 107 | 0.97 | 8.69** | 0.93 | 1.35 | 1.78 | 0.68 | 7.36 |
| JL 24 × ICGV 86699 | 83 | 1.23 | 8.48** | 0.71 | 1.68 | 2.23 | 0.59 | 6.21 |
| JL 24 × ICGV 99005 | 57 | 2.54 | 13.31** | 1.72 | 2.63 | 3.49 | 1.31 | 10.30 |

*Contd…….*

| Crosses | df | Mean sum of squares | | | Critical difference (CD) | | SEm± | CV (%) |
|---|---|---|---|---|---|---|---|---|
| | | Replication | Treatment | Error | 5% | 1% | | |
| **Test weight (gm)** | | | | | | | | |
| JL 24 × GPBD 4 | 107 | 0.12 | 34.49** | 4.28 | 4.10 | 5.42 | 1.46 | 3.95 |
| JL 24 × ICGV 86699 | 83 | 40.02 | 82.70** | 5.00 | 4.44 | 5.89 | 1.57 | 4.47 |
| JL 24 × ICGV 99005 | 57 | 15.94 | 38.28** | 4.43 | 4.21 | 5.60 | 1.48 | 3.98 |
| **Shelling percentage** | | | | | | | | |
| JL 24 × GPBD 4 | 107 | 20.17 | 32.23** | 4.73 | 4.30 | 5.70 | 1.53 | 3.02 |
| JL 24 × ICGV 86699 | 83 | 5.00 | 251.80** | 6.70 | 5.14 | 6.82 | 1.82 | 3.77 |
| JL 24 × ICGV 99005 | 57 | 0.70 | 132.65** | 3.77 | 3.88 | 5.17 | 1.36 | 2.73 |
| **Sound mature kernel percentage** | | | | | | | | |
| JL 24 × GPBD 4 | 107 | 0.70 | 13.07** | 1.84 | 2.68 | 3.55 | 0.95 | 1.39 |
| JL 24 × ICGV 86699 | 83 | 0.82 | 16.07** | 1.75 | 2.63 | 3.48 | 0.93 | 1.36 |
| JL 24 × ICGV 99005 | 57 | 0.00 | 19.50** | 1.54 | 2.48 | 3.30 | 0.87 | 1.27 |
| **Disease traits** | | | | | | | | |
| **R_80** | | | | | | | | |
| JL 24 × GPBD 4 | 107 | 0.00 | 0.38** | 0.02 | 0.27 | 0.35 | 0.10 | 4.18 |
| JL 24 × ICGV 86699 | 83 | 0.05 | 0.49** | 0.02 | 0.26 | 0.34 | 0.09 | 3.79 |
| JL 24 × ICGV 99005 | 57 | 0.01 | 0.37* | 0.03 | 0.32 | 0.43 | 0.11 | 4.95 |
| **LLS_80** | | | | | | | | |
| JL 24 × GPBD 4 | 107 | 0.23 | 2.81** | 0.14 | 0.74 | 0.99 | 0.27 | 6.84 |
| JL 24 × ICGV 86699 | 83 | 0.02 | 2.85** | 0.02 | 0.26 | 0.35 | 0.09 | 2.61 |
| JL 24 × ICGV 99005 | 57 | 0.14 | 2.70** | 0.19 | 0.87 | 1.16 | 0.31 | 7.96 |

*, **: Significance at 5% and 1%, respectively

**df:** degrees of freedom; **CV:** Coefficient of variation; **SEm±:** Standard error of mean; **R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS

**Table 26: Components of genetic variation for agronomic traits, productivity traits and response to LLS and rust among $BC_1F_4$ populations**

| Crosses | Mean | Range | | Variance | | Coefficient of variation (%) | | $h^2$ (%) | GA | GAM |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Min. | Max. | Vp | Vg | PCV | GCV | | | |
| **Agronomic traits** | | | | | | | | | | |
| **Plant height (cm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 34.19 | 25.20 | 39.08 | 15.90 | 12.05 | 11.66 | 10.15 | 75.80 | 6.23 | 18.20 |
| JL 24 × ICGV 86699 | 37.02 | 26.60 | 56.18 | 31.01 | 28.89 | 15.04 | 14.52 | 93.20 | 10.69 | 28.87 |
| JL 24 × ICGV 99005 | 30.88 | 20.18 | 41.70 | 28.83 | 24.85 | 17.39 | 16.14 | 86.20 | 9.53 | 30.87 |
| **Number of primary branches** | | | | | | | | | | |
| JL 24 × GPBD 4 | 5.75 | 4.00 | 7.50 | 1.26 | 0.94 | 19.45 | 16.85 | 75.10 | 1.73 | 30.08 |
| JL 24 × ICGV 86699 | 5.69 | 4.25 | 8.50 | 1.07 | 0.99 | 18.17 | 17.52 | 93.00 | 1.98 | 34.79 |
| JL 24 × ICGV 99005 | 5.28 | 6.75 | 4.00 | 0.65 | 0.56 | 15.25 | 14.13 | 85.80 | 1.43 | 26.95 |
| **Height of longest primary branches (cm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 37.11 | 28.75 | 40.88 | 17.41 | 13.22 | 11.24 | 9.80 | 75.90 | 6.52 | 17.58 |
| JL 24 × ICGV 86699 | 41.18 | 33.08 | 59.10 | 34.45 | 31.55 | 14.25 | 13.64 | 91.60 | 11.07 | 26.88 |
| JL 24 × ICGV 99005 | 33.98 | 20.18 | 41.70 | 33.96 | 29.19 | 17.15 | 15.90 | 86.00 | 10.32 | 30.36 |
| **Leaf length (cm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 6.33 | 4.80 | 7.30 | 0.48 | 0.37 | 10.87 | 9.64 | 78.60 | 1.12 | 17.60 |
| JL 24 × ICGV 86699 | 5.85 | 4.58 | 6.93 | 0.42 | 0.35 | 11.06 | 10.15 | 84.20 | 1.12 | 19.20 |
| JL 24 × ICGV 99005 | 6.34 | 4.83 | 7.80 | 0.37 | 0.28 | 9.57 | 8.31 | 75.40 | 0.94 | 14.87 |
| **Leaf width (cm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 3.27 | 2.75 | 3.65 | 0.07 | 0.05 | 8.18 | 7.12 | 75.80 | 0.42 | 12.77 |
| JL 24 × ICGV 86699 | 3.28 | 2.58 | 4.08 | 0.15 | 0.13 | 11.77 | 10.98 | 87.00 | 0.69 | 21.10 |
| JL 24 × ICGV 99005 | 3.26 | 2.55 | 4.08 | 0.16 | 0.13 | 12.04 | 11.18 | 86.20 | 0.70 | 21.39 |
| **Productivity traits** | | | | | | | | | | |
| **Pod yield per plant (gm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 13.10 | 8.38 | 17.92 | 4.81 | 3.88 | 16.74 | 15.03 | 80.60 | 3.64 | 27.81 |
| JL 24 × ICGV 86699 | 13.61 | 9.06 | 17.86 | 4.59 | 3.88 | 15.74 | 14.46 | 84.50 | 3.73 | 47.78 |
| JL 24 × ICGV 99005 | 12.72 | 7.41 | 18.66 | 7.51 | 5.79 | 21.54 | 18.92 | 77.13 | 4.35 | 34.23 |

*Contd…..*

| Crosses | Mean | Range | | Variance | | Coefficient of variation (%) | | $h^2$ (%) | GA | GAM |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Min. | Max. | Vp | Vg | PCV | GCV | | | |
| **Test weight (gm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 52.37 | 42.50 | 58.50 | 17.25 | 15.11 | 7.93 | 7.42 | 87.60 | 7.49 | 14.30 |
| JL 24 × ICGV 86699 | 49.93 | 37.00 | 60.00 | 41.35 | 38.85 | 12.88 | 12.48 | 94.00 | 12.45 | 24.93 |
| JL 24 × ICGV 99005 | 52.81 | 43.45 | 60.50 | 19.14 | 16.92 | 8.28 | 7.79 | 88.40 | 7.97 | 15.09 |
| **Shelling percentage** | | | | | | | | | | |
| JL 24 × GPBD 4 | 71.97 | 61.50 | 76.50 | 16.12 | 13.75 | 5.58 | 5.15 | 85.30 | 7.06 | 9.80 |
| JL 24 × ICGV 86699 | 68.66 | 37.00 | 60.00 | 125.90 | 122.55 | 16.34 | 16.12 | 97.30 | 22.50 | 32.77 |
| JL 24 × ICGV 99005 | 70.93 | 34.00 | 79.00 | 66.33 | 64.44 | 11.48 | 11.32 | 97.20 | 16.30 | 22.98 |
| **Sound mature kernel percentage** | | | | | | | | | | |
| JL 24 × GPBD 4 | 97.21 | 93.35 | 100.00 | 6.54 | 5.62 | 2.63 | 2.44 | 85.90 | 4.53 | 4.66 |
| JL 24 × ICGV 86699 | 96.75 | 90.58 | 100.00 | 8.04 | 7.16 | 2.93 | 2.77 | 89.10 | 5.20 | 5.38 |
| JL 24 × ICGV 99005 | 97.19 | 85.32 | 100.00 | 9.75 | 8.98 | 3.21 | 3.08 | 92.10 | 5.93 | 6.10 |
| | | | | | | **Disease traits** | | | | |
| **R_80** | | | | | | | | | | |
| JL 24 × GPBD 4 | 3.26 | 3.00 | 4.00 | 0.19 | 0.18 | 13.30 | 12.96 | 95.10 | 0.85 | 26.04 |
| JL 24 × ICGV 86699 | 3.48 | 3.00 | 4.00 | 0.24 | 0.24 | 14.17 | 13.92 | 96.40 | 0.98 | 28.16 |
| JL 24 × ICGV 99005 | 3.26 | 3.00 | 4.00 | 0.19 | 0.17 | 13.20 | 12.73 | 93.00 | 0.83 | 25.29 |
| **LLS_80** | | | | | | | | | | |
| JL 24 × GPBD 4 | 5.52 | 3.00 | 8.00 | 1.40 | 1.33 | 21.45 | 20.90 | 94.90 | 2.32 | 41.94 |
| JL 24 × ICGV 86699 | 5.10 | 3.00 | 7.00 | 1.43 | 1.42 | 23.41 | 23.34 | 99.40 | 2.45 | 47.93 |
| JL 24 × ICGV 99005 | 5.48 | 3.00 | 7.00 | 1.35 | 1.23 | 21.20 | 20.44 | 92.90 | 2.23 | 40.58 |

**Vp:** Phenotypic variance; **Vg:** Genotypic variance; **PCV:** Phenotypic coefficient of variation; **GCV:** Genotypic coefficient of variation; **$h^2$:** Heritability in broad sense; **GA:** Genetic advance; **GAM:** Genetic advance as percent of mean **R _80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS

**Table 27: Phenotypic correlation among rust and LLS scores (80 DAS), agronomic traits and productivity traits in $BC_1F_4$ populations**

| | | R_80 | LLS_80 | TW | SP | SMKP | NPB | PH | HLPB | LL | LW | PYPP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JL 24 × GPBD 4 | R_80 | 1 | | | | | | | | | | |
| | LLS_80 | 0.127 | 1 | | | | | | | | | |
| | TW | - 0.019 | - 0.095 | 1 | | | | | | | | |
| | SP | - 0.062 | 0.043 | 0.117 | 1 | | | | | | | |
| | SMKP | 0.220** | - 0.034 | 0.122 | - 0.084 | 1 | | | | | | |
| | NPB | 0.084 | 0.004 | 0.077 | - 0.111 | 0.025 | 1 | | | | | |
| | PH | 0.082 | 0.158* | - 0.090 | 0.045 | 0.075 | - 0.027 | 1 | | | | |
| | HLPB | 0.084 | 0.109 | - 0.100 | 0.036 | 0.115 | 0.005 | 0.869** | 1 | | | |
| | LL | 0.120 | 0.289** | - 0.094 | 0.095 | - 0.072 | - 0.044 | 0.433** | 0.412** | 1 | | |
| | LW | 0.195 | 0.121 | - 0.021 | - 0.007 | - 0.010 | 0.041 | 0.299** | 0.314** | 0.387** | 1 | |
| | PYPP | - 0.097 | - 0.440** | 0.122 | 0.102 | 0.042 | - 0.110 | 0.072 | 0.067 | 0.064 | 0.128 | 1 |

| | | R_80 | LLS_80 | TW | SP | SMKP | NPB | PH | HLPB | LL | LW | PYPP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JL 24 × ICGV 86699 | R_80 | 1 | | | | | | | | | | |
| | LLS_80 | 0.584** | 1 | | | | | | | | | |
| | TW | 0.121 | 0.285** | 1 | | | | | | | | |
| | SP | - 0.065 | 0.016 | 0.477** | 1 | | | | | | | |
| | SMKP | 0.064 | - 0.004 | 0.175* | 0.330** | 1 | | | | | | |
| | NPB | 0.062 | - 0.043 | - 0.017 | 0.004 | 0.109 | 1 | | | | | |
| | PH | 0.091 | 0.163* | - 0.103 | - 0.061 | - 0.047 | - 0.044 | 1 | | | | |
| | HLPB | 0.120 | 0.107 | - 0.040 | - 0.024 | 0.006 | 0.030 | 0.878** | 1 | | | |
| | LL | 0.015 | 0.090 | 0.080 | 0.006 | - 0.007 | - 0.048 | 0.146 | 0.162* | 1 | | |
| | LW | 0.039 | 0.097 | 0.023 | 0.033 | 0.060 | - 0.133 | 0.316** | 0.349** | 0.574** | 1 | |
| | PYPP | - 0.269** | - 0.295** | 0.335** | 0.370** | 0.054 | 0.088 | - 0.157 | - 0.151 | 0.068 | - 0.088 | 1 |

*Contd…..*

| JL 24 × ICGV 99005 | R_80 | LLS_80 | TW | SP | SMKP | NPB | PH | HLPB | LL | LW | PYPP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| R_80 | 1 | | | | | | | | | | |
| LLS_80 | 0.229* | 1 | | | | | | | | | |
| TW | - 0.373** | 0.019 | 1 | | | | | | | | |
| SP | 0.157 | 0.152 | 0.155 | 1 | | | | | | | |
| SMKP | - 0.014 | 0.106 | 0.074 | 0.036 | 1 | | | | | | |
| NPB | - 0.264* | 0.079 | 0.068 | - 0.041 | 0.127 | 1 | | | | | |
| PH | 0.423** | 0.438** | - 0.142 | 0.237* | - 0.062 | 0.061 | 1 | | | | |
| HLPB | 0.406** | 0.396** | - 0.206* | 0.270** | - 0.063 | 0.052 | 0.922** | 1 | | | |
| LL | 0.176 | 0.135 | - 0.157 | 0.209* | - 0.031 | - 0.049 | 0.466** | 0.451** | 1 | | |
| LW | 0.195* | 0.106 | - 0.064 | 0.262** | - 0.067 | 0.078 | 0.507** | 0.498** | 0.392** | 1 | |
| PYPP | - 0.131 | 0.099 | 0.214* | 0.209* | 0.078 | - 0.076 | 0.007 | - 0.028 | 0.231* | 0.141 | 1 |

*, **: Significant at 5% and 1%, respectively.

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** Height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm); **PYPP:** Pod yield per plant (gm)

**Table 28: Superior lines identified from $BC_1F_4$ of JL 24 × GPBD 4**

| Sl. No. | Superior lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | JG4_77 | 3.00 | 4.00 | 17.92 | 60.00 | 71.00 | 100.00 | 5.00 | 25.20 | 28.75 | 6.40 | 2.83 |
| 2 | JG4_57 | 3.00 | 4.00 | 17.65 | 59.00 | 71.00 | 94.69 | 4.50 | 32.65 | 34.00 | 6.30 | 3.55 |
| 3 | JG4_27 | 3.00 | 4.00 | 17.58 | 50.00 | 73.50 | 98.09 | 4.50 | 37.75 | 39.38 | 5.63 | 3.03 |
| 4 | JG4_24 | 3.00 | 3.00 | 17.54 | 58.50 | 74.50 | 96.50 | 5.50 | 29.75 | 39.50 | 5.25 | 2.95 |
| 5 | JG4_81 | 3.00 | 4.00 | 17.32 | 60.00 | 77.50 | 98.94 | 5.00 | 35.45 | 40.88 | 6.33 | 3.20 |
| 6 | JG4_14 | 3.00 | 4.00 | 16.94 | 54.00 | 75.50 | 100.00 | 6.50 | 36.50 | 37.13 | 6.53 | 3.23 |
| 7 | JG4_92 | 4.00 | 4.00 | 16.75 | 55.00 | 75.50 | 92.63 | 6.00 | 38.50 | 39.75 | 6.95 | 3.65 |
| 8 | JG4_90 | 3.00 | 4.00 | 16.55 | 50.50 | 76.50 | 96.54 | 7.00 | 30.00 | 33.25 | 6.03 | 2.95 |
| 9 | JG4_49 | 3.00 | 4.00 | 16.50 | 55.50 | 68.00 | 94.79 | 4.50 | 23.25 | 25.00 | 5.10 | 2.98 |
| 10 | JG4_30 | 4.00 | 4.00 | 16.49 | 49.00 | 67.00 | 100.00 | 5.00 | 29.75 | 36.05 | 5.70 | 2.75 |
| 11 | JG4_13 | 3.00 | 4.00 | 16.46 | 56.00 | 72.00 | 100.00 | 9.00 | 31.63 | 35.00 | 5.08 | 3.25 |
| 12 | JG4_59 | 3.00 | 3.00 | 16.25 | 45.00 | 72.50 | 100.00 | 7.50 | 32.50 | 37.00 | 6.05 | 3.43 |
| 13 | JG4_20 | 3.00 | 4.00 | 16.18 | 49.50 | 67.00 | 100.00 | 5.00 | 39.90 | 40.00 | 6.33 | 3.25 |
| 14 | JG4_37 | 3.00 | 4.00 | 15.78 | 51.50 | 71.50 | 93.41 | 5.50 | 31.00 | 32.43 | 5.75 | 3.08 |
| 15 | JG4_36 | 3.00 | 4.00 | 15.77 | 56.50 | 71.50 | 93.35 | 4.50 | 31.03 | 35.28 | 6.10 | 3.18 |
| 16 | JG4_43 | 3.00 | 3.00 | 15.54 | 53.00 | 75.00 | 100.00 | 7.50 | 29.80 | 31.83 | 5.80 | 3.13 |
| 17 | JG4_22 | 3.00 | 4.00 | 15.50 | 53.50 | 70.00 | 98.14 | 4.50 | 35.43 | 37.60 | 6.15 | 3.50 |
| 18 | JG4_52 | 3.00 | 4.00 | 15.38 | 45.00 | 76.50 | 96.32 | 5.50 | 32.95 | 35.88 | 6.53 | 3.20 |
| 19 | JG4_2 | 3.00 | 4.00 | 15.34 | 53.50 | 73.00 | 93.89 | 4.00 | 33.90 | 37.78 | 6.80 | 3.18 |
| 20 | JG4_31 | 4.00 | 3.00 | 15.25 | 51.50 | 72.00 | 95.62 | 6.00 | 38.05 | 43.58 | 6.65 | 3.30 |
| | JL 24 | 3.50 | 8.00 | 12.49 | 52.00 | 76.00 | 93.96 | 6.00 | 25.80 | 28.83 | 5.80 | 3.15 |
| | GPBD 4 | 3.00 | 3.00 | 12.81 | 42.50 | 76.50 | 100.00 | 5.00 | 30.80 | 32.83 | 4.93 | 2.98 |
| | CD (5%) | 0.27 | 0.75 | 1.91 | 4.10 | 4.31 | 2.69 | 1.57 | 5.50 | 5.74 | 0.89 | 0.37 |

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** Height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

exhibited significant superiority for PYPP and SMKP, and marginal superiority for TW and SP over JL 24 (Table 29).

Of the 56 $BC_1F_4$ backcross lines of JL 24 × ICGV 99005, 13 lines with high LLS resistance were selected. Ten lines recorded significantly superior PYPP over JL 24. Further, a few lines were also significantly superior for other productivity traits. Line J9-4_19 showed significant superiority for PYPP, TW, SP and SMKP over JL 24. Line J9-4_20 was significantly superior for PYPP, TW and SP, while marginally superior for SMKP over JL 24 (Table 30).

4.3.4.2 Second backcross

Seeds from 19 and 33 $BC_2F_1$ plants from JL 24 × GPBD 4 and JL 24 × ICGV 86699, respectively were harvested and sown in the field during the post-rainy season of 2012. Since $BC_2F_1$ seeds of JL 24 × ICGV 99005 failed to germinate, the selfed generations could not be developed. A total of 51 and 89 $BC_2F_2$ plants from JL 24 × GPBD 4 and JL 24 × ICGV 86699, respectively were evaluated under diseases epiphytotic condition. At harvest, JL 24 recorded a disease score of 8.0 for both rust and LLS, while the score ranged from 4.0-6.0 for rust and 5.0-8.0 for LLS among the plants of JL 24 × GPBD 4 and 3.0-6.0 for rust and 3.0-7.0 for LLS among the plants JL 24 × ICGV 86699. Plants resistant to either LLS or rust or both were selected. The number of selected plants was 20 in case of JL 24 × GPBD 4 and 48 in the case of JL 24 × ICGV 99005.

A total of twenty and forty six $BC_2F_3$ families from JL 24 × GPBD 4 and JL 24 × ICGV 86699, respectively were raised during the rainy season of 2013. The performance of these $BC_2F_3$ lines for LLS and rust resistance along with a few productivity and agronomic traits was evaluated. $BC_2F_3$ lines differed significantly for most of the traits studied (Table 31). A moderate to high PCV and GCV were recorded for most of the traits. However, reaction to rust recorded a low level of PCV and GCV when compared to LLS. Heritability ($h^2b$), GA and GAM for most of the traits were either low or moderate (Table 32). In general, pod yield per plant was positively associated with other productivity traits like TW, SP and SMKP. But they were negatively correlated with LLS incidence (Table 33).

**Table 29: Superior lines identified from $BC_1F_4$ of JL 24 × ICGV 86699**

| Sl. No. | Superior lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | J8-4_63 | 3.00 | 3.00 | 17.21 | 48.00 | 77.00 | 100.00 | 4.25 | 37.50 | 39.33 | 5.50 | 3.15 |
| 2 | J8-4_19 | 3.00 | 3.00 | 15.58 | 47.00 | 75.50 | 97.06 | 5.75 | 30.13 | 36.65 | 5.08 | 2.98 |
| 3 | J8-4_12 | 3.00 | 3.00 | 11.91 | 42.00 | 72.00 | 98.44 | 6.00 | 51.08 | 58.00 | 6.13 | 3.78 |
| 4 | J8-4_6 | 3.00 | 3.00 | 11.51 | 45.00 | 77.50 | 98.35 | 6.75 | 28.75 | 40.15 | 6.88 | 3.23 |
| 5 | J8-4_81 | 3.00 | 3.00 | 11.30 | 43.00 | 39.00 | 88.95 | 6.50 | 36.18 | 40.03 | 5.80 | 3.10 |
| 6 | J8-4_41 | 3.00 | 3.00 | 11.21 | 40.50 | 60.50 | 95.99 | 5.50 | 40.83 | 45.63 | 5.93 | 3.63 |
| 7 | J8-4_37 | 3.00 | 3.00 | 10.50 | 37.25 | 37.50 | 94.39 | 4.25 | 33.03 | 34.18 | 5.70 | 3.03 |
| 8 | J8-4_77 | 3.00 | 4.00 | 17.86 | 55.50 | 75.50 | 97.51 | 7.00 | 28.33 | 33.45 | 6.90 | 3.08 |
| 9 | J8-4_46 | 3.00 | 4.00 | 17.69 | 50.50 | 76.50 | 98.41 | 6.25 | 30.58 | 33.38 | 5.68 | 3.45 |
| 10 | J8-4_80 | 3.00 | 4.00 | 17.59 | 46.00 | 71.00 | 96.77 | 5.50 | 39.35 | 43.13 | 6.33 | 4.00 |
| 11 | J8-4_51 | 3.00 | 4.00 | 17.55 | 53.00 | 71.50 | 94.78 | 7.50 | 36.00 | 41.63 | 4.90 | 3.20 |
| 12 | J8-4_58 | 3.00 | 4.00 | 17.52 | 53.50 | 73.50 | 99.22 | 5.75 | 47.75 | 50.40 | 6.55 | 3.38 |
| 13 | J8-4_24 | 3.00 | 4.00 | 17.32 | 55.50 | 76.25 | 100.00 | 7.00 | 37.03 | 39.83 | 4.80 | 3.58 |
| 14 | J8-4_70 | 3.00 | 4.00 | 17.30 | 57.50 | 77.00 | 95.30 | 5.00 | 31.95 | 33.88 | 5.95 | 3.23 |
| 15 | J8-4_66 | 3.00 | 4.00 | 17.27 | 47.00 | 70.50 | 91.77 | 6.25 | 36.55 | 42.13 | 6.50 | 3.38 |
| 16 | J8-4_57 | 3.00 | 4.00 | 17.23 | 59.00 | 76.00 | 94.86 | 7.25 | 33.68 | 36.63 | 5.38 | 2.65 |
| 17 | J8-4_61 | 3.00 | 4.00 | 17.17 | 49.00 | 67.00 | 97.38 | 5.75 | 31.33 | 32.55 | 6.08 | 3.13 |
| 18 | J8-4_16 | 3.00 | 4.00 | 16.79 | 53.50 | 73.50 | 93.13 | 7.00 | 35.10 | 38.93 | 5.03 | 2.95 |
| 19 | J8-4_14 | 3.00 | 4.00 | 16.75 | 57.00 | 70.00 | 100.00 | 5.75 | 39.43 | 46.25 | 5.08 | 2.60 |
| 20 | J8-4_10 | 3.50 | 4.00 | 16.57 | 60.00 | 76.50 | 100.00 | 5.50 | 27.53 | 31.05 | 4.90 | 2.85 |
| 21 | J8-4_18 | 3.00 | 4.00 | 13.32 | 42.00 | 77.00 | 91.68 | 5.50 | 33.25 | 37.95 | 5.20 | 3.10 |
| 22 | J8-4_82 | 3.00 | 4.00 | 13.31 | 45.00 | 66.50 | 92.16 | 4.25 | 36.65 | 39.13 | 7.08 | 3.48 |
| 23 | J8-4_15 | 3.00 | 4.00 | 12.91 | 54.00 | 69.75 | 98.46 | 4.25 | 40.43 | 42.25 | 7.03 | 3.93 |
| 24 | J8-4_69 | 3.00 | 4.00 | 12.40 | 45.00 | 48.00 | 95.44 | 6.25 | 30.00 | 33.75 | 6.30 | 3.08 |
| 25 | J8-4_78 | 3.00 | 4.00 | 12.08 | 36.00 | 48.00 | 94.50 | 4.75 | 36.53 | 42.75 | 5.63 | 3.13 |
| 26 | J8-4_36 | 3.00 | 4.00 | 11.77 | 43.50 | 73.50 | 97.17 | 5.75 | 41.63 | 45.75 | 6.05 | 3.75 |
| 27 | J8-4_2 | 3.00 | 4.00 | 11.73 | 45.00 | 73.00 | 100.00 | 5.75 | 35.50 | 38.60 | 6.55 | 3.15 |
| | JL 24 | 4.00 | 6.75 | 12.50 | 52.50 | 72.50 | 93.53 | 5.75 | 36.55 | 40.08 | 6.25 | 3.35 |
| | ICGV 86699 | 3.00 | 3.00 | 12.50 | 42.00 | 67.50 | 94.49 | 8.50 | 40.83 | 43.95 | 4.58 | 2.58 |
| | CD (5%) | 0.26 | 0.27 | 1.68 | 4.45 | 5.15 | 2.63 | 0.77 | 4.09 | 4.79 | 0.72 | 0.39 |

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** Height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

**Table 30: Superior lines identified from $BC_1F_4$ of JL 24 × ICGV 99005**

| Sl. No. | Superior lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | J9-4_5 | 3.00 | 4.00 | 15.89 | 55.25 | 67.50 | 100.00 | 5.75 | 28.70 | 31.88 | 5.50 | 3.25 |
| 2 | J9-4_6 | 3.00 | 4.00 | 8.15 | 45.50 | 55.50 | 100.00 | 5.25 | 24.53 | 26.65 | 7.10 | 3.03 |
| 3 | J9-4_7 | 3.00 | 4.00 | 16.30 | 57.50 | 63.00 | 100.00 | 5.50 | 20.18 | 23.48 | 5.30 | 2.55 |
| 4 | J9-4_10 | 3.00 | 4.00 | 15.43 | 56.50 | 76.50 | 100.00 | 4.50 | 26.10 | 29.40 | 4.83 | 3.05 |
| 5 | J9-4_11 | 3.00 | 4.00 | 16.23 | 52.50 | 76.00 | 91.75 | 4.00 | 26.18 | 28.50 | 6.00 | 3.03 |
| 6 | J9-4_17 | 3.00 | 4.00 | 16.69 | 56.00 | 76.00 | 100.00 | 4.50 | 31.38 | 35.25 | 7.15 | 2.83 |
| 7 | J9-4_19 | 3.00 | 4.00 | 17.64 | 60.50 | 77.00 | 100.00 | 5.50 | 30.63 | 32.25 | 6.55 | 3.45 |
| 8 | J9-4_20 | 3.00 | 4.00 | 18.66 | 59.00 | 77.75 | 98.15 | 5.00 | 22.50 | 24.83 | 6.03 | 3.08 |
| 9 | J9-4_46 | 3.00 | 4.00 | 16.13 | 53.50 | 77.00 | 95.95 | 4.50 | 24.45 | 25.63 | 6.35 | 3.18 |
| 10 | J9-4_47 | 3.00 | 4.00 | 11.92 | 46.50 | 77.50 | 93.77 | 4.25 | 21.40 | 26.53 | 6.85 | 3.43 |
| 11 | J9-4_50 | 3.00 | 4.00 | 10.15 | 48.50 | 40.00 | 93.77 | 4.50 | 26.35 | 30.00 | 6.30 | 3.00 |
| 12 | J9-4_51 | 3.00 | 4.00 | 15.15 | 50.00 | 54.00 | 100.00 | 5.25 | 25.48 | 26.75 | 6.23 | 2.93 |
| 13 | J9-4_52 | 3.00 | 4.00 | 16.42 | 52.50 | 77.00 | 98.18 | 5.25 | 24.88 | 29.20 | 6.03 | 3.15 |
| | JL 24 | 3.50 | 6.50 | 12.59 | 52.50 | 73.00 | 98.20 | 5.00 | 28.10 | 30.88 | 6.58 | 2.68 |
| | ICGV 99005 | 3.50 | 3.00 | 12.06 | 49.50 | 63.50 | 93.87 | 5.50 | 31.28 | 32.70 | 6.18 | 2.63 |
| | CD (5%) | 0.32 | 0.87 | 2.59 | 4.22 | 3.89 | 2.48 | 0.86 | 5.65 | 6.19 | 0.85 | 0.41 |

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** Height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

**Table 31: ANOVA for agronomic traits, productivity traits and response to LLS and rust among $BC_2F_3$ populations**

| Crosses | df | Mean sum of squares | | | Critical difference (CD) | | SEm± | CV (%) |
|---|---|---|---|---|---|---|---|---|
| | | Replication | Treatment | Error | 5% | 1% | | |
| | | | | Agronomic traits | | | | |
| **Plant height (cm)** | | | | | | | | |
| JL 24 × GPBD 4 | 21 | 0.17 | 43.70** | 1.60 | 2.63 | 3.58 | 0.87 | 4.16 |
| JL 24 × ICGV 86699 | 47 | 90.19 | 29.37** | 4.30 | 4.17 | 5.57 | 1.45 | 5.78 |
| **Number of primary branches** | | | | | | | | |
| JL 24 × GPBD 4 | 21 | 0.28 | 1.13** | 0.20 | 0.92 | 1.25 | 0.31 | 8.77 |
| JL 24 × ICGV 86699 | 47 | 0.59 | 1.90** | 0.10 | 0.63 | 0.83 | 0.28 | 5.66 |
| **Height of longest primary branches (cm)** | | | | | | | | |
| JL 24 × GPBD 4 | 21 | 16.32 | 54.66** | 4.20 | 4.26 | 5.81 | 1.42 | 5.94 |
| JL 24 × ICGV 86699 | 47 | 65.75 | 30.52** | 2.50 | 3.18 | 4.25 | 1.11 | 4.00 |
| **Leaf length (cm)** | | | | | | | | |
| JL 24 × GPBD 4 | 21 | 0.93 | 3.66** | 0.03 | 0.34 | 0.46 | 0.11 | 2.65 |
| JL 24 × ICGV 86699 | 47 | 0.26 | 1.25** | 0.17 | 0.83 | 1.11 | 0.29 | 6.51 |
| **Leaf width (cm)** | | | | | | | | |
| JL 24 × GPBD 4 | 21 | 0.23 | 0.71** | 0.06 | 0.33 | 0.44 | 0.11 | 4.95 |
| JL 24 × ICGV 86699 | 47 | 0.07 | 0.26 | 0.04 | 0.42 | 0.56 | 0.15 | 6.44 |

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| Crosses | df | Mean sum of squares | | | Critical difference (CD) | | SEm± | CV (%) |
|---|---|---|---|---|---|---|---|---|
| | | Replication | Treatment | Error | 5% | 1% | | |
| | | | | Productivity traits | | | | |
| **Pod yield per plant(gm)** | | | | | | | | |
| JL 24 × GPBD 4 | 21 | 0.64 | 5.61** | 0.93 | 2.01 | 2.73 | 0.67 | 7.53 |
| JL 24 × ICGV 86699 | 47 | 2.79 | 9.45** | 1.22 | 2.22 | 2.96 | 0.77 | 8.63 |
| **Test weight (gm)** | | | | | | | | |
| JL 24 × GPBD 4 | 21 | 36.66 | 42.25** | 9.13 | 6.28 | 8.56 | 2.09 | 7.81 |
| JL 24 × ICGV 86699 | 47 | 4.38 | 53.28** | 5.87 | 4.87 | 6.50 | 1.69 | 5.87 |
| **Shelling percentage** | | | | | | | | |
| JL 24 × GPBD 4 | 21 | 14.56 | 34.07** | 9.53 | 5.30 | 7.64 | 1.75 | 7.79 |
| JL 24 × ICGV 86699 | 47 | 12.72 | 104.40** | 8.34 | 5.81 | 7.75 | 2.02 | 4.55 |
| **Sound mature kernel percentage** | | | | | | | | |
| JL 24 × GPBD 4 | 21 | 0.20 | 66.58** | 26.80 | 10.77 | 14.66 | 3.58 | 5.61 |
| JL 24 × ICGV 86699 | 47 | 0.36 | 35.68** | 3.73 | 3.88 | 5.18 | 1.35 | 2.11 |
| | | | | Disease traits | | | | |
| **R_80** | | | | | | | | |
| JL 24 × GPBD 4 | 21 | 0.02 | 0.32* | 0.02 | 0.31 | 0.43 | 0.10 | 4.70 |
| JL 24 × ICGV 86699 | 47 | 0.01 | 0.20 | 0.01 | 0.21 | 0.27 | 0.07 | 3.27 |
| **LLS_80** | | | | | | | | |
| JL 24 × GPBD 4 | 21 | 0.02 | 3.82** | 0.19 | 0.71 | 0.97 | 0.24 | 6.17 |
| JL 24 × ICGV 86699 | 47 | 0.01 | 3.41** | 0.14 | 0.75 | 1.00 | 0.26 | 6.55 |

*, **: Significance at 5% and 1%, respectively
**df:** degrees of freedom; **CV:** Coefficient of variation; **SEm±:** Standard error of mean; **R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS

**Table 32: Components of genetic variation for agronomic traits, productivity traits and response to LLS and rust among $BC_2F_3$ populations**

| Crosses | Mean | Range | | Variance | | Coefficient of variation (%) | | $h^2$ (%) | GA | GAM |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Min. | Max. | Vp | Vg | PCV | GCV | | | |
| | | | | | Agronomic traits | | | | | |
| **Plant height (cm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 30.45 | 22.30 | 42.50 | 21.85 | 21.05 | 15.35 | 15.07 | 96.30 | 9.28 | 30.46 |
| JL 24 × ICGV 86699 | 35.86 | 24.13 | 42.15 | 14.69 | 12.54 | 10.69 | 9.87 | 85.40 | 6.74 | 18.79 |
| **Number of primary branches** | | | | | | | | | | |
| JL 24 × GPBD 4 | 5.03 | 4.00 | 6.75 | 0.57 | 0.47 | 14.96 | 13.61 | 82.80 | 1.29 | 25.52 |
| JL 24 × ICGV 86699 | 5.48 | 4.00 | 7.75 | 0.95 | 0.90 | 17.75 | 17.29 | 94.90 | 1.90 | 34.71 |
| **Height of longest primary branches (cm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 34.51 | 26.15 | 42.65 | 27.33 | 25.23 | 15.15 | 14.55 | 92.30 | 9.94 | 28.81 |
| JL 24 × ICGV 86699 | 39.45 | 29.03 | 48.70 | 15.26 | 14.01 | 9.90 | 9.49 | 91.80 | 7.39 | 18.72 |
| **Leaf length (cm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 6.11 | 3.50 | 9.30 | 1.83 | 1.82 | 22.13 | 22.06 | 99.30 | 2.77 | 45.27 |
| JL 24 × ICGV 86699 | 6.33 | 4.55 | 8.05 | 0.63 | 0.54 | 12.51 | 11.63 | 86.40 | 1.41 | 22.27 |
| **Leaf width (cm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 3.17 | 2.05 | 4.25 | 0.36 | 0.34 | 18.81 | 18.48 | 96.50 | 1.19 | 37.41 |
| JL 24 × ICGV 86699 | 3.24 | 2.08 | 3.90 | 0.13 | 0.11 | 11.11 | 10.14 | 83.20 | 0.62 | 19.04 |

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| Crosses | Mean | Range | | Variance | | Coefficient of variation (%) | | $h^2$ (%) | GA | GAM |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Min. | Max. | Vp | Vg | PCV | GCV | | | |
| **Productivity traits** | | | | | | | | | | |
| **Pod yield per plant (gm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 12.81 | 9.39 | 16.28 | 3.27 | 2.34 | 14.11 | 11.94 | 71.55 | 2.66 | 20.80 |
| JL 24 × ICGV 86699 | 12.79 | 8.70 | 17.30 | 5.33 | 4.11 | 18.06 | 15.86 | 77.15 | 3.67 | 28.70 |
| **Test weight (gm)** | | | | | | | | | | |
| JL 24 × GPBD 4 | 38.70 | 32.11 | 49.50 | 21.12 | 16.56 | 11.88 | 10.51 | 78.40 | 7.42 | 19.18 |
| JL 24 × ICGV 86699 | 41.22 | 31.00 | 60.00 | 26.64 | 23.71 | 12.52 | 11.81 | 89.00 | 9.46 | 22.95 |
| **Shelling percentage** | | | | | | | | | | |
| JL 24 × GPBD 4 | 54.88 | 29.50 | 70.61 | 170.36 | 122.69 | 23.78 | 20.18 | 72.00 | 19.37 | 35.28 |
| JL 24 × ICGV 86699 | 63.41 | 44.75 | 73.50 | 52.20 | 48.03 | 11.39 | 10.93 | 92.00 | 13.69 | 21.60 |
| **Sound mature kernel percentage** | | | | | | | | | | |
| JL 24 × GPBD 4 | 92.34 | 73.02 | 98.70 | 33.29 | 19.89 | 6.25 | 4.83 | 59.70 | 7.10 | 7.69 |
| JL 24 × ICGV 86699 | 91.15 | 84.03 | 100.00 | 17.84 | 15.97 | 4.63 | 4.39 | 89.50 | 7.79 | 8.55 |
| **Disease traits** | | | | | | | | | | |
| **R_80** | | | | | | | | | | |
| JL 24 × GPBD 4 | 3.21 | 3.00 | 4.00 | 0.16 | 0.15 | 12.43 | 11.97 | 93.00 | 0.76 | 23.76 |
| JL 24 × ICGV 86699 | 3.11 | 3.00 | 4.00 | 0.10 | 0.09 | 10.33 | 9.80 | 89.90 | 0.60 | 19.14 |
| **LLS_80** | | | | | | | | | | |
| JL 24 × GPBD 4 | 3.08 | 3.00 | 4.00 | 1.91 | 1.85 | 24.83 | 24.45 | 97.00 | 2.76 | 49.58 |
| JL 24 × ICGV 86699 | 5.67 | 3.00 | 7.00 | 1.78 | 1.64 | 23.48 | 22.55 | 92.23 | 2.73 | 48.21 |

**Vp:** Phenotypic variance; **Vg:** Genotypic variance; **PCV:** Phenotypic coefficient of variation; **GCV:** Genotypic coefficient of variation; **$h^2$:** Heritability in broad sense; **GA:** Genetic advance; **GAM:** Genetic advance as percent of mean **R _80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS

**Table 33: Phenotypic correlation among rust and LLS scores (80 DAS), agronomic traits and productivity traits in $BC_2F_3$ populations**

| | | R_80 | LLS_80 | TW | SP | SMKP | NPB | PH | HLPB | LL | LW | PYPP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JL 24 × GPBD 4 | R_80 | 1 | | | | | | | | | | |
| | LLS_80 | - 0.170 | 1 | | | | | | | | | |
| | TW | 0.450** | 0.207 | 1 | | | | | | | | |
| | SP | 0.411** | - 0.228 | 0.253 | 1 | | | | | | | |
| | SMKP | 0.262 | - 0.218 | 0.075 | 0.268 | 1 | | | | | | |
| | NPB | - 0.134 | 0.168 | 0.158 | 0.020 | 0.088 | 1 | | | | | |
| | PH | 0.263 | 0.185 | 0.195 | 0.327* | 0.286 | 0.153 | 1 | | | | |
| | HLPB | - 0.059 | 0.264 | 0.018 | 0.214 | 0.334* | 0.135 | 0.566** | 1 | | | |
| | LL | - 0.108 | 0.183 | 0.114 | - 0.076 | 0.035 | - 0.240 | 0.273 | 0.255 | 1 | | |
| | LW | - 0.098 | 0.188 | 0.119 | - 0.011 | 0.017 | - 0.040 | 0.358 | 0.462** | 0.812** | 1 | |
| | PYPP | 0.131 | 0.313 | 0.179 | 0.016 | 0.264 | 0.196 | - 0.073 | 0.038 | 0.174 | - 0.313 | 1 |

| | | R_80 | LLS_80 | TW | SP | SMKP | NPB | PH | HLPB | LL | LW | PYPP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JL 24 × ICGV 86699 | R_80 | 1 | | | | | | | | | | |
| | LLS_80 | 0.141 | 1 | | | | | | | | | |
| | TW | - 0.041 | - 0.645** | 1 | | | | | | | | |
| | SP | - 0.198 | - 0.326** | 0.497** | 1 | | | | | | | |
| | SMKP | - 0.203* | 0.006 | 0.077 | - 0.231* | 1 | | | | | | |
| | NPB | 0.207* | - 0.125 | 0.308** | - 0.056 | 0.126 | 1 | | | | | |
| | PH | - 0.103 | 0.272** | - 0.184 | - 0.010 | 0.233* | 0.163 | 1 | | | | |
| | HLPB | - 0.045 | 0.207* | - 0.210* | - 0.139 | 0.230* | 0.156 | 0.846** | 1 | | | |
| | LL | 0.108 | 0.210* | - 0.178 | 0.062 | - 0.098 | - 0.006 | 0.219* | 0.238* | 1 | | |
| | LW | 0.128 | 0.331** | - 0.168 | - 0.000 | - 0.133 | - 0.171 | 0.197 | 0.280** | 0.627** | 1 | |
| | PYPP | - 0.028 | - 0.695** | 0.511** | 0.426** | 0.021 | 0.098 | - 0.174 | - 0.128 | - 0.004 | - 0.064 | 1 |

*, **: Significant at 5% and 1%, respectively

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** Height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm); **PYPP:** Pod yield per plant (gm)

Since the variability for rust incidence was less as compared to that of LLS during the rainy season of 2013, selection was exercised only for LLS resistance. From JL 24 × GPBD 4, six lines were selected which had LLS score of 3.0-4.0 in comparison to 6.5 score for JL 24. Three lines were significantly superior and two were marginally superior for PYPP over JL 24. Line JG2-3_13 and JG2-3_14 showed significant superiority for PYPP and SP, while marginal superiority for TW and SMKP over JL 24 (Table 34).

Eleven LLS resistant lines were selected from 46 $BC_2F_3$ lines of JL 24 × ICGV 86699. They were also significantly superior for PYPP over JL 24. Further, J8-2-3_5 and J8-2-3_41 showed significant superiority for PYPP, TW and SP. In addition, line J8-2-3_5 was marginally superior for SMKP over JL 24 (Table 35).

4.3.4.3 Third backcross

Seeds harvested from 11, 17 and 13 $BC_3F_1$ plants of JL 24 × GPBD 4, JL 24 × IGCV 86699 and JL 24 × IGCV 99005, respectively were sown during the rainy season of 2013 to raise $BC_3F_2$ plants. They were evaluated under diseases epiphytotic condition. At harvest, JL 24 recorded a disease score of 8.0 for LLS and 5.0 for rust. The scores ranged from 4.0-6.0 for LLS and 3.0-4.0 for rust among the plants of JL 24 × GPBD 4, 3.0-7.0 for LLS and 3.0-4.0 for rust among the plants JL 24 × ICGV 86699 and 4.0-7.0 for LLS and 3.0-4.0 for rust among the plants JL 24 × ICGV 99005. In an effort to identify the plants homozygous at the marker loci (IPAHM103, GM2301 and pPGPseq8D09) linked to LLS and rust resistance, genomic DNA was isolated from 30, 50 and 35 young seedlings of $BC_3F_2$ of JL 24 × GPBD 4, JL 24 × IGCV 86699 and JL 24 × IGCV 99005, respectively. One, seven and two plants were found to be homozygous at IPAHM103 and GM2301 in JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. These homozygous plants possessed pod features similar to those of JL 24.

Seeds harvested from one, seven and two homozygous $BC_3F_2$ plants from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively were grown as $BC_3F_3$ families during the post-rainy season of 2013. Genomic DNA was isolated

**Table 34: Superior lines identified from $BC_2F_3$ of JL 24 × GPBD 4**

| Sl. No. | Superior lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | JG2-3_1 | 3.00 | 3.50 | 15.54 | 35.71 | 41.27 | 91.52 | 4.75 | 32.00 | 30.60 | 5.35 | 2.65 |
| 2 | JG2-3_13 | 4.00 | 4.00 | 16.21 | 36.25 | 69.50 | 93.14 | 4.00 | 34.50 | 33.90 | 5.05 | 2.65 |
| 3 | JG2-3_14 | 3.00 | 4.00 | 16.28 | 42.19 | 59.20 | 92.99 | 5.25 | 24.85 | 29.90 | 7.10 | 3.50 |
| 4 | JG2-3_15 | 3.00 | 4.00 | 9.39 | 33.50 | 29.50 | 87.23 | 4.00 | 22.30 | 26.50 | 7.70 | 3.35 |
| 5 | JG2-3_19 | 3.00 | 4.00 | 13.25 | 32.11 | 67.50 | 94.47 | 4.50 | 22.65 | 26.15 | 4.30 | 2.35 |
| 6 | JG2-3_20 | 4.00 | 4.00 | 12.32 | 49.22 | 70.61 | 96.07 | 5.50 | 30.15 | 31.50 | 4.45 | 2.65 |
| | JL 24 | 3.00 | 6.00 | 12.44 | 36.16 | 34.25 | 94.25 | 6.00 | 25.30 | 38.50 | 5.20 | 3.20 |
| | GPBD 4 | 3.50 | 3.00 | 12.21 | 33.43 | 68.78 | 98.70 | 4.75 | 29.50 | 38.50 | 5.20 | 3.15 |
| | CD (5%) | 0.31 | 0.71 | 2.01 | 6.28 | 20.30 | 10.77 | 0.92 | 2.63 | 4.26 | 0.34 | 0.33 |

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** Height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

**Table 35: Superior lines identified from $BC_2F_3$ of JL 24 × ICGV 86699**

| Sl. No. | Superior lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | J82-3_10 | 3.00 | 3.00 | 17.30 | 48.00 | 70.25 | 93.30 | 5.25 | 36.35 | 41.03 | 7.45 | 3.48 |
| 2 | J82-3_19 | 3.00 | 3.00 | 16.80 | 45.00 | 68.00 | 90.71 | 4.25 | 33.03 | 36.55 | 6.25 | 3.18 |
| 3 | J82-3_18 | 3.00 | 4.00 | 16.80 | 45.00 | 66.96 | 90.71 | 6.00 | 42.15 | 48.70 | 6.73 | 3.48 |
| 4 | J82-3_5 | 3.00 | 4.00 | 16.61 | 49.50 | 72.00 | 94.28 | 5.25 | 39.95 | 42.65 | 6.53 | 3.53 |
| 5 | J82-3_27 | 3.00 | 4.00 | 16.49 | 47.00 | 73.50 | 89.82 | 6.25 | 32.58 | 40.50 | 6.90 | 3.38 |
| 6 | J82-3_41 | 3.00 | 3.00 | 16.33 | 50.50 | 70.50 | 89.60 | 5.25 | 35.78 | 41.13 | 6.25 | 3.33 |
| 7 | J82-3_28 | 3.00 | 4.00 | 15.96 | 44.00 | 69.00 | 89.74 | 7.25 | 31.70 | 33.83 | 4.55 | 2.08 |
| 8 | J82-3_17 | 3.00 | 4.00 | 15.83 | 46.00 | 69.50 | 85.99 | 5.25 | 33.78 | 34.75 | 5.30 | 3.08 |
| 9 | J82-3_20 | 3.00 | 4.00 | 15.72 | 46.00 | 72.00 | 88.06 | 4.50 | 32.48 | 40.05 | 5.98 | 3.13 |
| 10 | J82-3_7 | 3.00 | 4.00 | 15.65 | 45.50 | 68.50 | 94.36 | 6.50 | 35.58 | 39.68 | 6.03 | 3.13 |
| 11 | J82-3_25 | 3.00 | 4.00 | 15.46 | 45.00 | 66.50 | 92.31 | 6.25 | 41.05 | 43.93 | 6.85 | 3.23 |
| | JL 24 | 4.00 | 6.50 | 12.77 | 43.50 | 55.25 | 95.00 | 6.00 | 25.95 | 29.63 | 7.08 | 3.65 |
| | ICGV 86699 | 3.00 | 3.00 | 12.32 | 60.00 | 65.00 | 98.70 | 7.75 | 36.33 | 40.75 | 4.90 | 2.33 |
| | CD (5%) | 0.21 | 0.75 | 2.22 | 4.87 | 5.81 | 3.89 | 0.63 | 4.17 | 3.18 | 0.83 | 0.42 |

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** Height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

from at least 10 young seedlings within each family. All seedlings within these families were homozygous at IPAHM103 and GM2301 loci, confirming their true breeding behavior.

Homozygous lines from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005 were tested for the recovery of background genome from the recurrent parent (JL 24). For this, a total of 294 AhTE markers were screened for parental polymorphism between JL 24 versus GPBD 4, JL 24 versus ICGV 86699 and JL 24 versus ICGV 99005. It was observed that 30 markers between JL 24 and GPBD 4, seven between JL 24 and ICGV 86699, and 53 between JL 24 and ICGV 99005 were polymorphic. Line JG-18 from JL 24 × GPBD 4 showed the allelic pattern of JL 24 (recurrent pattern) at 26 marker loci out of total 30 polymorphic markers. Only 4 markers showed the allelic pattern of GPBD 4 (donor). This indicated that a large portion (87%) of the genome was similar to that of JL 24.

When the 30 markers polymorphic between JL 24 and GPBD 4 were studied for the map position, it was found that only 19 were mapped on the consensus map (Gautami *et al*., 2012). These 19 markers represented only 11 linkage groups of the total 20 LGs. There was only one marker representing LG A01, A04, A09, B02 and B03. But LG B01, B05, B07 and B09 were represented by two markers each and LG A03 was represented by six markers.

### 4.3.5 Field evaluation of $BC_3F_3$ homozygous lines

One, seven and two homozygous $BC_3F_3$ lines from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively were evaluated for LLS and rust resistance under disease epiphytotic condition, productivity traits and a few agronomic traits during the post-rainy season of 2013. ANOVA showed significant genotypic differences for most of the traits studied (Table 36). Yield attributing traits PYPP, TW and SP recorded high PCV and GCV (Table 37). But, response to LLS and rust recorded moderate level of variability. Productivity traits did not reveal any significant association with LLS and rust incidence. Though PYPP showed positive correlation with other productivity traits, the associations were not significant (Table 38).

**Table 36: ANOVA for agronomic traits, productivity traits and response to LLS and rust among homozygous lines ($BC_3F_3$)**

| Traits | df | Mean sum of squares | | | Critical difference (CD) | | SEm± | CV (%) |
|---|---|---|---|---|---|---|---|---|
| | | Replication | Treatment | Error | 5% | 1% | | |
| R_80 | 13 | 0.04 | 0.43** | 0.04 | 0.41 | 0.57 | 0.13 | 5.69 |
| R_90 | 13 | 0.04 | 0.48** | 0.04 | 0.41 | 0.57 | 0.13 | 5.82 |
| LLS_80 | 13 | 0.04 | 0.43** | 0.04 | 0.41 | 0.57 | 0.13 | 5.57 |
| LLS_90 | 13 | 0.04 | 0.43** | 0.04 | 0.41 | 0.57 | 0.13 | 5.69 |
| PYPP | 13 | 12.46 | 12.16** | 1.73 | 2.84 | 3.96 | 0.90 | 12.49 |
| TW | 13 | 0.75 | 156.24** | 16.12 | 8.68 | 12.10 | 2.84 | 10.30 |
| SP | 13 | 317.52 | 785.46** | 82.11 | 19.58 | 27.30 | 6.41 | 19.91 |
| SMKP | 13 | 28.35 | 73.37** | 13.86 | 8.04 | 11.21 | 2.63 | 4.29 |
| PL | 13 | 0.00 | 0.28** | 0.01 | 0.22 | 0.31 | 0.07 | 3.69 |
| PW | 13 | 0.00 | 0.01 | 0.00 | 0.04 | 0.06 | 0.01 | 1.72 |
| NPB | 13 | 14.29 | 2.00** | 0.29 | 1.16 | 1.61 | 0.38 | 8.91 |
| NSB | 13 | 0.14 | 12.99** | 1.91 | 2.99 | 4.17 | 0.98 | 22.78 |
| PH | 13 | 20.92 | 18.79** | 2.60 | 3.49 | 4.86 | 1.14 | 7.62 |
| HLPB | 13 | 21.09 | 16.85** | 2.30 | 3.27 | 4.57 | 1.07 | 6.17 |
| LL | 13 | 0.00 | 1.46** | 0.18 | 0.91 | 1.27 | 0.30 | 8.82 |
| LW | 13 | 0.00 | 0.18* | 0.02 | 0.28 | 0.40 | 0.09 | 5.04 |

*, **: Significance at 5% and 1%, respectively

**df:** degrees of freedom; **SEm±:** Standard error of mean; **CV:** Coefficient of variation; **R_80:** Rust score at 80 DAS; **R_90:** Rust score at 90 DAS; **LLS_80:** LLS score at 80 DAS; **LLS_90:** LLS score at 90 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **PL:** Pod length(cm); **PW:** Pod width (cm); **NPB**: Number of primary branch; **NSB**: Number of secondary branch; **PH:** Plant height (cm); **HLPB:** Height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

**Table 37: Components of genetic variation for agronomic traits, productivity traits and response to LLS and rust among homozygous ($BC_3F_3$) lines of JL 24 × GPBD4, JL 24 × ICGV 86699 and JL 24 × ICGV 990055**

| Traits | Mean | Min. | Max. | Vp | Vg | PCV | GCV | $h^2$ (%) | GA | GAM |
|---|---|---|---|---|---|---|---|---|---|---|
| R_80 | 3.32 | 3.00 | 4.00 | 0.23 | 0.20 | 14.55 | 13.39 | 84.71 | 0.84 | 25.39 |
| R_90 | 3.25 | 3.00 | 4.00 | 0.20 | 0.17 | 14.90 | 13.82 | 82.19 | 0.76 | 23.33 |
| LLS_80 | 3.39 | 3.00 | 4.00 | 0.26 | 0.22 | 14.55 | 13.39 | 86.02 | 0.90 | 26.40 |
| LLS_90 | 3.32 | 3.00 | 4.00 | 0.23 | 0.20 | 14.90 | 13.82 | 84.71 | 0.84 | 25.39 |
| PYPP | 10.53 | 5.72 | 13.09 | 6.95 | 5.22 | 25.03 | 21.70 | 75.12 | 4.08 | 38.74 |
| TW | 38.99 | 29.22 | 60.43 | 86.18 | 70.06 | 23.81 | 21.47 | 81.29 | 15.55 | 39.87 |
| SP | 45.52 | 12.47 | 70.44 | 433.79 | 351.67 | 45.75 | 41.20 | 81.07 | 34.78 | 76.41 |
| SMKP | 86.76 | 72.28 | 95.56 | 43.61 | 29.76 | 7.61 | 6.29 | 68.23 | 9.28 | 10.70 |
| PL | 2.80 | 2.30 | 3.35 | 0.15 | 0.14 | 13.69 | 13.18 | 92.74 | 0.73 | 26.15 |
| PW | 1.14 | 1.08 | 1.30 | 0.01 | 0.00 | 5.91 | 5.65 | 91.57 | 0.13 | 11.15 |
| NPB | 6.00 | 4.50 | 8.00 | 1.14 | 0.86 | 17.82 | 15.43 | 75.00 | 1.65 | 27.53 |
| NSB | 6.07 | 1.00 | 10.00 | 7.45 | 5.54 | 44.96 | 38.76 | 74.34 | 4.18 | 68.85 |
| PH | 21.16 | 15.60 | 24.90 | 10.69 | 8.09 | 15.45 | 13.44 | 75.66 | 5.10 | 24.08 |
| HLPB | 24.55 | 20.10 | 30.55 | 9.58 | 7.28 | 12.61 | 10.99 | 76.01 | 4.85 | 19.74 |
| LL | 4.80 | 3.05 | 5.85 | 0.82 | 0.64 | 18.89 | 16.71 | 78.22 | 1.46 | 30.44 |
| LW | 2.61 | 1.95 | 3.00 | 0.10 | 0.08 | 12.08 | 10.98 | 82.63 | 0.54 | 20.56 |

**Vp:** Phenotypic variance; **Vg:** Genotypic variance; **PCV:** Phenotypic coefficient of variation; **GCV:** Genotypic coefficient of variation; **$h^2$:** Heritability in broad sense (%); **GA:** Genetic advance; **GAM:** Genetic advance as percent of mean **R _80:** Rust score at 80 DAS; **R _90:** Rust score at 90 DAS; **LLS_80:** LLS score at 80 DAS; **LLS_90:** LLS score at 90 DAS

**Table 38: Phenotypic correlation among rust and LLS scores (80 and 90 DAS), productivity traits and agronomic traits in homozygous ($BC_3F_3$) lines of JL 24 × GPBD4, JL 24 × ICGV 86699 and JL 24 × ICGV 990055**

| | R_80 | R_90 | LLS_80 | LLS_90 | TW | SP | SMKP | PL | PW | NPB | NSB | PH | HLPB | LL | LW | PYPP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R_80 | 1 | | | | | | | | | | | | | | | |
| R_90 | 0.84** | 1 | | | | | | | | | | | | | | |
| LLS_80 | 0.85** | 0.85** | 1 | | | | | | | | | | | | | |
| LLS_90 | 0.71** | 0.73** | 0.85** | 1 | | | | | | | | | | | | |
| TW | 0.06 | - 0.15 | 0.04 | 0.02 | 1 | | | | | | | | | | | |
| SP | 0.04 | - 0.09 | 0.02 | 0.19 | 0.42* | 1 | | | | | | | | | | |
| SMKP | - 0.02 | 0.06 | 0.08 | 0.14 | 0.12 | 0.53** | 1 | | | | | | | | | |
| PL | - 0.21 | - 0.20 | - 0.19 | - 0.36 | 0.51** | 0.11 | 0.13 | 1 | | | | | | | | |
| PW | - 0.34 | - 0.38* | - 0.34 | - 0.19 | 0.47* | 0.54** | 0.35 | 0.48* | 1 | | | | | | | |
| NBP | - 0.24 | - 0.02 | - 0.20 | - 0.19 | - 0.16 | 0.00 | 0.18 | 0.08 | 0.27 | 1 | | | | | | |
| NSB | 0.22 | 0.27 | 0.24 | 0.14 | 0.27 | - 0.08 | - 0.11 | 0.16 | 0.15 | 0.31 | 1 | | | | | |
| PH | 0.15 | 0.06 | 0.07 | 0.13 | 0.00 | - 0.51** | - 0.46* | - 0.12 | - 0.45* | - 0.41* | 0.06 | 1 | | | | |
| HLPB | 0.09 | - 0.05 | 0.10 | 0.07 | 0.41* | - 0.25 | - 0.43* | 0.12 | - 0.15 | - 0.40* | 0.20 | 0.77** | 1 | | | |
| LL | - 0.06 | - 0.01 | - 0.01 | 0.05 | 0.06 | - 0.24 | - 0.24 | 0.06 | - 0.29 | - 0.54** | - 0.17 | 0.62** | 0.58** | 1 | | |
| LW | - 0.10 | - 0.16 | - 0.05 | - 0.08 | 0.36 | - 0.23 | - 0.26 | 0.29 | - 0.09 | - 0.32 | - 0.02 | 0.49** | 0.59** | 0.80** | 1 | |
| PYPP | 0.31 | - 0.40* | - 0.29 | - 0.31 | 0.18 | 0.31 | 0.20 | 0.10 | - 0.06 | 0.19 | 0.13 | - 0.26 | - 0.25 | - 0.18 | - 0.13 | 1 |

*, **: Significant at 5% and 1%, respectively

**R_80:** Rust score at 80 DAS; **R_90:** Rust score at 90 DAS; **L_80:** LLS score at 80 DAS; **L_90:** LLS score at 90 DAS; **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **PL:** Pod length(cm); **PW:** Pod width (cm); **NPB**: Number of primary branch; **NSB**: Number of secondary branch; **PH:** Plant height (cm); **HLPB:** Height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm); **PYPP:** Pod yield per plant (gm)

Overall, LLS and rust development was less during the post-rainy season of 2013. Of the ten homozygous lines, five from JL 24 × ICGV 86699 and one from JL 24 × ICGV 99005 exhibited significantly superior PYPP over JL 24. Line J9-08 from JL 24 × ICGV 99005 exhibited significant superiority for PYPP, TW, SP and SMKP over JL 24. Line J8-08 and line J8-11 of JL 24 × ICGV 86699 showed significant superiority for PYPP, TW and SP over JL 24 (Table 39).

**Table 39: Mean performance of the homozygous ($BC_3F_3$) lines of JL 24 × GPBD4, JL 24 × ICGV 86699 and JL 24 × ICGV 990055 for response to LLS and rust, productivity traits and agronomic traits**

| Lines | R (80) | R (90) | LLS (80) | LLS (90) | PYPP | TW | SP | SMKP | PL | PW | NPB | NSB | PH | HLPB | LL | LW | PC | PR | PB | KC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JG_18 (1) | 3.00 | 3.00 | 3.00 | 3.00 | 6.48 | 32.05 | 64.26 | 83.25 | 2.30 | 1.10 | 6.50 | 9.00 | 22.00 | 25.35 | 4.55 | 2.45 | M | M | S | Tan |
| J8_08 (2) | 3.00 | 3.00 | 3.00 | 3.00 | 13.09 | 41.90 | 59.45 | 86.70 | 3.00 | 1.13 | 4.50 | 7.00 | 22.10 | 25.25 | 5.70 | 2.80 | M | M | S | Tan |
| J8_10 (2) | 3.00 | 3.00 | 3.00 | 3.00 | 5.72 | 41.05 | 60.91 | 84.32 | 3.20 | 1.20 | 5.50 | 5.50 | 24.15 | 29.00 | 5.75 | 2.95 | D | M | P | Tan |
| J8_11 (2) | 4.00 | 3.50 | 4.00 | 4.00 | 11.94 | 60.43 | 67.89 | 85.04 | 2.90 | 1.10 | 4.50 | 7.00 | 24.80 | 30.55 | 5.50 | 3.00 | M | M | S | Tan |
| J8_13 (2) | 3.00 | 3.00 | 3.00 | 3.00 | 8.00 | 34.30 | 49.68 | 84.19 | 3.10 | 1.10 | 5.50 | 2.50 | 24.90 | 24.30 | 5.85 | 2.90 | D | M | P | Tan |
| J8_16 (2) | 3.00 | 3.00 | 3.00 | 3.00 | 11.09 | 36.24 | 52.14 | 72.29 | 2.80 | 1.10 | 6.50 | 6.50 | 22.50 | 27.15 | 5.45 | 2.95 | M | M | S | Tan |
| J8_17 (2) | 4.00 | 4.00 | 4.00 | 4.00 | 12.13 | 30.45 | 53.16 | 86.04 | 2.40 | 1.08 | 7.00 | 10.0 | 24.85 | 25.40 | 4.30 | 2.45 | M | M | S | Tan |
| J8_18 (2) | 4.00 | 4.00 | 4.00 | 4.00 | 11.58 | 30.40 | 60.35 | 80.41 | 2.50 | 1.10 | 5.50 | 5.50 | 19.25 | 21.40 | 4.20 | 2.30 | M | M | S | Tan |
| J9_08 (3) | 3.00 | 3.00 | 3.00 | 4.00 | 11.11 | 38.08 | 68.94 | 89.53 | 2.35 | 1.20 | 6.00 | 4.50 | 22.50 | 24.00 | 5.15 | 2.55 | M | M | S | Tan |
| J9_10 (3) | 3.50 | 3.00 | 3.50 | 3.50 | 7.67 | 40.50 | 70.29 | 89.48 | 2.55 | 1.20 | 5.00 | 3.50 | 18.75 | 23.90 | 4.05 | 2.40 | M | M | S | Tan |
| JL 24 | 4.00 | 4.00 | 4.00 | 4.00 | 12.88 | 36.68 | 70.44 | 95.56 | 3.05 | 1.13 | 6.00 | 7.00 | 19.30 | 23.00 | 5.35 | 2.65 | M | M | S | Tan |
| GPBD 4 | 3.00 | 3.00 | 3.00 | 3.00 | 11.33 | 29.22 | 74.54 | 93.47 | 2.40 | 1.08 | 6.50 | 1.00 | 17.20 | 20.10 | 4.40 | 2.45 | M | M | S | Tan |
| ICVG 86699 | 3.00 | 3.00 | 3.00 | 3.00 | 12.60 | 53.50 | 69.77 | 93.31 | 3.35 | 1.30 | 8.00 | 9.00 | 15.60 | 21.65 | 3.85 | 2.75 | M | M | S | Red |
| ICGV 99005 | 3.00 | 3.00 | 3.00 | 3.00 | 11.80 | 41.10 | 55.51 | 91.04 | 3.30 | 1.20 | 7.00 | 7.00 | 18.40 | 22.60 | 3.05 | 1.95 | M | M | S | Red |
| CD (5%) | 0.41 | 0.41 | 0.41 | 0.41 | 2.84 | 8.68 | 11.85 | 8.04 | 0.22 | 0.04 | 1.15 | 2.99 | 3.49 | 3.27 | 0.91 | 0.28 | | | | |

**R (80):** Rust score at 80 DAS; **R (90):** Rust score at 90 DAS; **LLS (80):** LLS score at 80 DAS; **LLS (90):** LLS score at 90 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **PL:** Pod length (cm); **PW:** Pod width (cm);**NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** Height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm); **PC:** Pod constriction (M:Moderate, D:Deep); **PR:** Pod reticulation (M:Moderate); **PB:** Pod beak (S:Slight, P: Prominent); **KC:** Kernel colour; **(1):** JL 24 × GPBD4; **(2):** JL 24 × ICGV 86699; **(3):** JL 24 × ICGV 990055

# 5. DISCUSSION

Groundnut is an important legume grown as oilseed, food and feed crop worldwide. The cultivated varieties largely belong to Spanish types, which are highly susceptible to foliar diseases namely, late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & Curt.) Van Arx. and rust caused by *Puccinia arachidis* Speg. Considering the severe yield loss of up to 70% due to the co-occurrence of LLS and rust in India (Subrahmanyam *et al.*, 1984; Subrahmanyam *et al.*, 1985; Waliyar, 1990; Shokes and Culbreath, 1997), a considerable effort is being made to breed for resistant genotypes.

The complex genetic nature of the resistance, interference between the two diseases and undesirable linkage drag between resistance and productivity traits and pod features have restricted the success in breeding for LLS and rust resistant genotypes. Use of genomic resources in the breeding programmes might alleviate some of these limitations. In this regard, extensive efforts, including genome sequencing (http://news.uga.edu/releases/article/first-peanut-genome-sequenced/), have been made to establish genomic resources in groundnut. Identification of molecular markers linked to LLS and rust resistance forms one such genomic resource which can provide a definite edge over conventional breeding methods (Bhat *et al.*, 2012; Pasupuleti *et al.*, 2013). Extensive linkage mapping and QTL analysis among the RILs of TAG 24 × GPBD 4 and TG 26 × GPBD 4 identified two major genomic regions governing resistance to LLS and rust (Khedikar *et al.*, 2010; Sujay *et al.*, 2012). One QTL region present on linkage group AhXV showed up to 67.98% and 82.96% phenotypic variance explained (PVE) towards resistance to LLS and rust, respectively. The other QTL region on AhXII showed PVE of up to 62.34% for LLS resistance.

QTL analysis reveals the number, location and contribution of QTL governing a trait along with the markers flanking them. However, some ‘significant’ QTL may be false positives and QTL responsible for significant variation within and between populations can be missed if the tested genotypes are fixed by chance for alleles with similar effects. Therefore, QTLs should be confirmed by repeated experiments using the same and different genotypes. Rust resistance-linked markers were identified and successfully validated using a set of resistant and susceptible

genotypes (Khedikar *et al.*, 2010). However, extensive validation across diverse germplasm and breeding material is necessary for successful use of markers in marker assisted selection.

Near Isogenic Lines (NILs), sharing a common genetic background, but differing only for a small region of the genome (Paterson *et al.*, 1990; Kaeppler *et al.*, 1993), offer a great opportunity for validating the QTL, in addition to allowing fine mapping and characterization of individual loci (Borevitz and Chory, 2004; Brouwer and Clair, 2004). NILs are generally developed from advanced backcross lines. NILs differing for nematode resistance have been developed from backcross lines (Holbrook *et al.*, 2008). Alternatively, Heterogeneous Inbred Families (HIFs), developed from selfing and selection scheme (Allard, 1960), that segregate only for a small portion of the genome of interest have been utilized as the source of NILs for rust resistance in common bean (Haley *et al.*, 1994) and seed weight in sorghum (Tuinstra *et al.*, 1997). In this study, an attempt was made to develop NILs from heterogeneous inbred families (HIFs) for use in validating LLS and rust resistance QTL such that the candidate QTL can be transferred from resistant donors to susceptible cultivars.

## 5.1 Validation of QTL/markers using NILs

Inbred lines that are not entirely homozygous can be selected from selfing and selection scheme of plant breeding (Allard, 1960; Fehr, 1987; Haley *et al.*, 1994). Such lines segregating for the loci/trait that are not yet fixed are referred to as Heterogeneous Inbred Families (HIFs). Plants with contrasting alleles/phenotypes can be selected from such HIFs and developed into true breeding lines, which are called as Near Isogenic Lines (NILs). HIFs have been successfully exploited in extracting the NILs in sorghum (Tuinstra *et al.*, 1997), Arabidopsis (Loudet *et al.*, 2005) and chick pea (Castro *et al.*, 2010).

Previously at the Department of Genetics and Plant Breeding at UAS, Dharwad, two crosses, TAG 24 × GPBD 4 and TG 26 × GPBD 4 were made and advanced. Majority of the families from the advanced generations ($F_5$, $F_6$ and $F_7$) of both these crosses bred true, while a few families segregated for rust and/or LLS resistance (Khedikar *et al.*, 2010; Sarvamangala *et al.*, 2011; Sujay *et al.*, 2012).

Plants showing contrasting phenotypes for LLS and rust within such HIFs were selected and developed into true breeding lines by selfing. True breeding lines with contrasting phenotype for rust and LLS were maintained separately in Set I and Set II, respectively. In total, 30 rust resistant and 34 rust susceptible true breeding lines formed Set I. Similarly, 21 LLS resistant and 25 LLS susceptible true breeding lines constituted Set II. There was at least one resistant and one susceptible true breeding line isolated from each HIF of both the crosses based on rust and LLS reaction.

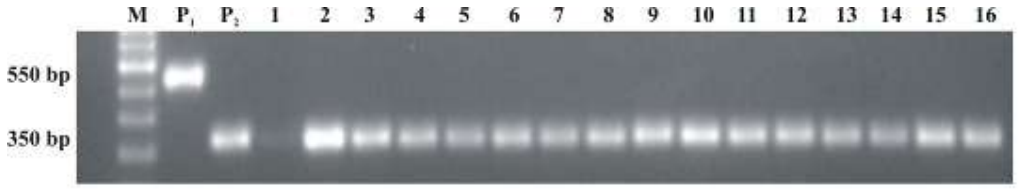
Pooled ANOVA for the response to rust and LLS, agronomic and productivity traits for Set I and Set II showed significant differences among the lines. The sister lines originating from the same HIF differed significantly for reaction to rust in Set I and for LLS in Set II. The resistant lines had score of less than 5.0, while the susceptible lines had a score of more than 5.0 for both LLS and rust. Thus, it was observed that the sister lines from each of the HIFs had contrasting phenotypes for rust resistance in Set I and for LLS resistance in Set II. But the sister lines originating from the common HIFs did not differ significantly for the phenotype with respect to the agronomic and productivity traits, indicating that these sister lines differed significantly for disease resistance but not for other traits observed in this study.

An effort was also made to compare the genomic regions of the sister lines using the DNA markers. For this purpose, a large number of *Arachis hypogaea* Genome-SSR (AHGS) (Shirasawa *et al.*, 2012b), *Arachis hypogaea* EST-SSR (AHES) (Koilkonda *et al.*, 2012) and *Arachis hypogaea* transposable element (AhTE) (Shirasawa *et al.*, 2012a) markers covering all 20 linkage groups of groundnut (Shirasawa *et al.*, 2013) were employed. In addition to these markers, previously identified 89 polymorphic SSR markers (Sujay *et al.*, 2012) were also employed for genotyping the lines of Set I. Of the 1079 AHGS, 470 AHS, 89 SSRs and 405 TE markers used for screening, 168 and 252 were found to be polymorphic between TAG 24 versus GPBD 4 and TG 26 versus GPBD 4, respectively. These polymorphic markers covered all the linkage groups of groundnut genome with number of markers per linkage group ranging from 2 (AhIII) to 14 (AhXIV) in TAG 24 × GPBD 4, while 6 (AhXII) to 22 (AhIII) in TG 26 × GPBD 4 based on the

consensus maps (Gautami *et al*., 2012; Shirasawa *et al*., 2013). The extent of genome similarity (indicated by monomorphic markers) ranged from 51.7% (among the lines of HIF 77) to 86.4% (among the lines of HIF 14) in TAG 24 × GPBD 4. Similarly, it varied from 54.8% (among the lines of HIF 1) to 83.1% (among the lines of HIF 53) in TG 26 × GPBD 4. This DNA marker data indicated that a large portion of the genome between the sister lines was isogenic (Plate 1). Overall, the sister lines selected from each HIF were similar for agronomic and productivity traits, but differed significantly for rust and LLS resistance, and a major portion of the genome was similar (isogenic/monomorphic). These features qualify the sister lines to be regarded as the near isogenic lines (NILs).

In the present study, the NILs differing for LLS and rust resistance were used for validating the markers linked to both LLS and rust resistance (IPAHM103, GM2301 and GM1536 and GM2009) (Khedikar *et al*., 2010; Sujay *et al*., 2012) and those linked to only LLS resistance (GM1009, GM1573 and pPGPseq8D09) (Sujay *et al*., 2012). For the validation of IPAHM103, GM2301 and GM1536 markers, the NILs of Set I with contrasting phenotypes for rust resistance were employed (Plate 2). Of the 64 NILs within Set I, those showing co-segregation between allele and rust reaction were more in number than those showing no co-segregation. High proportion of individuals showing co-segregation and a significant z value was considered as a good case of marker validation. IPAHM103, GM2301 and GM1536 showed strong validation within both the crosses in Set I. However, the markers (IPAHM103, GM2301, GM1536, GM2009, GM1009, GM1573 and pPGPseq8D09) linked to LLS resistance failed to show significant co-segregation between the allele and LLS reaction among the NILs of either TAG 24 × GPBD 4 or 24 NILs of TG 26 × GPBD 4 in Set II. Hence, all the seven markers showed weak validation among the NILs of Set II.

Backcross-derived NILs generally represent a high and uniform contribution from the recurrent parent. But the NILs derived from different HIFs represent varying genomic contribution from the two parents due to an array of recombination events. Hence, HIF-derived NILs in comparison with backcross-derived NILs provide a unique opportunity of ascertaining the expression of the QTL in question over a wide range of constituent background genome. Such a situation would also




(M: 100 bp DNA ladder, $P_1$: TAG 24, $P_2$: GPBD 4, 1: 14-1, 2: 14-2, 3: 77-1, 4: 77-2, 5: 9-1, 6: 9-2, 7: 9-3, 8: 50-1, 9: 50-2, 10: 60-1, 11: 60-2, 12: 60-3, 13: 89-1, 14: 89-2, 15: 101-1 and 16: 101-2)

Plate 1. AhTE0268 marker profile among the HIF-derived lines of TAG 24 × GPBD 4 in Set I

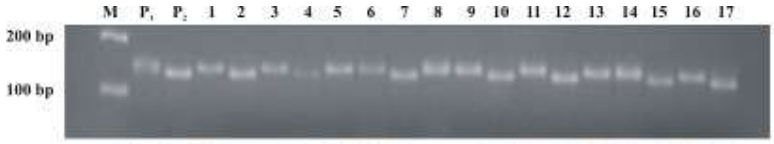



Plate 2a: SSR profile for GM2301 among the NILs of TAG 24 × GPBD 4

(M: 100 bp DNA ladder, $P_1$: TAG 24, $P_2$: GPBD 4, 1: 9-1, 2: 9-2, 3: 9-3, 4: 30-1, 5: 30-2, 6: 46-1, 7: 46-2, 8: 46-3, 9: 50-1, 10: 50-2, 11: 59-1, 12: 59-2, 13: 60-1, 14: 60-2, 15: 60-3, 16: 65-1 and 17:65-2)

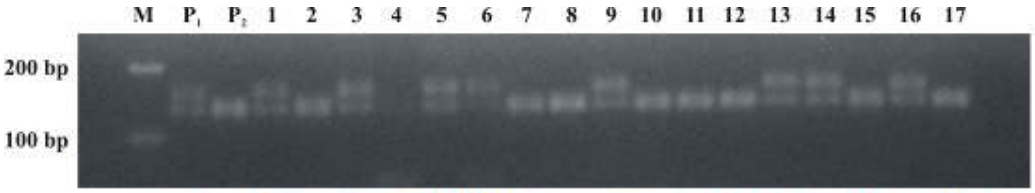



Plate 2b: SSR profile for IPAHM103 among the NILs of TAG 24 × GPBD 4

(M: 100 bp DNA ladder, $P_1$: TAG 24, $P_2$: GPBD 4, 1: 9-1, 2: 9-2, 3: 9-3, 4: 30-1, 5: 30-2, 6: 46-1, 7: 46-2, 8: 46-3, 9: 50-1, 10: 50-2, 11: 59-1, 12: 59-2, 13: 60-1, 14: 60-2, 15: 60-3, 16: 65-1 and 17:65-2)

**Plate 2: SSR profile for rust resistance-linked markers amoung the NILs of Set from TAG24 x GPBD 4**

enable ascertaining epistatic interactions and degree of penetrance for the genomic region in question (Tuinstra *et al*., 1997). In turn, this also gives the advantage of selecting the ideal genetic background that gives maximum expression of the QTL. An effort was made to determine the relative representation of the two parental genomes among the NILs of Set I using the background markers. Average number of alleles from TAG 24 ranged from 41.1-62.7% among the lines of TAG 24 × GPBD 4. Likewise, the average number of TG 26 type of alleles ranged from 41.9-64.4% among the lines of TG 26 × GPBD 4. This wide range of allele representation is the indication of diverse recombination events among the HIFs. Despite varying background genome constitution, NILs showed significant phenotypic difference for LLS and rust resistance and significant marker-trait association, indicating that the QTL governing the resistance were not influenced by the constituent background. This also confirms the negligible epistatic effect and maximum expressivity of the QTL governing rust and LLS resistance. Such QTL with maximum direct effect are ideal for marker assisted introgression.

5.2 Validation of markers using the RILs of VL 1 × 110

An attempt was also made to validate the LLS and rust resistance-linked markers using a new RIL population derived from VL 1 × 110. The RIL population of VL 1 × 110 was already available at the Department of Genetics and Plant Breeding of UAS, Dharwad. The population consisting of 114 RILs was developed by crossing VL 1 (LLS susceptible Valencia type EMS mutant from DER) and 110 (LLS resistant Spanish bunch EMS mutant of VL 1) (Gowda *et al*., 1996).

The pooled analysis of variance across four rainy seasons (2010, 2011, 2012 and 2013) showed significant differences among the RILs for LLS and rust resistance. High PCV and GCV were observed only for LLS at 80 DAS and rust at 80 and 90 DAS. The RILs were used to validate the LLS and rust resistance-linked markers. IPAHM103 and GM2009 linked to both LLS and rust resistance, and GM1009 and *AhMITE1*-PCR associated with only LLS resistance were polymorphic between the parents. The RILs were genotyped with these four markers (Plate 3 and 4) and the marker validation was attempted using single marker analysis, where F statistic and $R^2$ were worked out using WinQTL Cartographer version 2.5 (Wang *et al*., 2007). The F statistic was non-significant for IPAHM103 and GM2009 when the phenotypic classes were grouped based on rust score, indicating that these two markers were not associated with rust resistance among the RILs of VL 1

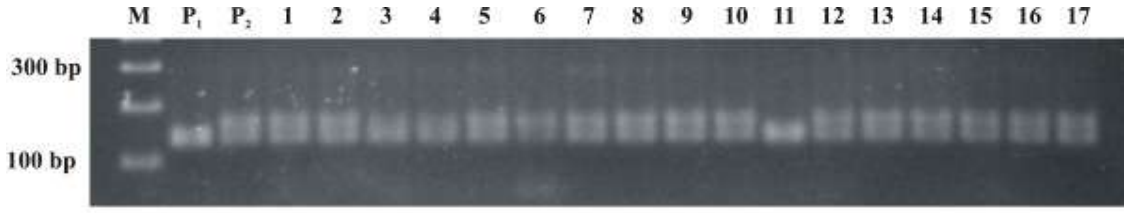



Plate 3a: SSR marker profile for IPAHM103 among the RILs of VL1 × 110

(M: 100 bp DNA ladder, $P_1$ VL1, $P_2$: 110, 1-17: Selected RILs of VL1 × 110)

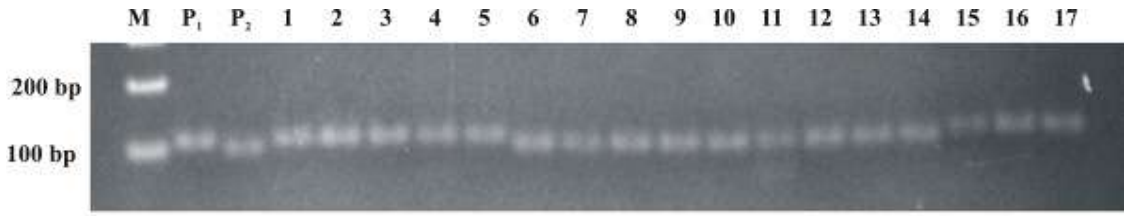


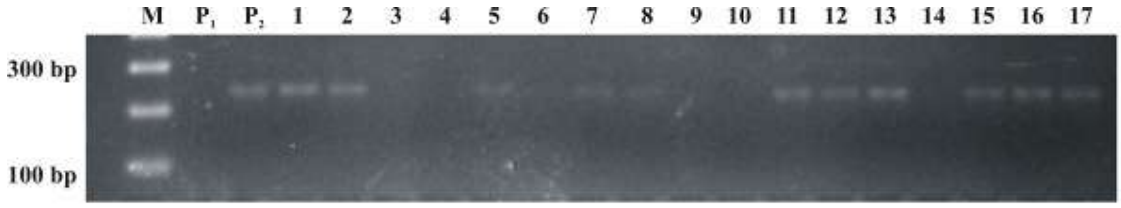

Plate 3b: SSR marker profile for GM2009 among the RILs of VL1 × 110

(M: 100 bp DNA ladder, $P_1$ VL1, $P_2$: 110, 1-17: Selected RILs of VL1 × 110)


Plate 3c: SSR marker profile for GM1009 among the RILs of VL1 × 110

(M: 100 bp DNA ladder, $P_1$ VL1, $P_2$: 110, 1-17: Selected RILs of VL1 × 110)

**Plate 3: SSR profile for LLS and rust resistance-linked markers among the RILs of VL1 × 110**




(M: 100 bp DNA ladder, $P_1$ VL1, $P_2$: 110, 1-17: Selected RILs of VL1 × 110)

**Plate 4. AhMITE1-PCR profile among the RILs of VL 1 × 110**

× 110. However, all the four markers (IPAHM103, GM2009, GM1009 and *AhMITE1*-PCR) showed significant association with LLS resistance only at 70 DAS, but not at 80 and 90 DAS. This can be attributed to the co-occurrence of LLS and rust leading to defoliation at advanced stages.

5.3 Marker assisted backcrossing in JL 24

Marker assisted backcrossing (MABC) is a method of molecular breeding involving the use of markers to transfer one or a few genes/QTL of interest from one genetic source (serving as the donor parent) into a superior cultivar or elite breeding line (serving as the recurrent parent) to improve the targeted trait. It is a precise and effective method to introgress a QTL controlling a trait of interest while retaining the essential characteristics of the recurrent parent (Hospital and Charcosset, 1997; Hospital, 2001; Collard and Mackill, 2008). MABC has three main advantages over conventional backcrossing. Firstly, DNA markers can be used for simple and efficient selection of the target locus ('foreground selection'). Secondly, the size of the donor chromosome segment containing the target locus can be minimized ('recombinant selection'). Thirdly, the recovery of the recurrent parent can be accelerated by selecting backcross lines with a higher proportion of recurrent parent genome ('background selection').

In the present study, efforts were made to develop a series of backcross populations in the genetic background of JL 24 which is highly praised for its wide adaptation and superior market acceptable qualities. But JL 24 is highly susceptible LLS and rust diseases. GPBD 4 is an ideal source of QTL governing LLS and rust resistance since it is resistant to these diseases and it is an improved variety with desirable agronomic and productivity traits (Gowda *et al.*, 2002). Therefore, a previous MABC employed GPBD 4 as the donor of resistance (Varshney *et al.*, 2014).

GPBD 4 is suited for cultivation only in its niche area particularly during rainy season. Also, it is susceptible to groundnut bud necrosis virus disease. Therefore, in the present study, in addition to GPBD 4, two interspecific derivatives ICGV 86699 and ICGV 99005 were used as the donors for transferring the resistance to LLS and rust. ICGV 86699 shows multiple resistance/tolerance to early and late leaf spots, rust, groundnut bud necrosis and groundnut mottle viruses, stem and pod rots (*Sclerotium rolfsii*). It is less susceptible to the tobacco caterpillar and jassids than popular Indian cultivars (Reddy *et al.*, 1996). ICGV 99005 is immune or highly

resistant to rust and/or LLS (Singh *et al*., 2003). GPBD 4 has the contribution from *Arachis cardenasii* (Gowda *et al*., 2002), while the two interspecific derivatives have the contribution of *Arachis batizocoi* in addition to the two progenitor diploids, *Arachis duranensis* and *Arachis ipaensis*. Considering the varying levels of LLS and rust resistance and diverse diploid genomes involved in their origin, a possible allelic variation at the QTL regions was assumed among GPBD 4, ICGV 86699 and ICGV 99005.

For marker assisted selection, two genomic regions (one each on linkage group AhXV and AhXII) were considered. In contrast, the previous attempt on MABC in JL 24 by Varshney *et al*. (2014) employed only the genomic region on AhXV for MAS. In the present study, two SSR markers, IPAHM103 and GM2301 linked to LLS and rust resistance QTL on AhXV, and pPGPseq8D09 linked to only LLS resistance on AhXII were employed. The markers were polymorphic between the parents (JL 24 versus GPBD 4, JL24 versus ICGV 86699 and JL 24 versus ICGV 99005).

With the objective of introgressing the QTL located on the genomic regions of AhXV and AhXII controlling LLS and rust resistance, MABC was initiated with the hybridization of JL 24 with the three donors during the rainy season of 2011. A large number of plants were confirmed to be $F_1$ by marker-specific PCR or by phenotyping in all the three crosses. $BC_1F_1$s were produced during the post-rainy season of 2011 by pollinating JL 24 with the pollens collected from the $F_1$ plants of three crosses separately. Nine, 13 and 11 $BC_1F_1$ plants were confirmed by marker PCR from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. Second backcross plants ($BC_2F_1$) were generated during the rainy season of 2012 by crossing JL 24 with the PCR positive $BC_1F_1$ plants. Five, seven and nine $BC_2F_1$ plants were confirmed by marker PCR from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. Third backcross plants ($BC_3F_1$) were generated during the post-rainy season of 2012 by crossing JL 24 with the PCR positive $BC_2F_1$ plants. Eleven, 17 and 13 $BC_3F_1$ plants were confirmed by marker PCR from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively (Plate 5). Since the donors (GPBD 4, ICGV 86699 and ICGV 99005) had varying levels of relatedness to JL 24, the recurrent parent, backcrossing was continued for three cycles (Fig. 3). Genetic relatedness of the recurrent parent with the donor, number of backcrosses to be attempted, size of the

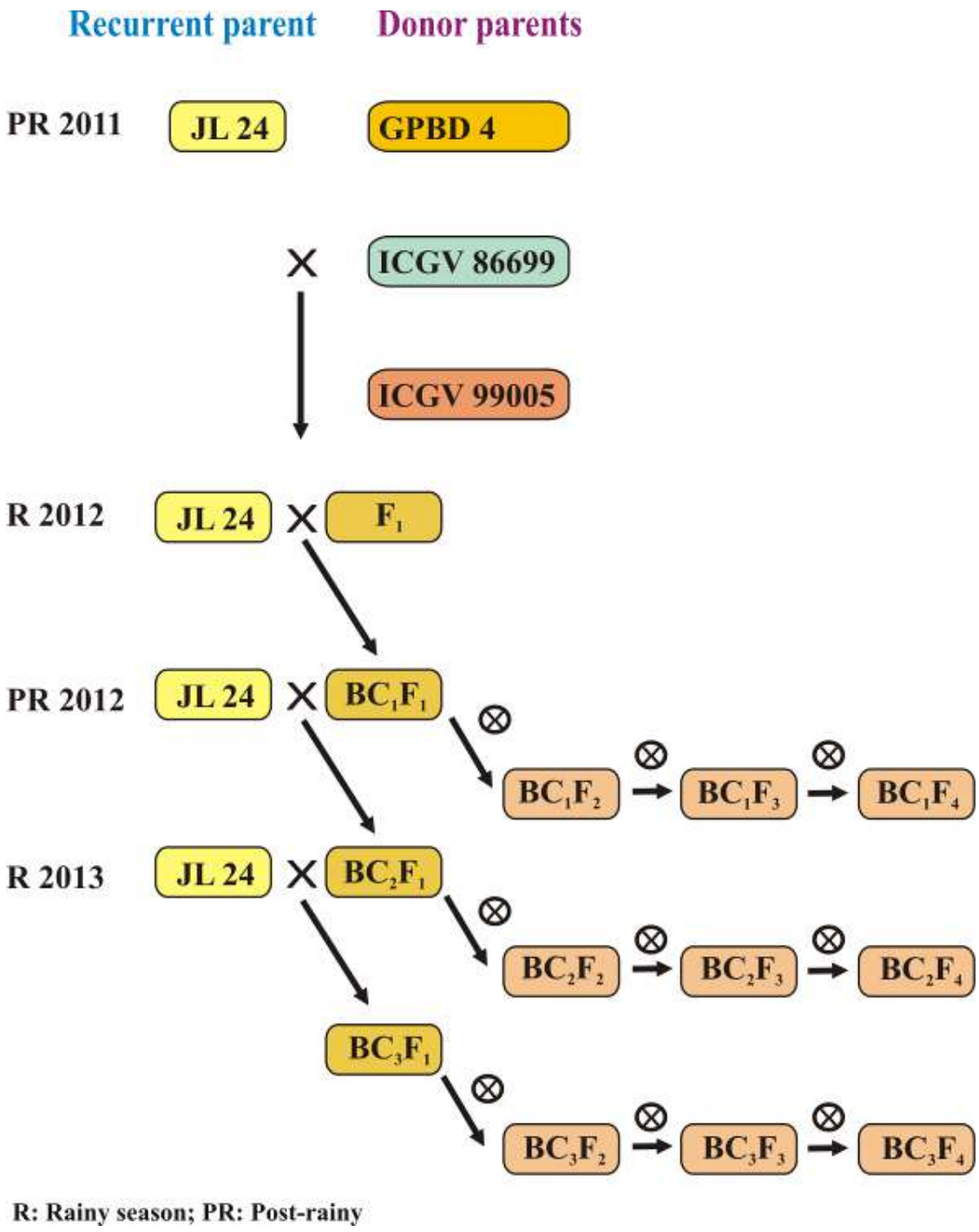



**Fig. 4. Backcross breeding scheme followed in JL 24**

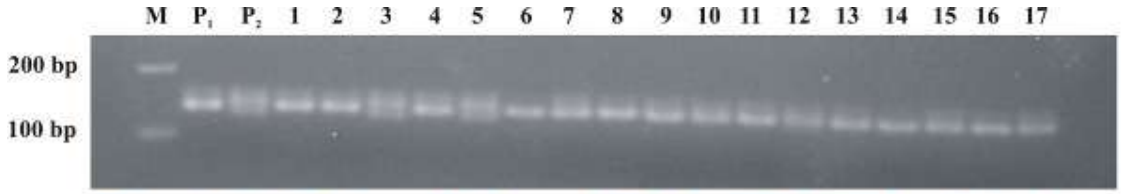



**Plate 5a: Identification of $F_1$ plants in JL 24 × ICGV 86699 by LLS and rust resistance-linked marker (GM2301)**

(M: 100 bp DNA ladder, $P_1$: JL 24; $P_2$: ICGV 86699, 1-17: Selected plants)


**Plate 5b: Identification of $BC_1F_1$ plants in JL 24 × ICGV 86699 by LLS and rust resistance-linked marker (GM2301)**

(M: 100 bp DNA ladder, $P_1$: JL 24; $P_2$: ICGV 86699, 1-17: Selected plants)

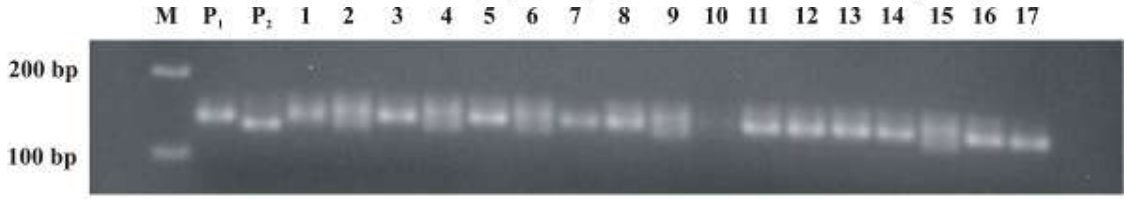



**Plate 5c: Identification of $BC_2F_1$ plants in JL 24 × ICGV 86699 by LLS and rust resistance-linked marker (GM2301)**

(M: 100 bp DNA ladder, $P_1$: JL 24; $P_2$: ICGV 86699, 1-17: Selected plants)

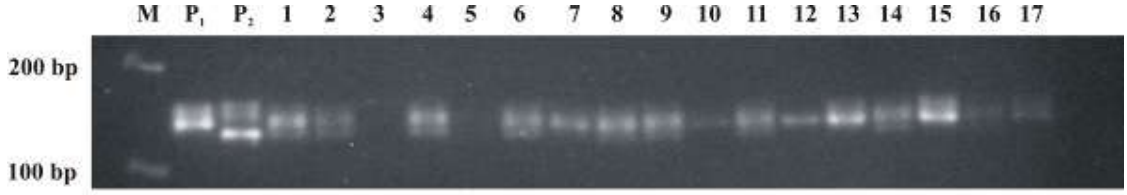



**Plate 5d: Identification of $BC_3F_1$ plants in JL 24 × ICGV 86699 by LLS and rust resistance-linked marker (GM2301)**

(M: 100 bp DNA ladder, $P_1$: JL 24; $P_2$: ICGV 86699, 1-17: Selected plants)

**Plate 5: Identification of $F_1$, $BC_1F_1$, $BC_2F1$ and $BC_3F_1$ in JL 24 × ICGV 86699**

backcross population to be developed and the number of markers to be used are the issue under discussion (Frisch and Melchinger, 2005; Collard and Mackill, 2008; Gupta *et al.*, 2010).

5.3.1 Evaluation of backcross lines

With repeated backcrossing, the frequency of desirable homozygote (like the recurrent parent) increases rapidly. Selection for the plant type of recurrent parent would result in further faster rate of return of the backcross progenies to the genotype of the recurrent parent (Tanksley and Nelson, 1996; Frisch and Melchinger, 2001). Though the frequency of homozygotes for the allele from the recurrent parent would decrease more rapidly with selfing than with backcrossing, selfing leads to homozygosity at the same rate as repeated backcrossing. Therefore, selfed progenies of backcross plants were evaluated for LLS and rust resistance and productivity traits. In all these selfed generations, a large number of plants/families were employed for evaluation to identify the superior lines.

5.3.2 Selfed generations of first backcross

When the recurrent parent is closely related to the donor or when the donor has an improved and elite genetic background (like that of GPBD 4), the MABC may be accelerated by using only one backcross followed by selfing instead of two or three backcross generations (Neeraja *et al.*, 2007; Septiningsih *et al.*, 2009). The increased genetic similarity between the recurrent and donor parents allows for faster conversion to the recurrent parent due to the segments with shared ancestry. At the same time, any remaining background introgressions will be less likely to cause significant differences due to the elite background of the improved donor; thereby the new developed variety is more likely to be phenotypically similar to the original parent. However, large populations are required to allow for reconstitution of most of the recurrent parent genome and exercise selection for such genotypes in as early as the $BC_1F_2$ generation.

By advancing a large number of $BC_1F_1$ plants based on phenotypic/genotypic selection in this study, a total of 106, 82 and 56 $BC_1F_4$ families were developed from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. They were evaluated for LLS and rust resistance, and a few productivity as well as agronomic traits during the rainy season of 2013. The genotypes differed significantly for most of the traits, and the variability observed for most of the traits was moderate to high in all the three crosses. Since, rust

incidence was relatively less as compared to LLS incidence, backcross lines superior to JL 24 for LLS resistance were selected based on the normality concept (Khan *et al.*, 2013) where top 20% was considered for selection.

In total, 20, 27 and 13 lines from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively were found to be superior for LLS resistance over JL 24. Selected lines showed LLS scores of 3.0 to 4.0 while JL 24 had a mean score of 7.0 to 8.0. Many of such lines possessed either marginally or significantly higher pod yield per plant over JL 24. Due to positive correlation among the productivity traits, many of the selected lines also recorded superior TW, SP and SMKP. JG4_81 and JG4_43 were identified as promising backcross lines from JL 24×GPBD 4. JG4_81 was significantly superior for PYPP and TW, while it was marginally superior for SP and SMKP over JL 24. JG4_43 was significantly superior for PYPP and SMKP, while it was marginally superior for TW and SP over JL 24. Similarly, J8-4_10 and J8-4_24 were identified as the promising genotypes from JL 24 × ICGV 86699. J8-4_10 had significant superiority for PYPP, TW and SMKP, and marginal superiority for SP over JL 24. J8-4_24 exhibited significant superiority for PYPP and SMKP, and marginal superiority for TW and SP over JL 24. From JL 24 × ICGV 99005, J9-4_19 and J9-4_20 were found to be promising backcross lines. J9-4_19 recorded significant superiority for PYPP, TW, SP and SMKP over JL 24. J9-4_20 was significantly superior for PYPP, TW and SP, while marginally superior for SMKP over JL 24 (Plate 6).

Submergence tolerant genotypes were developed in rice where transfer of *Sub1* and recovery of the recurrent genome was successfully accomplished in a short time at the $BC_1F_2$ generation (Septiningsih *et al.*, 2015). This was possible because the donor (IR64-Sub1) was related to the recurrent parent (Ciherang) by ancestry. On the other hand, the development of submergence tolerant genotypes in PSB Rc18, a recurrent parent less related to IR64-Sub1 required one more generation of selfing ($BC_1F_3$).

5.3.3 Selfed generations of second backcross

As described in an earlier simulation study, using the two-stage selection maximizes the recurrent parent genome faster than three or four stage selection when negative linkage drag is not a problem (Frisch *et al.*, 1999). The population size can be increased in order to recover the genetic background of the recurrent parent by only two backcrosses in the $BC_2F_2$ generation. If a small portion of the

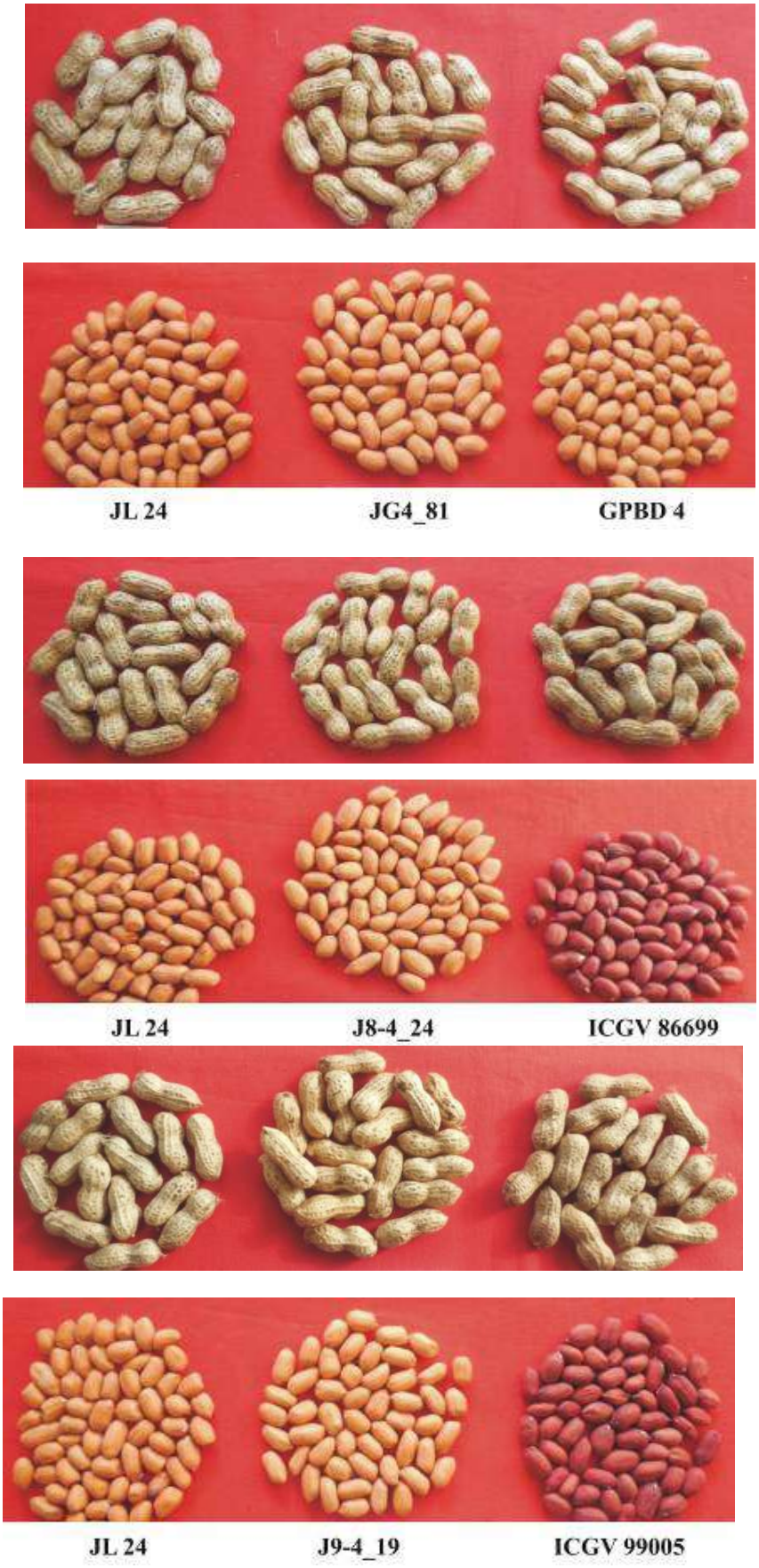



**Plate 6: Pod and kernel features of superior $BC_1F_4$ backcross lines**

donor genome still persists, another backcrossing is usually needed to recover the recurrent parent genome by $BC_3F_2$. Otherwise, an additional selfing generation can also be used. Also, a large population size may not be required when the donor is an adapted, high-yielding variety, and introgression of deleterious alleles would not be so likely (Iftekharuddaula *et al.*, 2011).

In this study, a large number of $BC_2F_1$ plants were advanced based on phenotypic/genotypic selection to get 20 and 48 $BC_2F_3$ families from JL 24 × GPBD 4 and JL 24 × ICGV 86699, respectively. Since the donors used in this study were improved genotypes, they were evaluated during the rainy season of 2013 for the performance under disease epiphytotic condition. $BC_2F_3$ lines of JL 24 × GPBD 4 recorded higher PCV and GCV for shelling percentage and LLS_90 resistance when compared to those of JL 24 × ICGV 86699. Since rust incidence was less during the season, selection was exercised for LLS resistance. Six lines selected from JL 24 × GPBD 4 had LLS score of 3.0-4.0 in comparison to 6.5 score for JL 24. Three lines were significantly superior and two were marginally superior for PYPP over JL 24. Line JG2-3_13 and JG2-3_14 showed significant superiority for PYPP and SP, while marginal superiority for TW and SMKP over JL 24. Similarly, 11 LLS resistant lines were selected from 46 $BC_2F_3$ lines of JL 24 × ICGV 86699. They were also significantly superior for PYPP over JL 24. Further, J8-2-3_5 and J8-2-3_41 showed significant superiority for PYPP, TW and SP. In addition, line J8-2-3_5 was marginally superior for SMKP over JL 24.

### 5.3.4 Selfed generations of third backcross

Seeds harvested from 11, 17 and 13 $BC_3F_1$ plants of JL 24 × GPBD 4, JL 24 × IGCV 86699 and JL 24 × IGCV 99005, respectively were sown during the rainy season of 2013 to raise $BC_3F_2$ plants. They were evaluated under diseases epiphytotic condition. The plants showed a wide range of reaction to both LLS and rust. An effort was also made to identify the plants homozygous for LLS and rust resistance-linked marker loci (IPAHM103 and GM2301). One, seven and two $BC_3F_2$ plants were found to be homozygous at these marker loci from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively (Plate 7). Overall, the plant and pod features in these lines resembled those of JL 24.


**Plate 7a: Identification of homozygous $BC_3F_2$ plants of JL 24 × GPBD 4 using LLS and rust resistance-linked marker (IPAHM103)**

(M: 100 bp DNA ladder, $P_1$: JL 24; $P_2$: GPBD 4, 1-17: $BC_3F_2$ plants)

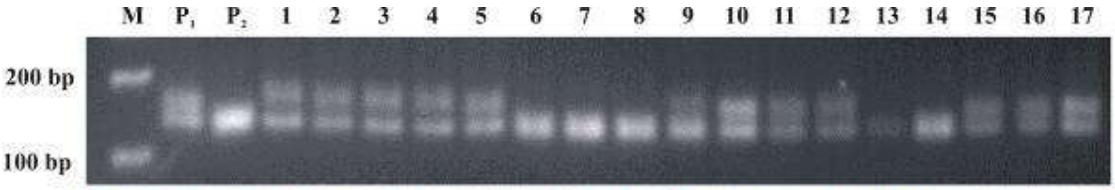



**Plate 7b: Identification of homozygous $BC_3F_2$ plants of JL 24 × ICGV 86699 using LLS and rust resistance-linked marker (IPAHM103)**

(M: 100 bp DNA ladder, $P_1$: JL 24; $P_2$: GPBD 4, 1-17: $BC_3F_2$ plants)


**Plate 7c: Identification of homozygous $BC_3F_2$ plants of JL 24 × ICGV 99005 using LLS and rust resistance-linked marker (IPAHM103)**

(M: 100 bp DNA ladder, $P_1$: JL 24; $P_2$: GPBD 4, 1-17: $BC_3F_2$ plants)

**Plate 7: PCR analysis for identification of homozygous lines among the BC3F2 plants**

$BC_3F_3$ families were established from the $BC_3F_2$ homozygous plants. Evaluation of these families during the post-rainy season of 2013 for LLS and rust resistance under disease epiphytotic condition, along with important agronomic and productivity traits led to the identification of superior lines. Homozygous lines differed significantly for most of the traits studied. PYPP, TW and SP recorded high PCV and GCV. But, response to LLS and rust recorded moderate level of variability. Productivity traits did not reveal any significant association with LLS and rust incidence. Though PYPP showed positive correlation with other productivity traits, the associations were not significant. With these parameters favouring selection, five homozygous lines from JL 24 × ICGV 86699 and one from JL 24 × ICGV 99005 were selected. They exhibited significantly superior PYPP over JL 24. Line J9-08 from JL 24 × ICGV 99005 exhibited significant superiority for PYPP, TW, SP and SMKP over JL 24. Line J8-08 and line J8-11 of JL 24 × ICGV 86699 showed significant superiority for PYPP, TW and SP over JL 24 (Plate 8).

Compared to the first and second backcross progenies, the third backcross progenies are expected to carry higher background genome of the recurrent parent. The extent of genome recovered from JL 24 was tested among $BC_3F_3$. A total of 294 markers were screened for parental polymorphism between JL 24 versus GPBD 4, JL 24 versus ICGV 86699 and JL 24 versus ICGV 99005. Thirty markers were polymorphic between JL 24 and GPBD 4, 7 were polymorphic between JL 24 and ICGV 86699, and 53 were polymorphic between JL 24 and ICGV 99005. Of the 30 polymorphic markers between JL 24 and GPBD 4, only 19 were previously mapped (Gautami *et al.*, 2012). Of the total 20 linkage groups, 11 linkage groups (LG) were represented by the 19 markers. LG AhXI, AhVII, AhXIII, AhII and AhXV were represented by one marker each. LG AhI, AhIV, AhXVIII, AHIX and AhIX were represented by two markers each and LG AhIII was represented by six markers. All the 30 marker loci were in homozygous state among the $BC_3F_2$ plants of JL 24 × GPBD 4. Of the 30 marker loci, 26 showed the allelic pattern of JL 24 (recurrent parent), while 4 markers showed the allelic pattern of GPBD 4 (donor parent). This indicated that a large portion (87%) of the genome was similar to that of JL 24 (Plate 9). Generally, screening with a large number of markers represents the extent of background genome of the recurrent parent. Use of 30 markers for background screening in this study could be less. However, in a backcross

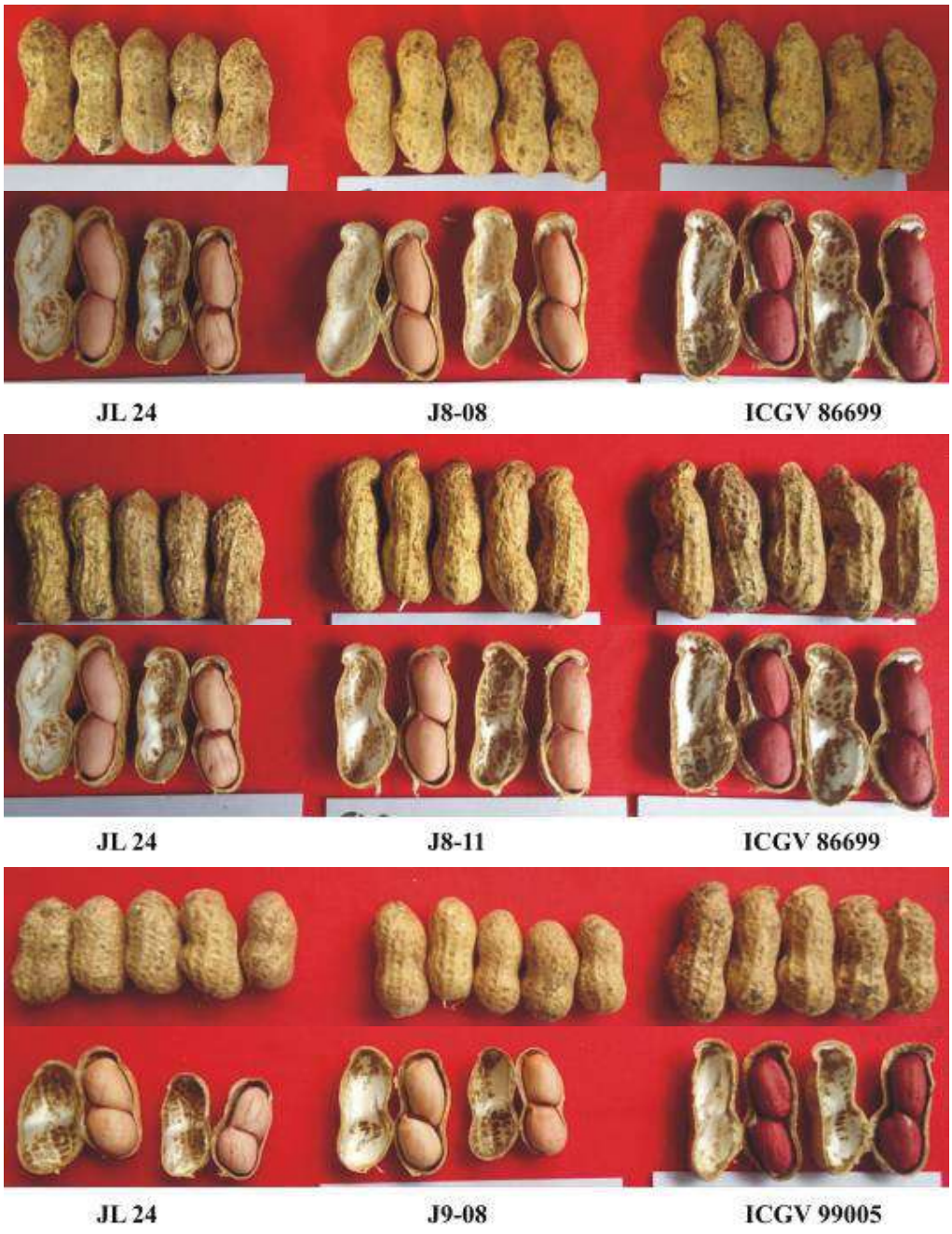
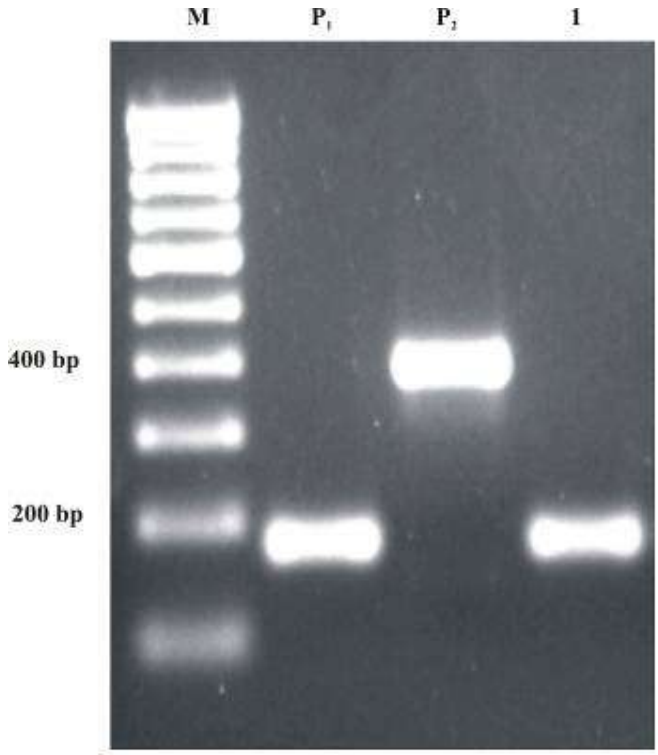



**Plate 8: Pod and kernel features of $BC_3F_3$ homozygous lines**




(M: 100 bp DNA ladder, $P_1$: JL 24; $P_2$: GPBD 4; 1: JG_18)

**Plate 9. Background selection using AhTE0268 marker in a $BC_3F_3$ line of JL 24 × GPBD 4**

programme involving two improved and released varieties aided with phenotypic selection might ensure the recovery of desirable genomic regions. In previous MABC programmes, background selection has been done with as low as 13 markers in groundnut (Varshney *et al.*, 2014) to as high as 88 markers in rice (Vu *et al.*, 2012).

Overall, MABC in JL 24 could identify six superior lines (JG4_81, JG4_43, J8-4_10, J8-4_24, J9-4_19 and J8-4_20) from $BC_1F_4$, four superior lines (JG2-3_13, JG2-3_14, J8-2-3_5 and J8-2-3_41) from $BC_2F_3$ and five superior homozygous lines (JG-18, J8-08, J8-11, J8-18 and J9-08) of $BC_3F_3$ which were more tolerant to LLS than JL 24, while marginally or significantly superior to JL 24 for productivity traits. In addition, the pod and kernel features of these selected lines resembled those of JL 24. Though recombinant selection was not attended in the present study because the donors were in improved genetic background, recovery of the phenotypes of the recurrent parent without any linkage drag is very significant. However, the superior backcross lines need to be evaluated for rust resistance at field level.

Apart from groundnut, similar success with MABC has been reported for improving rust resistance in sunflower (Lawson *et al.*, 1998; Bulos *et al.*, 2013) and wheat (Bariana *et al.*, 2007; Mago *et al.*, 2009; Randhawa *et al.*, 2009). In rice, successful introgression of submergence tolerance QTL *Sub1* to a widely grown cultivars was achieved by MABC (Neeraja *et al.*, 2007; Iftekharuddaula *et al.*, 2011; Septiningsih *et al.*, 2015). A pre-harvest sprouting (PHS) tolerant and leaf rust resistant wheat genotype was developed in HD2329 (Kumar *et al.*, 2010). MABC was used to improve drought adaptation in maize (Ribaut and Ragot, 2007), resistance to white mold caused by *Sclerotinia sclerotiorum* (Lib.) in dry bean (*Phaseolus vulgaris* L.) (Miklas, 2007).

In groundnut, with the validation of LLS and rust resistance-linked markers using the RILs of VL 1 × 110 and the NILs of TAG 24 × GPBD 4 and TG 26 × GPBD 4, now these genomic resources can be employed for MABC. NILs differing for rust resistance are the ideal resources for expression analysis and fine mapping of the gene(s). Using LLS and rust resistance-linked markers, a large number of backcross lines were developed in an elite groundnut variety, JL 24 by transferring

two major genomic regions from three donors (GPBD 4, ICGV 86699 and ICGV 99005). The superior backcross lines with higher LLS resistance need to be evaluated for rust resistance. Also, the backcross lines can be tested for the number and combination of LLS and rust resistance-linked QTL in order to check the contribution of these QTL.

# 6. SUMMARY AND CONCLUSIONS

The lines with contrasting phenotype for LLS and rust resistance, which were previously extracted from heterogeneous inbreed families (HIFs) of TAG 24 × GPBD 4 and TG 26 × GPBD 4 were characterized and regarded as the Near Isogenic Lines (NILs). These HIF-derived NILs and recombinant inbred lines (RILs) of VL 1 × 110 were used to validate LLS and rust resistance linked markers. Using these markers, a marker assisted backcross breeding programme was attempted in JL 24 to develop and select LLS and rust resistant lines. The major findings of the study are presented here.

- Lines breeding true for reaction to LLS and rust, which were selected from the heterogeneous inbred families of TAG 24 × GPBD 4 and TG 26 × GPBD 4, were obtained from the Department of Genetics and Plant Breeding, UAS, Dharwad. True breeding lines with contrasting phenotype for rust and LLS were maintained separately in two Sets (I and II). In total, 30 rust resistant and 34 rust susceptible true breeding lines formed Set I. Similarly, 21 LLS resistant and 25 LLS susceptible true breeding lines constituted Set II. There was at least one resistant and one susceptible true breeding line isolated from each HIF of both the crosses based on rust and LLS reaction.

- Pooled ANOVA for the five rainy seasons of 2008, 2009, 2010, 2011 and 2012 and one post-rainy season of 2010 showed significant genotypic differences for most of the traits. Lines originating from the common HIFs differed significantly for rust resistance in Set I, and for LLS resistance in Set II. But, the lines belonging to the same HIF were *on par* for majority of agronomic and productivity traits.

- In Set I, the lines derived from each HIF of both the crosses cross showed high background similarity. The extent of genome similarity (indicated by monomorphic markers) ranged from 51.7% to 86.4% in TAG 24 × GPBD 4. Similarly, it varied from 54.8% to 83.1% in TG 26 × GPBD 4.

- The lines selected from each HIF were phenotypically similar for agronomic and productivity traits, but differed significantly for rust and LLS

resistance, and a major portion of the genome was similar (isogenic/monomorphic). Hence, these lines were regarded as Near Isogenic Lines (NILs).

- These NILs were used to validate rust resistance-linked markers. IPAHM103, GM1536 and GM2301 linked to rust resistance showed significant co-segregation between the marker allele and the disease phenotype in Set I, indicating their strong validation.

- IPAHM103, GM2301, GM1536, GM2009, GM1009, GM1573 and pPGPseq8D09 linked to LLS resistance failed to show significant co-segregation with the phenotype in Set II, so could not be validated using these NILs.

- Average number of alleles from TAG 24 ranged from 41.1-62.7% among the lines of TAG 24 × GPBD 4. Likewise, the average number of TG 26 type of alleles ranged from 41.9-64.4% among the lines of TG 26 × GPBD 4. This wide range of allele representation did not influence the phenotypes at LLS and rust response, indicating negligible epistatic effect and maximum expressivity of the QTL governing rust and LLS resistance.

- Single marker analysis among RILs of VL 1 × 110 failed to show significant association of IPAHM103, GM2009, GM1009 and *AhMITE1*-PCR with rust resistance. But GM1009, GM2009, IPAHM103 and *AhMITE1*-PCR showed a significant association with LLS resistance only at 70 DAS but not at 80 and 90 DAS. The phenotypic variance explained (PVE) at 70 DAS was highest for GM1009 (5.04%) followed by *AhMITE1*-PCR (3.56%), GM2009 (1.47%) and IPAHM103 (1.33%).

- Marker assisted backcrossing was undertaken to develop backcross lines in JL 24 for LLS and rust resistance using three donors (GPBD 4, ICGV 86699 and ICGV 99005). Backcross populations were developed by crossing JL 24 with the donors separately, and backcrossing the $F_1$ with JL 24. Backcrossing was continued for two more cycles.

- $F_1$, $BC_1F_1$, $BC_2F_1$ and $BC_3F_1$ were confirmed by foreground selection with IPAHM103 and GM2301 (both linked to LLS and rust resistance) and pPGPseq8D09 (linked to only LLS resistance) in addition to phenotypic confirmation. They were advanced to get the stabilized genotypes by selfing.

- Advanced generations of a large number of backcrosses lines were evaluated for agronomic and yield attributing traits along with resistance to LLS and rust under disease epiphytotic condition during the rainy season of 2013. The genotypes differed significantly for most of the traits. Since rust incidence was low during the season, genotypes were selected only for LLS resistance.

- With top 20 per cent selection among $BC_1F_4$ families, 20, 27 and 13 lines that were resistant to LLS were selected from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. Among them, JG4_81 from JL 24 × GPBD 4 was significantly superior for PYPP and TW, while it was marginally superior for SP and SMK% over JL 24. JG4_43 was significantly superior for PYPP and SMKP, while it was marginally superior for TW and SP over JL 24. Similarly, J8-4_10 from JL 24 × ICGV 86699 had significant superiority for PYPP, TW and SMK%, and marginal superiority for SP over JL 24. J8-4_24 exhibited significant superiority for PYPP and SMKP, and marginal superiority for TW and SP over JL 24. J9-4_19 from JL 24 × ICGV 99005 recorded significant superiority for PYPP, TW, SP and SMKP over JL 24. J9-4_20 was significantly superior for PYPP, TW and SP, while marginally superior for SMKP over JL 24.

- Of the four lines selected from $BC_2F_3$ of JL 24 × GPBD 4. Line JG2-3_13 and JG2-3_14 showed significant superiority for PYPP and SP, while marginal superiority for TW and SMKP over JL 24. Similarly, J8-2-3_5 and J8-2-3_41 from JL 24 × ICGV 86699 showed significant superiority for PYPP, TW and SP. In addition, line J8-2-3_5 was marginally superior for SMKP over JL 24.

- Genotyping of $BC_3F_2$ plants with LLS and rust resistance linked markers identified one, seven and two homozygous plants at IPAHM103 and GM2301 from JL 24 × GPBD 4, JL 24 × ICGV 86699 and JL 24 × ICGV 99005, respectively. Overall, the plant and pod features in these lines resembled those of JL 24. Of the 294 markers screened, 30 were polymorphic between JL 24 and GPBD 4, 7 between JL 24 and ICGV 86699, and 53 between JL 24 and ICGV 99005. All the 30 marker loci were in homozygous state among the $BC_3F_2$ plants of JL 24 × GPBD 4. Data from 30 polymorphic marker data indicated that a large portion (87%) of the genome was similar to that of JL 24.

- Five homozygous lines from JL 24 × ICGV 86699 and one from JL 24 × ICGV 99005 exhibited significantly superior PYPP over JL 24. Line J9-08 from JL 24 × ICGV 99005 exhibited significant superiority for PYPP, TW, SP and SMKP over JL 24. Line J8-08 and line J8-11 of JL 24 × ICGV 86699 showed significant superiority for PYPP, TW and SP over JL 24.

- Overall, MABC in JL 24 could identify six superior lines (JG4_81, JG4_43, J8-4_10, J8-4_24, J9-4_19 and J8-4_20) from $BC_1F_4$, four superior lines (JG2-3_13, JG2-3_14, J8-2-3_5 and J8-2-3_41) from $BC_2F_3$ and five superior homozygous lines (JG-18, J8-08, J8-11, J8-18 and J9-08) of $BC_3F_3$. These lines showed higher level of resistance to LLS as compared to JL 24 (recurrent parent). In addition, they were either *on par* or significantly superior to the recurrent parent for the productivity traits. The selected lines need to be evaluated in larger plots for the overall performance.

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**Appendix I: List of AhTE markers used for background selection among the backcross lines**

| Sl. No. | Markers | JL 24 | GPBD 4 | ICGV 86699 | ICGV 99005 |
|---|---|---|---|---|---|
| 1 | AhTE0107 | A | A | A | A |
| 2 | AhTE0113 | A | B | A | B |
| 3 | AhTE0116 | A | A | A | A |
| 4 | AhTE0119 | A | A | A | A |
| 5 | AhTE0121 | A | A | A | A |
| 6 | AhTE0130 | A | A | A | A |
| 7 | AhTE0148 | A | A | A | B |
| 8 | AhTE0159 | A | A | A | A |
| 9 | AhTE0164 | A | A | A | B |
| 10 | AhTE0180 | A | B | A | B |
| 11 | AhTE0181 | A | A | A | A |
| 12 | AhTE0184 | A | A | A | A |
| 13 | AhTE0188 | A | A | A | A |
| 14 | AhTE0190 | A | A | A | A |
| 15 | AhTE0202 | A | A | A | A |
| 16 | AhTE0205 | A | B | A | B |
| 17 | AhTE0211 | A | A | A | A |
| 18 | AhTE0213 | A | A | A | A |
| 19 | AhTE0218 | A | A | A | A |
| 20 | AhTE0222 | A | A | A | A |
| 21 | AhTE0231 | A | A | A | A |
| 22 | AhTE0248 | A | A | A | A |
| 23 | AhTE0254 | A | A | A | A |
| 24 | AhTE0268 | A | A | A | A |
| 25 | AhTE0278 | A | A | A | A |
| 26 | AhTE0282 | - | A | - | A |
| 27 | AhTE0287 | A | A | A | A |
| 28 | AhTE0296 | A | A | A | A |
| 29 | AhTE0300 | A | A | A | A |
| 30 | AhTE0302 | A | B | B | B |
| 31 | AhTE0303 | A | A | A | B |
| 32 | AhTE0319 | A | B | A | A |
| 33 | AhTE0323 | A | A | A | A |
| 34 | AhTE0324 | A | A | A | A |
| 35 | AhTE0333 | A | A | A | A |
| 36 | AhTE0335 | A | A | A | A |
| 37 | AhTE0343 | A | A | A | A |
| 38 | AhTE0347 | A | A | A | A |
| 39 | AhTE0359 | A | A | A | A |
| 40 | AhTE0360 | A | A | A | A |
| 41 | AhTE0361 | A | A | A | A |
| 42 | AhTE0363 | A | A | A | A |

*Contd….*

| Sl. No. | Markers | JL 24 | GPBD 4 | ICGV 86699 | ICGV 99005 |
|---|---|---|---|---|---|
| 43 | AhTE0372 | A | A | A | B |
| 44 | AhTE0373 | A | A | A | A |
| 45 | AhTE0376 | A | A | A | A |
| 46 | AhTE0388 | A | A | A | A |
| 47 | AhTE0398 | A | A | - | A |
| 48 | AhTE0416 | A | A | A | A |
| 49 | AhTE0422 | A | - | A | - |
| 50 | AhTE0426 | A | A | A | A |
| 51 | AhTE0437 | A | A | A | A |
| 52 | AhTE0442 | A | A | A | A |
| 53 | AhTE0445 | A | A | A | A |
| 54 | AhTE0446 | A | A | A | A |
| 55 | AhTE0459 | A | A | A | A |
| 56 | AhTE0467 | A | B | A | B |
| 57 | AhTE0471 | A | A | A | A |
| 58 | AhTE0477 | A | A | A | A |
| 59 | AhTE0489 | A | A | A | A |
| 60 | AhTE0491 | A | A | A | A |
| 61 | AhTE0496 | A | A | A | A |
| 62 | AhTE0500 | A | A | A | A |
| 63 | AhTE0501 | A | A | A | A |
| 64 | AhTE0502 | A | A | A | A |
| 65 | AhTE0504 | A | B | A | B |
| 66 | AhTE0001 | A | A | A | A |
| 67 | AhTE0002 | A | B | A | B |
| 68 | AhTE0003 | A | A | A | A |
| 69 | AhTE0004 | A | A | A | A |
| 70 | AhTE0005 | A | A | A | A |
| 71 | AhTE0006 | A | A | A | - |
| 72 | AhTE0007 | A | A | A | - |
| 73 | AhTE0008 | A | A | A | A |
| 74 | AhTE0009 | A | A | A | A |
| 75 | AhTE0010 | A | B | A | B |
| 76 | AhTE0011 | A | A | A | A |
| 77 | AhTE0012 | A | A | A | A |
| 78 | AhTE0013 | A | B | A | B |
| 79 | AhTE0014 | A | A | A | A |
| 80 | AhTE0015 | A | A | A | A |
| 81 | AhTE0016 | A | A | A | A |
| 82 | AhTE0017 | A | A | A | A |
| 83 | AhTE0018 | A | A | A | A |
| 84 | AhTE0019 | A | A | A | A |
| 85 | AhTE0020 | A | A | A | A |
| 86 | AhTE0021 | A | A | A | A |

*Contd.…*

| Sl. No. | Markers | JL 24 | GPBD 4 | ICGV 86699 | ICGV 99005 |
|---|---|---|---|---|---|
| 87 | AhTE0022 | A | A | A | A |
| 88 | AhTE0023 | A | B | A | B |
| 89 | AhTE0024 | A | B | A | B |
| 90 | AhTE0025 | A | A | A | A |
| 91 | AhTE0026 | A | A | A | B |
| 92 | AhTE0027 | A | A | A | A |
| 93 | AhTE0028 | A | A | A | A |
| 94 | AhTE0029 | - | - | - | - |
| 95 | AhTE0030 | - | - | - | - |
| 96 | AhTE0031 | - | - | - | - |
| 97 | AhTE0032 | - | - | - | - |
| 98 | AhTE0033 | A | A | A | A |
| 99 | AhTE0034 | A | A | A | A |
| 100 | AhTE0035 | A | A | A | A |
| 101 | AhTE0036 | A | A | A | A |
| 102 | AhTE0037 | A | A | A | A |
| 103 | AhTE0038 | A | A | A | A |
| 104 | AhTE0039 | A | A | A | A |
| 105 | AhTE0040 | A | B | A | A |
| 106 | AhTE0041 | A | A | A | A |
| 107 | AhTE0042 | A | A | A | A |
| 108 | AhTE0043 | A | A | A | A |
| 109 | AhTE0044 | A | A | A | A |
| 110 | AhTE0045 | A | B | A | B |
| 111 | AhTE0046 | A | A | A | A |
| 112 | AhTE0047 | A | A | A | A |
| 113 | AhTE0048 | A | A | A | A |
| 114 | AhTE0049 | A | A | A | A |
| 115 | AhTE0050 | A | B | A | B |
| 116 | AhTE0051 | A | A | A | A |
| 117 | AhTE0052 | A | A | A | A |
| 118 | AhTE0053 | - | - | - | - |
| 119 | AhTE0054 | A | - | - | - |
| 120 | AhTE0055 | A | A | A | B |
| 121 | AhTE0056 | A | A | A | A |
| 122 | AhTE0057 | A | A | A | A |
| 123 | AhTE0058 | A | A | A | A |
| 124 | AhTE0059 | A | A | A | A |
| 125 | AhTE0060 | A | A | A | A |
| 126 | AhTE0061 | A | A | A | A |
| 127 | AhTE0062 | A | A | A | A |
| 128 | AhTE0063 | - | - | - | - |
| 129 | AhTE0064 | A | A | A | B |
| 130 | AhTE0065 | A | A | A | A |

*Contd….*

| Sl. No. | Markers | JL 24 | GPBD 4 | ICGV 86699 | ICGV 99005 |
|---|---|---|---|---|---|
| 131 | AhTE0066 | A | A | A | A |
| 132 | AhTE0067 | A | A | A | A |
| 133 | AhTE0068 | - | - | - | - |
| 134 | AhTE0069 | A | A | A | A |
| 135 | AhTE0070 | A | A | A | A |
| 136 | AhTE0071 | A | A | A | A |
| 137 | AhTE0072 | A | A | A | A |
| 138 | AhTE0073 | A | A | A | A |
| 139 | AhTE0074 | A | A | A | B |
| 140 | AhTE0075 | A | A | A | A |
| 141 | AhTE0076 | A | A | A | A |
| 142 | AhTE0077 | A | A | A | A |
| 143 | AhTE0078 | A | A | A | A |
| 144 | AhTE0079 | A | A | A | A |
| 145 | AhTE0080 | A | A | A | A |
| 146 | AhTE0081 | A | A | A | A |
| 147 | AhTE0082 | A | A | A | A |
| 148 | AhTE0083 | A | A | A | A |
| 149 | AhTE0084 | A | A | A | A |
| 150 | AhTE0085 | A | A | A | A |
| 151 | AhTE0086 | A | A | A | A |
| 152 | AhTE0087 | A | A | A | A |
| 153 | AhTE0088 | A | A | A | A |
| 154 | AhTE0089 | A | A | A | A |
| 155 | AhTE0090 | A | A | A | A |
| 156 | AhTE0091 | A | A | A | A |
| 157 | AhTE0092 | A | A | A | A |
| 158 | AhTE0093 | A | A | A | A |
| 159 | AhTE0094 | A | A | A | A |
| 160 | AhTE0095 | A | A | A | A |
| 161 | AhTE0096 | A | A | A | A |
| 162 | AhTE0097 | A | A | A | B |
| 163 | AhTE0098 | A | A | A | B |
| 164 | AhTE0099 | A | A | A | A |
| 165 | AhTE0100 | A | A | A | A |
| 166 | AhTE0101 | A | A | A | A |
| 167 | AhTE0111 | A | A | A | A |
| 168 | AhTE0122 | A | A | A | A |
| 169 | AhTE0129 | A | A | A | A |
| 170 | AhTE0136 | A | A | A | A |
| 171 | AhTE0143 | A | A | A | B |
| 172 | AhTE0149 | - | - | - | - |
| 173 | AhTE0162 | A | A | A | A |
| 174 | AhTE0163 | A | A | A | A |

*Contd.…*

| Sl. No. | Markers | JL 24 | GPBD 4 | ICGV 86699 | ICGV 99005 |
|---|---|---|---|---|---|
| 175 | AhTE0167 | A | A | A | A |
| 176 | AhTE0178 | A | A | A | A |
| 177 | AhTE0189 | A | A | A | A |
| 178 | AhTE0191 | A | A | A | A |
| 179 | AhTE0194 | A | B | A | A |
| 180 | AhTE0197 | A | A | A | A |
| 181 | AhTE0198 | A | A | A | A |
| 182 | AhTE0200 | A | A | A | A |
| 183 | AhTE0203 | A | A | A | A |
| 184 | AhTE0206 | A | A | A | A |
| 185 | AhTE0212 | A | A | A | A |
| 186 | AhTE0217 | A | A | A | A |
| 187 | AhTE0227 | A | A | A | A |
| 188 | AhTE0229 | A | A | A | A |
| 189 | AhTE0232 | A | A | A | - |
| 190 | AhTE0233 | A | A | A | A |
| 191 | AhTE0237 | A | A | A | A |
| 192 | AhTE0245 | A | A | - | - |
| 193 | AhTE0246 | A | A | A | A |
| 194 | AhTE0249 | A | A | A | B |
| 195 | AhTE0251 | A | A | A | B |
| 196 | AhTE0261 | A | A | A | A |
| 197 | AhTE0271 | A | A | A | A |
| 198 | AhTE0283 | A | A | A | A |
| 199 | AhTE0294 | A | A | A | A |
| 200 | AhTE0295 | A | A | A | A |
| 201 | AhTE0305 | A | A | A | A |
| 202 | AhTE0313 | A | A | A | A |
| 203 | AhTE0317 | A | A | A | A |
| 204 | AhTE0328 | A | A | A | A |
| 205 | AhTE0332 | A | A | A | A |
| 206 | AhTE0352 | A | A | A | A |
| 207 | AhTE0356 | A | A | A | A |
| 208 | AhTE0357 | A | A | A | A |
| 209 | AhTE0362 | A | A | A | A |
| 210 | AhTE0369 | A | A | A | A |
| 211 | AhTE0371 | A | A | A | A |
| 212 | AhTE0378 | A | A | A | A |
| 213 | AhTE0381 | A | A | A | A |
| 214 | AhTE0387 | A | A | A | A |
| 215 | AhTE0389 | A | A | A | A |
| 216 | AhTE0391 | A | A | A | A |
| 217 | AhTE0392 | A | A | A | A |
| 218 | AhTE0403 | A | A | A | A |

*Contd….*

| Sl. No. | Markers | JL 24 | GPBD 4 | ICGV 86699 | ICGV 99005 |
|---|---|---|---|---|---|
| 219 | AhTE0410 | A | A | A | A |
| 220 | AhTE0417 | A | A | A | A |
| 221 | AhTE0419 | A | A | A | A |
| 222 | AhTE0423 | A | A | A | A |
| 223 | AhTE0429 | A | A | A | A |
| 224 | AhTE0433 | A | A | A | A |
| 225 | AhTE0443 | A | A | A | A |
| 226 | AhTE0444 | A | A | A | A |
| 227 | AhTE0457 | A | A | A | B |
| 228 | AhTE0465 | A | A | A | A |
| 229 | AhTE0469 | A | A | A | A |
| 230 | AhTE0470 | A | A | A | A |
| 231 | AhTE0475 | A | A | A | A |
| 232 | AhTE0476 | A | A | A | A |
| 233 | AhTE0478 | A | A | A | B |
| 234 | AhTE0479 | A | A | A | A |
| 235 | AhTE0481 | A | A | A | A |
| 236 | AhTE0482 | A | A | A | A |
| 237 | AhTE0483 | A | A | A | A |
| 238 | AhTE0486 | A | A | A | A |
| 239 | AhTE0487 | A | A | A | A |
| 240 | AhTE0488 | - | - | - | - |
| 241 | AhTE0498 | A | A | A | A |
| 242 | AhTE0499 | A | A | A | A |
| 243 | AhTE0503 | A | A | A | A |
| 244 | AhTE0491 | A | A | A | A |
| 245 | AhTE0498 | A | A | A | A |
| 246 | AhTE0129 | A | A | A | A |
| 247 | AhTE0569 | A | A | A | A |
| 248 | AhTE0584 | A | A | A | B |
| 249 | AhTE0893 | - | - | - | - |
| 250 | AhTE0628 | A | A | A | A |
| 251 | AhTE0101 | A | A | A | A |
| 252 | AhTE0571 | A | A | A | A |
| 253 | AhTE0556 | A | A | B | B |
| 254 | AhTE0534 | A | B | A | B |
| 255 | AhTE0074 | A | A | A | A |
| 256 | AhTE0607 | A | A | A | A |
| 257 | AhTE0623 | A | A | A | A |
| 258 | AhTE0913 | - | - | - | - |
| 259 | AhTE0565 | A | B | A | B |
| 260 | AhTE0010 | A | B | A | B |
| 261 | AhTE0013 | A | B | A | B |
| 262 | AhTE0590 | A | A | A | A |

*Contd.…*

| Sl. No. | Markers | JL 24 | GPBD 4 | ICGV 86699 | ICGV 99005 |
|---|---|---|---|---|---|
| 263 | AhTE0647 | A | A | - | A |
| 264 | AhTE1016 | A | A | A | - |
| 265 | AHTE0634 | A | B | A | B |
| 266 | AhTE0621 | A | A | A | A |
| 267 | AhTE0573 | A | A | A | A |
| 268 | AhTE0600 | A | B | A | B |
| 269 | AhTE0658 | A | A | A | A |
| 270 | AhTE0599 | A | A | A | A |
| 271 | AhTE0032 | A | A | A | A |
| 272 | AhTE0826 | A | A | A | - |
| 273 | AhTE0586 | A | A | A | A |
| 274 | AhTE0006 | A | A | A | A |
| 275 | AhTE0520 | A | B | A | B |
| 276 | AhTE0552 | A | A | A | A |
| 277 | AhTE0251 | A | A | A | B |
| 278 | AhTE0615 | A | A | A | B |
| 279 | AhTE0566 | A | A | A | B |
| 280 | AhTE0887 | 0 | A | A | B |
| 281 | AhTE0633 | A | B | A | A |
| 282 | AhTE0547 | A | A | A | A |
| 283 | AhTE0570 | A | B | A | B |
| 284 | AhTE0577 | - | - | - | - |
| 285 | AhTE0654 | A | A | A | A |
| 286 | AhTE0047 | A | A | A | A |
| 287 | AhTE0305 | A | A | A | A |
| 288 | AhTE0636 | A | B | A | B |
| 289 | AhTE0212 | A | A | A | A |
| 290 | AhTE0200 | A | A | A | A |
| 291 | AhTE0162 | A | A | A | A |
| 292 | AhTE0163 | A | A | A | A |
| 293 | AhTE0544 | A | A | A | A |
| 294 | AhTE0567 | A | A | A | A |

A: allele with *AhMITE1* insertion; B: allele without *AhMITE1*; - : No amplification

**Appendix II: Mean performance of $BC_1F_4$ lines from JL24 × GPBD 4 for disease response, agronomic traits and productivity traits**

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **JL 24** | 3.50 | 8.00 | 12.49 | 52.00 | 76.00 | 93.96 | 6.00 | 25.80 | 28.83 | 5.80 | 3.15 |
| **GPBD 4** | 3.00 | 3.00 | 12.81 | 42.50 | 76.50 | 100.00 | 5.00 | 30.80 | 32.83 | 4.93 | 2.98 |
| 1 | 3.00 | 6.50 | 12.37 | 44.00 | 71.50 | 96.43 | 4.00 | 30.75 | 34.88 | 6.90 | 2.75 |
| 2 | 3.00 | 4.00 | 15.34 | 53.50 | 73.00 | 93.89 | 5.00 | 25.20 | 28.75 | 6.40 | 2.83 |
| 3 | 3.50 | 5.50 | 13.59 | 55.00 | 71.00 | 94.39 | 6.50 | 27.88 | 29.50 | 5.75 | 3.14 |
| 4 | 3.50 | 5.50 | 12.30 | 49.50 | 72.50 | 95.21 | 5.50 | 30.38 | 31.50 | 7.30 | 3.40 |
| 5 | 3.00 | 6.50 | 12.57 | 43.00 | 61.50 | 93.36 | 6.50 | 31.75 | 33.58 | 6.20 | 3.30 |
| 6 | 3.00 | 5.00 | 13.66 | 49.50 | 75.50 | 96.36 | 7.50 | 28.75 | 31.75 | 6.58 | 3.40 |
| 7 | 3.00 | 6.50 | 13.30 | 53.50 | 73.00 | 94.96 | 4.00 | 39.08 | 40.75 | 6.28 | 3.65 |
| 8 | 3.00 | 7.50 | 11.54 | 48.00 | 74.50 | 100.00 | 4.50 | 38.50 | 39.80 | 6.45 | 3.03 |
| 9 | 4.00 | 5.50 | 12.54 | 52.50 | 71.00 | 100.00 | 6.50 | 33.50 | 37.15 | 6.20 | 3.55 |
| 10 | 4.00 | 5.00 | 11.91 | 54.00 | 74.00 | 100.00 | 5.50 | 31.58 | 36.50 | 6.10 | 3.23 |
| 11 | 3.00 | 5.00 | 11.50 | 52.00 | 74.50 | 95.48 | 4.00 | 29.75 | 31.25 | 6.15 | 3.15 |
| 12 | 3.00 | 5.00 | 10.64 | 57.50 | 72.00 | 96.01 | 4.50 | 28.75 | 30.75 | 4.80 | 3.03 |
| 13 | 3.00 | 4.00 | 16.46 | 56.00 | 72.00 | 100.00 | 4.50 | 32.65 | 34.00 | 6.30 | 3.55 |
| 14 | 3.00 | 4.00 | 16.94 | 54.00 | 75.50 | 100.00 | 4.50 | 37.75 | 39.38 | 5.63 | 3.03 |
| 15 | 4.00 | 6.50 | 13.32 | 52.00 | 72.50 | 100.00 | 6.50 | 28.63 | 29.10 | 5.40 | 3.23 |
| 16 | 3.00 | 6.50 | 13.28 | 54.00 | 71.50 | 95.28 | 7.50 | 31.85 | 33.25 | 5.78 | 3.20 |
| 17 | 4.00 | 6.50 | 12.31 | 54.00 | 71.50 | 100.00 | 6.00 | 35.00 | 38.00 | 6.88 | 3.43 |
| 18 | 3.00 | 5.00 | 8.57 | 46.00 | 73.00 | 93.38 | 6.00 | 27.50 | 31.00 | 4.93 | 2.98 |
| 19 | 4.00 | 5.00 | 12.84 | 52.50 | 74.50 | 99.37 | 6.50 | 33.53 | 38.28 | 6.03 | 3.20 |
| 20 | 3.00 | 4.00 | 16.18 | 49.50 | 67.00 | 100.00 | 5.50 | 29.75 | 39.50 | 5.25 | 2.95 |
| 21 | 4.00 | 5.00 | 12.14 | 52.00 | 65.00 | 100.00 | 4.50 | 27.55 | 29.75 | 5.08 | 3.00 |
| 22 | 3.00 | 4.00 | 15.50 | 53.50 | 70.00 | 98.14 | 5.00 | 35.45 | 40.88 | 6.33 | 3.20 |
| 23 | 4.00 | 6.50 | 13.98 | 53.50 | 70.50 | 100.00 | 7.00 | 35.75 | 38.90 | 6.45 | 3.45 |
| 24 | 3.00 | 3.00 | 17.54 | 58.50 | 74.50 | 96.50 | 6.50 | 36.50 | 37.13 | 6.53 | 3.23 |

*Contd…..*

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | 3.00 | 6.50 | 12.56 | 52.50 | 75.50 | 94.92 | 5.50 | 32.53 | 38.10 | 6.00 | 3.05 |
| 26 | 3.00 | 6.50 | 13.93 | 55.00 | 74.00 | 94.43 | 4.00 | 36.28 | 38.00 | 7.05 | 2.98 |
| 27 | 3.00 | 4.00 | 17.58 | 50.00 | 73.50 | 98.09 | 6.00 | 38.50 | 39.75 | 6.95 | 3.65 |
| 28 | 3.00 | 6.50 | 10.77 | 53.50 | 72.00 | 94.96 | 4.00 | 30.25 | 31.50 | 6.13 | 2.88 |
| 29 | 3.00 | 6.50 | 12.27 | 53.50 | 76.00 | 98.07 | 7.50 | 31.50 | 33.40 | 5.98 | 3.30 |
| 30 | 4.00 | 4.00 | 16.49 | 49.00 | 67.00 | 100.00 | 6.50 | 34.33 | 37.40 | 6.23 | 3.23 |
| 31 | 4.00 | 3.00 | 15.25 | 51.50 | 72.00 | 95.62 | 8.50 | 36.50 | 38.50 | 6.00 | 3.25 |
| 32 | 3.00 | 6.50 | 12.10 | 57.50 | 74.50 | 100.00 | 7.00 | 31.15 | 32.50 | 6.95 | 2.65 |
| 33 | 3.00 | 6.50 | 10.98 | 48.00 | 75.50 | 96.67 | 6.50 | 28.78 | 30.50 | 5.88 | 3.08 |
| 34 | 3.00 | 5.00 | 10.70 | 47.00 | 74.25 | 91.80 | 4.50 | 34.28 | 39.75 | 6.05 | 3.20 |
| 35 | 3.00 | 5.00 | 13.00 | 51.00 | 63.50 | 93.41 | 5.50 | 37.75 | 40.75 | 6.93 | 3.43 |
| 36 | 3.00 | 4.00 | 15.77 | 56.50 | 71.50 | 93.35 | 7.00 | 30.00 | 33.25 | 6.03 | 2.95 |
| 37 | 3.00 | 4.00 | 15.78 | 51.50 | 71.50 | 93.41 | 4.50 | 23.25 | 25.00 | 5.10 | 2.98 |
| 38 | 3.00 | 6.50 | 10.75 | 53.50 | 70.50 | 94.16 | 4.00 | 39.00 | 39.28 | 6.05 | 3.15 |
| 39 | 4.00 | 6.50 | 11.49 | 59.00 | 71.00 | 98.58 | 6.00 | 37.75 | 41.00 | 7.08 | 2.98 |
| 40 | 3.00 | 7.00 | 13.33 | 45.50 | 65.50 | 98.71 | 6.50 | 36.58 | 37.25 | 6.80 | 2.90 |
| 41 | 3.00 | 6.00 | 11.58 | 57.00 | 62.50 | 98.98 | 6.00 | 34.10 | 35.58 | 5.78 | 3.23 |
| 42 | 3.00 | 6.50 | 13.68 | 55.00 | 74.00 | 95.78 | 4.00 | 30.50 | 33.28 | 6.13 | 3.70 |
| 43 | 3.00 | 3.00 | 15.54 | 53.00 | 75.00 | 100.00 | 5.00 | 29.75 | 36.05 | 5.70 | 2.75 |
| 44 | 3.00 | 6.50 | 15.00 | 49.50 | 61.00 | 99.46 | 6.00 | 40.25 | 40.75 | 7.28 | 3.80 |
| 45 | 3.00 | 5.00 | 13.67 | 53.00 | 70.50 | 95.81 | 7.00 | 34.40 | 36.75 | 6.20 | 3.45 |
| 46 | 3.00 | 3.00 | 12.43 | 57.00 | 66.00 | 95.91 | 9.00 | 31.63 | 35.00 | 5.08 | 3.25 |
| 47 | 3.00 | 5.50 | 11.94 | 49.00 | 65.50 | 100.00 | 5.50 | 32.03 | 34.98 | 5.10 | 3.43 |
| 48 | 3.00 | 6.00 | 11.78 | 50.50 | 66.00 | 100.00 | 7.00 | 35.08 | 40.25 | 6.20 | 3.80 |
| 49 | 3.00 | 4.00 | 16.50 | 55.50 | 68.00 | 94.79 | 7.50 | 32.50 | 37.00 | 6.05 | 3.43 |
| 50 | 3.00 | 6.50 | 13.76 | 41.50 | 75.00 | 97.11 | 5.50 | 35.05 | 39.78 | 6.33 | 3.35 |
| 51 | 4.00 | 6.50 | 11.05 | 50.50 | 75.00 | 95.47 | 8.00 | 38.65 | 41.53 | 7.98 | 3.33 |
| 52 | 3.00 | 4.00 | 15.38 | 45.00 | 76.50 | 96.32 | 5.00 | 39.90 | 40.00 | 6.33 | 3.25 |

*Contd.....*

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 53 | 3.00 | 7.00 | 12.78 | 49.00 | 70.00 | 94.48 | 7.00 | 36.60 | 39.03 | 7.70 | 3.18 |
| 54 | 4.00 | 6.00 | 13.00 | 52.50 | 70.00 | 100.00 | 5.50 | 35.50 | 37.50 | 7.20 | 3.43 |
| 55 | 3.00 | 5.00 | 12.18 | 56.00 | 74.00 | 95.28 | 5.50 | 37.25 | 40.75 | 6.18 | 3.28 |
| 56 | 3.00 | 5.00 | 14.01 | 55.50 | 71.50 | 100.00 | 6.00 | 37.10 | 39.85 | 6.40 | 3.00 |
| 57 | 3.00 | 4.00 | 17.65 | 59.00 | 71.00 | 94.69 | 5.50 | 31.00 | 32.43 | 5.75 | 3.08 |
| 58 | 4.00 | 5.00 | 13.10 | 60.50 | 67.00 | 100.00 | 4.50 | 31.28 | 34.28 | 5.98 | 3.28 |
| 59 | 3.00 | 3.00 | 16.25 | 45.00 | 72.50 | 100.00 | 4.50 | 31.03 | 35.28 | 6.10 | 3.18 |
| 60 | 3.00 | 5.00 | 13.24 | 49.50 | 73.50 | 98.68 | 5.50 | 37.30 | 41.75 | 7.10 | 3.70 |
| 61 | 3.00 | 5.00 | 11.18 | 56.00 | 73.00 | 98.65 | 5.00 | 28.83 | 32.75 | 6.70 | 3.08 |
| 62 | 3.00 | 6.50 | 13.56 | 49.00 | 71.50 | 98.32 | 5.00 | 37.25 | 40.75 | 7.10 | 3.20 |
| 63 | 3.00 | 6.00 | 9.70 | 54.00 | 73.00 | 100.00 | 5.50 | 34.60 | 36.75 | 6.00 | 3.15 |
| 64 | 3.00 | 6.50 | 12.59 | 51.50 | 77.00 | 98.28 | 6.50 | 34.65 | 38.08 | 7.43 | 3.58 |
| 65 | 3.00 | 4.00 | 9.36 | 50.50 | 67.00 | 91.61 | 7.50 | 29.80 | 31.83 | 5.80 | 3.13 |
| 66 | 4.00 | 6.00 | 9.76 | 49.00 | 74.50 | 94.86 | 5.00 | 35.43 | 32.75 | 7.13 | 3.53 |
| 67 | 4.00 | 6.00 | 9.35 | 50.00 | 71.00 | 100.00 | 6.00 | 32.00 | 38.00 | 5.95 | 3.05 |
| 68 | 4.00 | 6.00 | 10.45 | 53.00 | 74.50 | 100.00 | 7.50 | 28.38 | 32.40 | 5.63 | 3.40 |
| 69 | 3.00 | 6.00 | 13.00 | 56.00 | 74.25 | 100.00 | 5.50 | 28.03 | 30.45 | 5.95 | 3.13 |
| 70 | 3.00 | 5.00 | 13.78 | 56.00 | 75.50 | 100.00 | 6.00 | 40.05 | 41.38 | 6.55 | 3.28 |
| 71 | 3.00 | 6.00 | 12.83 | 54.00 | 78.00 | 98.39 | 7.50 | 36.35 | 42.13 | 7.05 | 3.50 |
| 72 | 3.00 | 7.00 | 10.35 | 53.50 | 73.50 | 93.10 | 7.00 | 31.68 | 33.25 | 6.20 | 2.90 |
| 73 | 3.00 | 5.00 | 11.06 | 59.50 | 74.00 | 100.00 | 7.00 | 40.05 | 40.60 | 5.50 | 3.05 |
| 74 | 4.00 | 6.50 | 12.33 | 59.50 | 75.00 | 95.40 | 6.00 | 30.75 | 35.90 | 7.60 | 3.55 |
| 75 | 4.00 | 5.00 | 14.06 | 52.50 | 78.00 | 98.25 | 4.00 | 40.50 | 44.00 | 6.53 | 3.60 |
| 76 | 3.00 | 5.00 | 12.46 | 49.00 | 70.00 | 96.03 | 5.50 | 42.53 | 46.25 | 7.33 | 2.90 |
| 77 | 3.00 | 4.00 | 17.92 | 60.00 | 71.00 | 100.00 | 4.50 | 35.43 | 37.60 | 6.15 | 3.50 |
| 78 | 3.00 | 6.50 | 13.96 | 57.00 | 72.00 | 98.15 | 6.00 | 36.03 | 38.25 | 6.28 | 3.55 |
| 79 | 3.00 | 6.00 | 9.80 | 58.50 | 68.00 | 96.17 | 5.00 | 28.90 | 32.25 | 6.33 | 3.33 |
| 80 | 3.00 | 6.50 | 11.09 | 62.00 | 75.50 | 100.00 | 7.00 | 33.78 | 35.78 | 5.63 | 3.10 |

*Contd…..*

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 81 | 3.00 | 4.00 | 17.32 | 60.00 | 77.50 | 98.94 | 5.50 | 32.95 | 35.88 | 6.53 | 3.20 |
| 82 | 3.00 | 6.00 | 12.72 | 49.00 | 71.50 | 94.59 | 4.00 | 38.83 | 39.33 | 5.95 | 2.95 |
| 83 | 3.00 | 7.00 | 12.55 | 54.50 | 75.00 | 98.61 | 4.00 | 34.70 | 38.95 | 6.68 | 3.00 |
| 84 | 3.00 | 6.00 | 12.11 | 48.50 | 72.50 | 96.14 | 6.00 | 38.18 | 42.38 | 7.73 | 3.75 |
| 85 | 3.00 | 6.50 | 11.50 | 55.00 | 70.50 | 94.34 | 5.00 | 37.00 | 41.03 | 7.23 | 3.80 |
| 86 | 3.00 | 7.00 | 11.92 | 49.00 | 71.00 | 100.00 | 6.00 | 31.90 | 36.28 | 5.85 | 3.45 |
| 87 | 3.00 | 7.00 | 14.48 | 54.00 | 76.00 | 98.45 | 6.00 | 36.53 | 38.25 | 5.73 | 3.35 |
| 88 | 3.00 | 5.00 | 13.75 | 47.50 | 75.50 | 92.13 | 5.50 | 31.68 | 35.25 | 6.90 | 3.78 |
| 89 | 3.00 | 6.00 | 13.86 | 50.50 | 76.00 | 95.36 | 6.00 | 36.70 | 42.00 | 7.45 | 3.83 |
| 90 | 3.00 | 4.00 | 16.55 | 50.50 | 76.50 | 96.54 | 4.00 | 33.90 | 37.78 | 6.80 | 3.18 |
| 91 | 3.50 | 4.00 | 14.39 | 56.00 | 61.00 | 100.00 | 6.50 | 31.85 | 37.25 | 6.00 | 3.15 |
| 92 | 4.00 | 4.00 | 16.75 | 55.00 | 75.50 | 92.63 | 5.00 | 39.05 | 40.50 | 6.10 | 3.95 |
| 93 | 4.00 | 7.00 | 13.94 | 51.50 | 76.00 | 93.35 | 4.00 | 39.10 | 41.83 | 7.80 | 3.63 |
| 94 | 4.00 | 7.00 | 13.30 | 48.50 | 61.00 | 98.25 | 4.50 | 39.23 | 40.75 | 6.83 | 3.68 |
| 95 | 4.00 | 6.00 | 12.38 | 51.00 | 72.50 | 100.00 | 6.00 | 36.75 | 38.00 | 7.15 | 3.35 |
| 96 | 4.00 | 5.50 | 13.48 | 48.00 | 73.00 | 98.17 | 4.50 | 38.18 | 41.50 | 6.15 | 3.25 |
| 97 | 4.00 | 7.00 | 9.35 | 49.00 | 73.50 | 100.00 | 6.00 | 38.00 | 39.58 | 5.65 | 3.13 |
| 98 | 4.00 | 6.00 | 11.72 | 48.50 | 70.50 | 100.00 | 5.50 | 38.65 | 40.95 | 6.78 | 3.35 |
| 99 | 3.00 | 6.00 | 14.67 | 50.00 | 75.50 | 96.66 | 6.00 | 38.40 | 40.00 | 7.85 | 3.45 |
| 100 | 3.00 | 4.00 | 8.38 | 51.50 | 62.50 | 100.00 | 6.00 | 38.05 | 43.58 | 6.65 | 3.30 |
| 101 | 3.00 | 7.00 | 13.91 | 53.50 | 76.50 | 94.77 | 6.50 | 39.03 | 45.98 | 6.55 | 3.35 |
| 102 | 3.00 | 6.00 | 12.07 | 53.50 | 78.00 | 94.79 | 5.50 | 38.33 | 41.08 | 5.95 | 3.10 |
| 103 | 3.00 | 6.00 | 12.01 | 52.50 | 71.00 | 93.18 | 6.50 | 36.63 | 40.38 | 5.78 | 3.18 |
| 104 | 4.00 | 6.00 | 13.46 | 51.50 | 73.00 | 100.00 | 6.50 | 37.68 | 43.55 | 7.20 | 3.65 |
| 105 | 3.00 | 6.00 | 13.54 | 60.50 | 74.00 | 98.39 | 6.50 | 35.73 | 37.83 | 5.85 | 3.18 |
| 106 | 4.00 | 7.00 | 13.61 | 47.50 | 65.00 | 95.40 | 7.50 | 37.68 | 42.75 | 6.50 | 3.50 |

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

**Appendix III: Mean performance of $BC_1F_4$ lines from JL24 × ICGV 86699 for disease response, agronomic traits and productivity traits**

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| JL 24 | 4.00 | 6.75 | 12.50 | 55.00 | 72.50 | 93.53 | 5.75 | 36.55 | 40.08 | 6.25 | 3.35 |
| ICGV 86699 | 3.00 | 3.00 | 12.50 | 42.00 | 67.50 | 94.49 | 8.50 | 40.83 | 43.95 | 4.58 | 2.58 |
| 1 | 3.50 | 5.50 | 11.77 | 45.00 | 72.50 | 90.58 | 4.25 | 30.03 | 33.08 | 5.35 | 3.15 |
| 2 | 3.00 | 4.00 | 11.73 | 45.00 | 73.00 | 100.00 | 4.25 | 37.50 | 39.33 | 5.50 | 3.15 |
| 3 | 3.00 | 5.25 | 13.67 | 46.00 | 74.00 | 98.42 | 4.50 | 41.08 | 44.43 | 5.98 | 3.53 |
| 4 | 3.50 | 4.50 | 16.70 | 51.00 | 78.00 | 93.96 | 4.50 | 40.65 | 47.55 | 6.83 | 3.50 |
| 5 | 4.00 | 6.75 | 12.35 | 49.50 | 75.25 | 100.00 | 7.00 | 33.33 | 37.38 | 5.90 | 2.80 |
| 6 | 3.00 | 3.00 | 11.51 | 45.00 | 77.50 | 98.35 | 5.75 | 30.13 | 36.65 | 5.08 | 2.98 |
| 7 | 4.00 | 5.00 | 13.43 | 37.00 | 69.50 | 98.55 | 5.50 | 41.85 | 46.65 | 5.48 | 3.10 |
| 8 | 4.00 | 6.75 | 9.06 | 48.00 | 58.50 | 100.00 | 6.00 | 45.53 | 49.70 | 6.30 | 3.68 |
| 9 | 3.00 | 6.50 | 13.18 | 50.00 | 73.00 | 96.17 | 5.00 | 33.53 | 36.40 | 4.75 | 2.83 |
| 10 | 3.50 | 4.00 | 16.57 | 60.00 | 76.50 | 100.00 | 5.75 | 43.03 | 47.58 | 5.70 | 3.35 |
| 11 | 4.00 | 6.50 | 13.12 | 46.00 | 77.50 | 98.42 | 6.50 | 32.58 | 44.75 | 5.08 | 3.18 |
| 12 | 3.00 | 3.00 | 11.91 | 42.00 | 72.00 | 98.44 | 6.00 | 51.08 | 58.00 | 6.13 | 3.78 |
| 13 | 3.00 | 5.00 | 13.26 | 52.50 | 72.50 | 100.00 | 6.25 | 26.60 | 34.28 | 6.93 | 3.65 |
| 14 | 3.00 | 4.00 | 16.75 | 57.00 | 70.00 | 100.00 | 6.75 | 28.75 | 40.15 | 6.88 | 3.23 |
| 15 | 3.00 | 4.00 | 12.91 | 54.00 | 69.75 | 98.46 | 6.50 | 36.18 | 40.03 | 5.80 | 3.10 |
| 16 | 3.00 | 4.00 | 16.79 | 53.50 | 73.50 | 93.13 | 5.50 | 40.83 | 45.63 | 5.93 | 3.63 |
| 17 | 3.00 | 5.00 | 14.69 | 54.00 | 75.50 | 98.50 | 5.50 | 39.75 | 43.18 | 6.83 | 3.75 |
| 18 | 3.00 | 4.00 | 13.32 | 42.00 | 77.00 | 91.68 | 4.25 | 33.03 | 34.18 | 5.70 | 3.03 |
| 19 | 3.00 | 3.00 | 15.58 | 47.00 | 75.50 | 97.06 | 7.00 | 28.33 | 33.45 | 6.90 | 3.08 |
| 20 | 4.00 | 6.50 | 12.50 | 39.00 | 35.25 | 100.00 | 5.75 | 41.78 | 43.13 | 5.55 | 3.13 |
| 21 | 4.00 | 7.00 | 12.42 | 60.00 | 75.50 | 93.24 | 5.75 | 35.18 | 39.25 | 5.50 | 3.10 |
| 22 | 4.00 | 5.00 | 13.41 | 53.50 | 75.50 | 98.36 | 7.50 | 32.23 | 45.40 | 5.33 | 3.10 |
| 23 | 4.00 | 6.00 | 12.65 | 56.50 | 71.00 | 98.43 | 5.50 | 38.50 | 41.00 | 5.88 | 4.08 |
| 24 | 3.00 | 4.00 | 17.32 | 55.50 | 76.25 | 100.00 | 6.25 | 30.58 | 33.38 | 5.68 | 3.45 |

*Contd.....*

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | 4.00 | 6.50 | 12.34 | 52.50 | 43.50 | 92.87 | 5.75 | 35.60 | 38.15 | 6.13 | 2.90 |
| 26 | 4.00 | 5.00 | 13.40 | 47.25 | 40.00 | 92.71 | 6.50 | 40.08 | 47.25 | 5.78 | 3.13 |
| 27 | 3.00 | 7.00 | 15.36 | 54.50 | 75.50 | 95.13 | 4.25 | 43.55 | 49.25 | 6.85 | 3.90 |
| 28 | 3.00 | 7.00 | 13.35 | 53.00 | 76.00 | 98.21 | 5.75 | 56.18 | 59.10 | 5.83 | 3.53 |
| 29 | 4.00 | 6.50 | 11.39 | 52.50 | 70.50 | 98.49 | 7.75 | 40.30 | 44.25 | 6.55 | 4.15 |
| 30 | 4.00 | 6.50 | 13.82 | 53.00 | 76.50 | 96.45 | 4.25 | 41.20 | 44.00 | 5.28 | 2.90 |
| 31 | 4.00 | 6.00 | 11.69 | 50.00 | 73.00 | 93.53 | 4.25 | 37.35 | 40.75 | 5.95 | 3.30 |
| 32 | 4.00 | 5.00 | 15.28 | 41.00 | 55.00 | 94.12 | 4.25 | 40.38 | 42.70 | 5.60 | 3.50 |
| 33 | 4.00 | 7.00 | 12.93 | 50.00 | 39.00 | 92.97 | 5.75 | 39.28 | 43.25 | 6.65 | 3.80 |
| 34 | 4.00 | 5.00 | 13.25 | 47.50 | 76.00 | 100.00 | 6.50 | 36.73 | 39.38 | 5.75 | 3.48 |
| 35 | 4.00 | 6.00 | 14.17 | 56.00 | 78.00 | 95.48 | 6.75 | 26.03 | 33.45 | 5.68 | 3.08 |
| 36 | 3.00 | 4.00 | 11.77 | 43.50 | 73.50 | 97.17 | 5.50 | 39.35 | 43.13 | 6.33 | 4.00 |
| 37 | 3.00 | 3.00 | 10.50 | 36.25 | 37.50 | 94.39 | 7.50 | 36.00 | 41.63 | 4.90 | 3.20 |
| 38 | 4.00 | 7.00 | 11.97 | 55.50 | 70.50 | 92.56 | 7.00 | 37.75 | 42.03 | 5.93 | 3.58 |
| 39 | 4.00 | 6.00 | 13.07 | 54.00 | 71.00 | 98.14 | 6.00 | 51.10 | 53.65 | 5.20 | 2.75 |
| 40 | 4.00 | 5.00 | 15.33 | 56.50 | 72.75 | 96.21 | 6.25 | 35.05 | 39.08 | 7.03 | 4.00 |
| 41 | 3.00 | 3.00 | 11.21 | 40.50 | 60.50 | 95.99 | 5.75 | 47.75 | 50.40 | 6.55 | 3.38 |
| 42 | 4.00 | 5.00 | 11.55 | 53.00 | 74.00 | 100.00 | 5.00 | 42.75 | 56.08 | 5.90 | 3.83 |
| 43 | 4.00 | 6.00 | 15.21 | 48.00 | 75.50 | 100.00 | 5.75 | 31.45 | 34.58 | 5.60 | 3.05 |
| 44 | 4.00 | 5.00 | 12.96 | 54.50 | 75.00 | 100.00 | 5.75 | 35.20 | 36.40 | 5.70 | 2.70 |
| 45 | 4.00 | 5.00 | 13.38 | 45.50 | 61.50 | 100.00 | 4.50 | 36.55 | 40.15 | 6.10 | 4.05 |
| 46 | 3.00 | 4.00 | 17.69 | 50.50 | 76.50 | 98.41 | 7.00 | 37.03 | 39.83 | 4.80 | 3.58 |
| 47 | 4.00 | 5.00 | 15.14 | 64.50 | 75.50 | 95.59 | 4.25 | 41.25 | 45.90 | 4.93 | 2.88 |
| 48 | 3.00 | 7.00 | 12.88 | 57.50 | 76.00 | 98.31 | 5.50 | 36.00 | 39.23 | 5.43 | 3.15 |
| 49 | 3.00 | 5.00 | 14.26 | 50.00 | 76.00 | 98.49 | 5.50 | 34.50 | 39.50 | 5.23 | 3.08 |
| 50 | 4.00 | 6.00 | 14.14 | 67.50 | 75.50 | 100.00 | 5.75 | 33.70 | 36.40 | 5.70 | 3.35 |
| 51 | 3.00 | 4.00 | 17.55 | 53.00 | 71.50 | 94.78 | 5.00 | 31.95 | 33.88 | 5.95 | 3.23 |
| 52 | 3.00 | 5.00 | 13.39 | 57.50 | 74.50 | 100.00 | 5.00 | 32.90 | 35.75 | 4.55 | 2.65 |

*Contd…..*

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 53 | 3.00 | 6.00 | 11.38 | 43.75 | 49.00 | 92.70 | 6.00 | 41.45 | 43.25 | 5.28 | 3.20 |
| 54 | 4.00 | 6.00 | 12.01 | 55.00 | 68.50 | 98.35 | 4.00 | 39.53 | 44.25 | 6.30 | 3.70 |
| 55 | 4.00 | 6.00 | 12.43 | 53.00 | 76.25 | 98.24 | 5.00 | 43.75 | 49.00 | 6.00 | 4.15 |
| 56 | 4.00 | 6.00 | 13.56 | 56.00 | 74.50 | 100.00 | 6.25 | 38.65 | 45.10 | 6.43 | 3.28 |
| 57 | 3.00 | 4.00 | 17.23 | 59.00 | 76.00 | 94.86 | 6.25 | 36.55 | 42.13 | 6.50 | 3.38 |
| 58 | 3.00 | 4.00 | 17.52 | 53.50 | 73.50 | 99.22 | 7.25 | 33.68 | 36.63 | 5.38 | 2.65 |
| 59 | 4.00 | 6.00 | 13.87 | 55.00 | 73.50 | 98.61 | 5.75 | 37.83 | 44.30 | 6.88 | 3.05 |
| 60 | 3.00 | 5.00 | 11.62 | 58.00 | 66.00 | 98.76 | 4.50 | 27.08 | 30.13 | 5.50 | 3.10 |
| 61 | 3.00 | 4.00 | 17.17 | 49.00 | 67.00 | 97.38 | 5.75 | 31.33 | 32.55 | 6.08 | 3.13 |
| 62 | 3.00 | 5.00 | 11.11 | 50.00 | 77.50 | 98.77 | 4.25 | 38.03 | 42.13 | 6.13 | 3.28 |
| 63 | 3.00 | 3.00 | 17.21 | 48.00 | 77.00 | 100.00 | 7.00 | 35.10 | 38.93 | 5.03 | 2.95 |
| 64 | 4.00 | 7.00 | 11.85 | 50.50 | 62.00 | 90.99 | 6.25 | 36.03 | 37.25 | 5.33 | 2.75 |
| 65 | 4.00 | 6.00 | 13.13 | 43.50 | 72.00 | 98.08 | 8.50 | 39.93 | 41.88 | 5.55 | 2.98 |
| 66 | 3.00 | 4.00 | 17.27 | 47.00 | 70.50 | 91.77 | 5.75 | 39.43 | 46.25 | 5.08 | 2.60 |
| 67 | 4.00 | 5.00 | 12.00 | 41.00 | 74.00 | 100.00 | 5.00 | 29.08 | 30.63 | 4.98 | 3.03 |
| 68 | 4.00 | 5.00 | 12.90 | 52.50 | 70.00 | 95.79 | 4.25 | 33.25 | 35.43 | 5.23 | 3.13 |
| 69 | 3.00 | 4.00 | 12.40 | 45.00 | 48.00 | 95.44 | 5.50 | 27.53 | 31.05 | 4.90 | 2.85 |
| 70 | 3.00 | 4.00 | 17.30 | 57.50 | 77.00 | 95.30 | 5.50 | 33.25 | 37.95 | 5.20 | 3.10 |
| 71 | 4.00 | 6.00 | 12.78 | 51.50 | 77.50 | 93.94 | 6.75 | 36.53 | 40.25 | 6.65 | 3.43 |
| 72 | 4.00 | 6.00 | 13.40 | 39.00 | 47.00 | 93.58 | 6.50 | 36.88 | 38.38 | 6.70 | 3.10 |
| 73 | 4.00 | 5.00 | 13.05 | 42.50 | 71.50 | 97.06 | 4.50 | 39.38 | 40.88 | 5.73 | 3.08 |
| 74 | 4.00 | 5.00 | 11.43 | 43.75 | 56.00 | 100.00 | 5.75 | 33.55 | 40.00 | 5.50 | 2.80 |
| 75 | 3.00 | 6.00 | 14.04 | 55.00 | 76.00 | 96.52 | 5.25 | 35.53 | 36.63 | 6.78 | 3.25 |
| 76 | 3.00 | 5.00 | 10.47 | 44.50 | 46.50 | 97.13 | 4.25 | 30.75 | 38.00 | 5.95 | 3.23 |
| 77 | 3.00 | 4.00 | 17.86 | 55.50 | 75.50 | 97.51 | 4.25 | 36.65 | 39.13 | 7.08 | 3.48 |
| 78 | 3.00 | 4.00 | 12.08 | 36.00 | 48.00 | 94.50 | 4.25 | 40.43 | 42.25 | 7.03 | 3.93 |
| 79 | 3.00 | 6.00 | 14.03 | 44.50 | 76.50 | 95.25 | 6.50 | 44.50 | 45.75 | 6.85 | 3.80 |
| 80 | 3.00 | 4.00 | 17.59 | 46.00 | 71.00 | 96.77 | 6.25 | 30.00 | 33.75 | 6.30 | 3.08 |
| 81 | 3.00 | 3.00 | 11.30 | 43.00 | 39.00 | 88.95 | 4.75 | 36.53 | 42.75 | 5.63 | 3.13 |
| 82 | 3.00 | 4.00 | 13.31 | 45.00 | 66.50 | 92.16 | 5.75 | 41.63 | 45.75 | 6.05 | 3.75 |

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

**Appendix IV: Mean performance of $BC_1F_4$ lines from JL24 × ICGV 99005 for disease response, agronomic traits and productivity traits**

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| JL 24 | 3.50 | 6.50 | 12.59 | 52.50 | 73.00 | 98.20 | 5.00 | 28.10 | 30.88 | 6.58 | 2.68 |
| ICGV 99005 | 3.50 | 3.00 | 12.06 | 49.50 | 63.50 | 93.87 | 5.50 | 31.28 | 32.70 | 6.18 | 2.63 |
| 1 | 3.50 | 6.50 | 11.78 | 46.00 | 57.50 | 100.00 | 5.50 | 31.68 | 36.75 | 6.95 | 3.05 |
| 2 | 4.00 | 4.50 | 12.96 | 46.00 | 76.00 | 91.34 | 5.50 | 37.45 | 41.70 | 6.60 | 3.40 |
| 3 | 3.00 | 4.50 | 11.21 | 52.00 | 75.00 | 93.25 | 5.75 | 28.70 | 31.88 | 5.50 | 3.25 |
| 4 | 3.00 | 5.00 | 10.85 | 52.50 | 76.50 | 94.04 | 5.25 | 27.75 | 29.68 | 5.28 | 3.40 |
| 5 | 3.00 | 4.00 | 15.89 | 55.25 | 67.50 | 100.00 | 5.25 | 24.53 | 26.65 | 7.10 | 3.03 |
| 6 | 3.00 | 4.00 | 8.15 | 45.50 | 55.50 | 100.00 | 5.50 | 20.18 | 23.48 | 5.30 | 2.55 |
| 7 | 3.00 | 4.00 | 16.30 | 57.50 | 63.00 | 100.00 | 4.50 | 26.10 | 29.40 | 4.83 | 3.05 |
| 8 | 3.00 | 6.50 | 10.56 | 50.50 | 72.50 | 98.60 | 6.50 | 25.48 | 27.05 | 6.33 | 3.03 |
| 9 | 3.00 | 5.00 | 12.07 | 56.25 | 65.25 | 92.30 | 4.00 | 30.58 | 31.10 | 5.85 | 3.25 |
| 10 | 3.00 | 4.00 | 15.43 | 56.50 | 76.50 | 100.00 | 4.00 | 26.18 | 28.50 | 6.00 | 3.03 |
| 11 | 3.00 | 4.00 | 16.23 | 52.50 | 76.00 | 91.75 | 4.50 | 31.38 | 35.25 | 7.15 | 2.83 |
| 12 | 4.00 | 5.00 | 9.86 | 50.00 | 67.00 | 100.00 | 6.50 | 34.28 | 36.90 | 5.98 | 3.50 |
| 13 | 4.00 | 6.50 | 14.10 | 56.50 | 74.50 | 100.00 | 4.75 | 33.70 | 37.93 | 6.90 | 2.88 |
| 14 | 3.00 | 5.00 | 9.08 | 52.00 | 72.00 | 100.00 | 5.75 | 23.40 | 26.55 | 6.05 | 2.98 |
| 15 | 4.00 | 6.50 | 12.72 | 47.00 | 69.50 | 100.00 | 4.25 | 34.95 | 40.88 | 7.18 | 3.80 |
| 16 | 4.00 | 6.50 | 9.30 | 53.00 | 66.50 | 100.00 | 5.75 | 31.40 | 33.73 | 5.48 | 3.28 |
| 17 | 3.00 | 4.00 | 16.69 | 56.00 | 76.00 | 100.00 | 5.50 | 30.63 | 32.25 | 6.55 | 3.45 |
| 18 | 4.00 | 5.00 | 12.50 | 55.00 | 74.00 | 92.67 | 4.00 | 28.38 | 30.90 | 6.53 | 3.08 |
| 19 | 3.00 | 4.00 | 17.64 | 60.50 | 77.00 | 100.00 | 5.00 | 22.50 | 24.83 | 6.03 | 3.08 |
| 20 | 3.00 | 4.00 | 18.66 | 59.00 | 77.75 | 98.15 | 4.50 | 24.45 | 25.63 | 6.35 | 3.18 |
| 21 | 3.00 | 6.50 | 11.84 | 53.50 | 51.50 | 92.84 | 5.00 | 30.83 | 34.58 | 6.40 | 3.18 |
| 22 | 3.00 | 6.50 | 13.40 | 59.50 | 77.50 | 100.00 | 6.25 | 28.33 | 29.50 | 5.78 | 3.38 |
| 23 | 3.00 | 6.50 | 14.07 | 53.00 | 64.75 | 98.29 | 6.75 | 33.20 | 35.90 | 6.55 | 2.77 |
| 24 | 3.00 | 6.50 | 11.00 | 52.50 | 75.50 | 100.00 | 4.50 | 27.88 | 35.70 | 5.88 | 3.15 |
| 25 | 3.00 | 6.50 | 13.20 | 47.50 | 76.75 | 98.44 | 5.75 | 40.58 | 41.75 | 7.80 | 3.98 |
| 26 | 4.00 | 6.50 | 12.14 | 47.50 | 76.00 | 98.49 | 4.75 | 35.00 | 37.40 | 6.40 | 3.13 |

*Contd…..*

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | NPB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 27 | 4.00 | 6.50 | 11.70 | 57.50 | 79.00 | 94.24 | 5.50 | 41.70 | 41.95 | 7.50 | 4.00 |
| 28 | 4.00 | 6.50 | 11.58 | 48.00 | 76.00 | 98.25 | 4.75 | 39.33 | 41.50 | 7.28 | 4.08 |
| 29 | 4.00 | 6.50 | 12.41 | 47.00 | 78.50 | 94.69 | 4.50 | 42.33 | 49.23 | 6.98 | 3.55 |
| 30 | 4.00 | 4.50 | 12.55 | 46.50 | 75.00 | 98.06 | 4.50 | 42.00 | 48.25 | 6.55 | 3.80 |
| 31 | 3.00 | 6.50 | 14.30 | 54.00 | 73.50 | 100.00 | 6.00 | 33.00 | 39.25 | 6.05 | 3.13 |
| 32 | 3.00 | 6.50 | 13.17 | 60.50 | 71.00 | 93.44 | 6.00 | 36.75 | 39.10 | 6.75 | 3.90 |
| 33 | 3.00 | 5.00 | 11.75 | 56.25 | 78.00 | 95.33 | 7.50 | 31.55 | 36.25 | 6.58 | 3.35 |
| 34 | 3.00 | 5.00 | 13.93 | 50.50 | 71.00 | 95.73 | 7.00 | 30.83 | 35.25 | 6.83 | 3.50 |
| 35 | 3.00 | 5.00 | 10.21 | 56.00 | 76.50 | 98.59 | 6.50 | 29.98 | 33.50 | 6.03 | 3.98 |
| 36 | 3.00 | 5.00 | 13.80 | 54.00 | 57.50 | 100.00 | 4.75 | 30.25 | 31.25 | 7.03 | 3.45 |
| 37 | 3.00 | 6.50 | 14.30 | 56.00 | 66.00 | 94.73 | 5.50 | 28.03 | 29.25 | 6.05 | 3.13 |
| 38 | 3.00 | 5.00 | 11.99 | 51.50 | 76.00 | 95.74 | 6.00 | 26.95 | 31.83 | 6.20 | 3.25 |
| 39 | 3.00 | 5.00 | 11.25 | 56.00 | 63.00 | 100.00 | 6.50 | 36.75 | 39.00 | 6.03 | 3.30 |
| 40 | 3.00 | 5.00 | 12.28 | 56.75 | 73.50 | 100.00 | 5.00 | 32.40 | 36.03 | 7.00 | 4.00 |
| 41 | 4.00 | 6.50 | 12.57 | 51.00 | 74.50 | 89.95 | 4.00 | 38.28 | 41.50 | 6.93 | 4.03 |
| 42 | 4.00 | 6.00 | 12.30 | 51.00 | 76.50 | 100.00 | 5.00 | 30.55 | 32.00 | 5.43 | 3.05 |
| 43 | 3.00 | 7.00 | 11.77 | 53.50 | 78.00 | 100.00 | 5.50 | 35.15 | 35.50 | 7.05 | 2.48 |
| 44 | 3.00 | 7.00 | 9.84 | 63.50 | 69.00 | 100.00 | 4.50 | 32.25 | 33.55 | 6.00 | 2.78 |
| 45 | 3.00 | 7.00 | 12.18 | 57.00 | 73.50 | 98.14 | 5.75 | 30.75 | 29.58 | 5.85 | 3.25 |
| 46 | 3.00 | 4.00 | 16.13 | 53.50 | 77.00 | 95.95 | 4.25 | 21.40 | 26.53 | 6.85 | 3.43 |
| 47 | 3.00 | 4.00 | 11.92 | 46.50 | 77.50 | 93.77 | 4.50 | 26.35 | 30.00 | 6.30 | 3.00 |
| 48 | 4.00 | 7.00 | 12.94 | 45.50 | 73.50 | 100.00 | 4.50 | 25.38 | 27.90 | 6.15 | 3.15 |
| 49 | 3.00 | 7.00 | 12.85 | 58.50 | 76.00 | 100.00 | 6.25 | 36.38 | 44.08 | 6.38 | 3.43 |
| 50 | 3.00 | 4.00 | 10.15 | 48.50 | 40.00 | 93.77 | 5.25 | 25.48 | 26.75 | 6.23 | 2.93 |
| 51 | 3.00 | 4.00 | 15.15 | 50.00 | 54.00 | 100.00 | 5.25 | 24.88 | 29.20 | 6.03 | 3.15 |
| 52 | 3.00 | 4.00 | 16.42 | 52.50 | 77.00 | 98.18 | 5.25 | 29.58 | 35.00 | 6.88 | 3.85 |
| 53 | 3.00 | 6.00 | 7.41 | 53.50 | 55.00 | 90.52 | 5.25 | 25.78 | 27.63 | 5.25 | 3.15 |
| 54 | 3.00 | 5.00 | 14.82 | 55.00 | 74.50 | 94.22 | 5.25 | 26.25 | 32.53 | 6.08 | 2.98 |
| 55 | 3.00 | 7.00 | 13.61 | 53.50 | 77.00 | 93.59 | 4.75 | 31.23 | 35.78 | 5.83 | 3.03 |
| 56 | 3.00 | 7.00 | 14.30 | 45.00 | 72.50 | 98.49 | 5.75 | 40.93 | 42.55 | 6.75 | 3.68 |

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

**Appendix V: Mean performance of $BC_2F_3$ from JL24 × GPBD for disease response, agronomic traits and productivity traits**

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | PB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| JL 24 | 3.00 | 6.00 | 12.44 | 36.16 | 34.25 | 94.25 | 6.00 | 25.30 | 38.50 | 5.20 | 3.20 |
| GPBD 4 | 3.50 | 3.00 | 12.21 | 33.43 | 68.78 | 98.70 | 4.75 | 29.50 | 38.50 | 5.20 | 3.15 |
| 1 | 3.00 | 3.50 | 15.54 | 35.71 | 41.27 | 91.52 | 4.75 | 32.00 | 30.60 | 5.35 | 2.65 |
| 2 | 3.00 | 7.00 | 11.84 | 37.93 | 32.23 | 73.02 | 4.75 | 24.65 | 27.55 | 5.05 | 2.65 |
| 3 | 3.00 | 6.50 | 12.54 | 35.33 | 51.40 | 93.75 | 4.00 | 32.50 | 42.00 | 9.30 | 4.15 |
| 4 | 3.00 | 5.00 | 13.55 | 35.10 | 69.00 | 95.03 | 5.50 | 42.50 | 41.50 | 7.55 | 3.85 |
| 5 | 3.00 | 6.50 | 11.45 | 38.34 | 51.71 | 83.47 | 5.50 | 30.00 | 32.00 | 6.30 | 3.55 |
| 6 | 3.00 | 7.00 | 13.20 | 38.27 | 60.40 | 84.83 | 5.50 | 32.50 | 38.50 | 7.20 | 4.25 |
| 7 | 3.00 | 6.50 | 11.92 | 41.40 | 54.40 | 96.49 | 4.50 | 30.65 | 33.50 | 6.00 | 2.85 |
| 8 | 3.00 | 7.00 | 13.11 | 35.75 | 58.09 | 91.69 | 6.00 | 30.40 | 40.50 | 3.50 | 2.05 |
| 9 | 4.00 | 6.50 | 13.76 | 49.50 | 59.34 | 97.22 | 5.00 | 35.55 | 34.95 | 7.95 | 3.65 |
| 10 | 3.00 | 7.00 | 12.04 | 37.92 | 33.42 | 95.45 | 5.50 | 35.20 | 33.00 | 5.70 | 3.25 |
| 11 | 3.00 | 6.00 | 10.63 | 38.26 | 64.09 | 91.25 | 4.50 | 28.65 | 30.50 | 6.05 | 2.85 |
| 12 | 4.00 | 7.00 | 11.93 | 43.73 | 62.40 | 95.90 | 4.75 | 32.65 | 33.00 | 6.20 | 3.30 |
| 13 | 4.00 | 4.00 | 16.21 | 36.25 | 69.50 | 93.14 | 4.00 | 34.50 | 33.90 | 5.05 | 2.65 |
| 14 | 3.00 | 4.00 | 16.28 | 42.19 | 59.20 | 92.99 | 5.25 | 24.85 | 29.90 | 7.10 | 3.50 |
| 15 | 3.00 | 4.00 | 9.39 | 33.50 | 29.50 | 87.23 | 4.00 | 22.30 | 26.50 | 7.70 | 3.35 |
| 16 | 3.00 | 5.00 | 14.32 | 42.73 | 63.50 | 94.09 | 5.75 | 33.50 | 42.00 | 6.30 | 3.95 |
| 17 | 3.00 | 6.00 | 11.74 | 39.14 | 56.10 | 94.37 | 6.75 | 29.95 | 32.00 | 6.15 | 2.50 |
| 18 | 3.00 | 7.00 | 12.25 | 39.51 | 50.77 | 96.67 | 4.00 | 30.00 | 42.65 | 6.80 | 3.45 |
| 19 | 3.00 | 4.00 | 13.25 | 32.11 | 67.50 | 94.47 | 4.50 | 22.65 | 26.15 | 4.30 | 2.35 |
| 20 | 4.00 | 4.00 | 12.32 | 49.22 | 70.61 | 96.07 | 5.50 | 30.15 | 31.50 | 4.45 | 2.65 |

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

**Appendix VI: Mean performance of $BC_2F_3$ from JL24 × ICGV 86699 for disease response, agronomic traits and productivity traits**

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | PB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| JL 24 | 4.00 | 3.00 | 12.77 | 43.50 | 55.25 | 95.00 | 6.00 | 25.95 | 29.63 | 7.08 | 3.65 |
| ICGV 86699 | 3.00 | 3.00 | 12.32 | 42.50 | 65.00 | 98.70 | 7.75 | 36.33 | 40.75 | 4.90 | 2.33 |
| 1 | 3.00 | 3.00 | 16.61 | 49.50 | 72.00 | 94.28 | 5.25 | 36.35 | 41.03 | 7.45 | 3.48 |
| 2 | 4.00 | 3.00 | 12.70 | 38.50 | 52.25 | 85.17 | 5.50 | 33.00 | 39.63 | 5.75 | 3.20 |
| 3 | 3.00 | 3.00 | 11.53 | 39.00 | 68.00 | 92.87 | 4.25 | 33.03 | 36.55 | 6.25 | 3.18 |
| 4 | 3.00 | 3.00 | 12.22 | 36.75 | 57.50 | 100.00 | 6.00 | 42.15 | 48.70 | 6.73 | 3.48 |
| 5 | 3.00 | 3.00 | 11.99 | 38.00 | 61.50 | 94.27 | 5.25 | 39.95 | 42.65 | 6.53 | 3.53 |
| 6 | 3.00 | 3.00 | 12.69 | 40.50 | 53.50 | 88.82 | 6.25 | 32.58 | 40.50 | 6.90 | 3.38 |
| 7 | 3.00 | 3.00 | 11.67 | 39.00 | 62.00 | 94.42 | 5.25 | 35.78 | 41.13 | 6.25 | 3.33 |
| 8 | 3.00 | 3.00 | 10.81 | 36.50 | 51.75 | 93.40 | 7.25 | 31.70 | 33.83 | 4.55 | 2.08 |
| 9 | 3.00 | 3.00 | 9.74 | 40.00 | 61.50 | 84.03 | 5.25 | 33.78 | 34.75 | 5.30 | 3.08 |
| 10 | 3.00 | 3.00 | 9.94 | 33.50 | 56.50 | 88.54 | 4.50 | 32.48 | 40.05 | 5.98 | 3.13 |
| 11 | 3.00 | 3.00 | 11.58 | 44.00 | 54.50 | 93.72 | 6.50 | 35.58 | 39.68 | 6.03 | 3.13 |
| 12 | 3.00 | 3.00 | 12.57 | 31.50 | 44.75 | 93.94 | 6.25 | 41.05 | 43.93 | 6.85 | 3.23 |
| 13 | 3.00 | 3.00 | 13.72 | 43.50 | 63.00 | 95.25 | 5.75 | 38.38 | 42.25 | 5.98 | 3.30 |
| 14 | 3.00 | 3.00 | 10.07 | 39.50 | 55.00 | 89.71 | 4.25 | 31.45 | 33.18 | 5.25 | 3.08 |
| 15 | 3.00 | 3.00 | 10.87 | 38.50 | 69.82 | 85.94 | 6.50 | 34.83 | 37.33 | 6.63 | 3.23 |
| 16 | 3.00 | 3.00 | 15.65 | 45.50 | 68.50 | 94.36 | 5.75 | 29.03 | 32.18 | 5.40 | 3.03 |
| 17 | 3.00 | 3.00 | 10.25 | 39.50 | 59.50 | 100.00 | 4.50 | 24.13 | 29.03 | 5.00 | 3.00 |
| 18 | 3.50 | 3.50 | 12.15 | 43.50 | 69.50 | 86.05 | 5.50 | 32.50 | 37.75 | 6.35 | 3.25 |
| 19 | 3.00 | 3.00 | 11.40 | 39.50 | 65.50 | 88.95 | 4.25 | 37.08 | 40.93 | 5.73 | 3.18 |
| 20 | 3.00 | 3.00 | 15.84 | 46.00 | 69.50 | 85.99 | 5.25 | 32.65 | 36.88 | 6.03 | 3.05 |
| 21 | 3.00 | 3.00 | 17.30 | 48.00 | 70.25 | 93.30 | 4.25 | 37.75 | 40.58 | 7.33 | 3.75 |
| 22 | 3.00 | 3.00 | 11.69 | 37.00 | 68.50 | 90.65 | 5.50 | 37.28 | 38.20 | 8.05 | 2.83 |
| 23 | 3.00 | 3.00 | 13.57 | 37.50 | 61.50 | 95.28 | 4.25 | 34.63 | 36.05 | 6.00 | 2.70 |
| 24 | 3.00 | 3.00 | 16.80 | 45.00 | 66.96 | 90.71 | 5.75 | 38.50 | 40.63 | 6.28 | 3.53 |

*Contd……*

| Lines | R_80 | LLS_80 | PYPP | TW | SP | SMKP | PB | PH | HLPB | LL | LW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | 3.00 | 3.00 | 11.77 | 37.00 | 63.00 | 84.88 | 4.50 | 34.00 | 36.23 | 6.50 | 2.78 |
| 26 | 3.00 | 3.00 | 16.80 | 45.00 | 68.00 | 90.71 | 4.00 | 38.88 | 45.53 | 6.38 | 3.90 |
| 27 | 3.00 | 3.00 | 16.50 | 47.00 | 73.50 | 89.82 | 5.75 | 35.13 | 40.28 | 6.63 | 3.20 |
| 28 | 3.00 | 3.00 | 15.97 | 44.00 | 69.00 | 89.75 | 5.75 | 36.80 | 41.75 | 7.88 | 3.90 |
| 29 | 3.00 | 3.00 | 12.99 | 38.00 | 69.50 | 90.94 | 4.25 | 38.20 | 40.03 | 5.80 | 3.18 |
| 30 | 3.00 | 3.00 | 11.82 | 42.00 | 70.50 | 88.60 | 6.50 | 37.95 | 40.50 | 6.65 | 3.65 |
| 31 | 3.00 | 3.00 | 15.46 | 45.00 | 66.50 | 92.31 | 4.75 | 36.30 | 37.13 | 5.53 | 3.30 |
| 32 | 3.00 | 3.00 | 10.90 | 38.50 | 71.50 | 87.31 | 4.25 | 38.10 | 38.88 | 7.55 | 3.25 |
| 33 | 3.00 | 3.00 | 12.37 | 39.50 | 58.50 | 85.76 | 5.75 | 37.65 | 40.50 | 7.00 | 3.53 |
| 34 | 4.00 | 4.00 | 11.61 | 43.50 | 66.00 | 85.13 | 7.25 | 41.03 | 44.25 | 7.15 | 3.33 |
| 35 | 3.00 | 3.00 | 13.11 | 42.00 | 73.00 | 88.78 | 4.25 | 32.50 | 37.43 | 7.98 | 3.85 |
| 36 | 3.00 | 3.00 | 11.42 | 37.00 | 53.00 | 94.43 | 5.25 | 32.38 | 37.65 | 5.88 | 2.90 |
| 37 | 4.00 | 4.00 | 12.07 | 40.00 | 63.50 | 85.00 | 6.75 | 37.00 | 39.93 | 6.80 | 3.30 |
| 38 | 4.00 | 4.00 | 12.09 | 36.00 | 55.00 | 92.83 | 5.00 | 37.38 | 42.40 | 6.35 | 3.50 |
| 39 | 3.00 | 3.00 | 13.99 | 44.50 | 69.50 | 88.58 | 5.75 | 37.35 | 39.38 | 7.05 | 3.60 |
| 40 | 3.00 | 3.00 | 11.07 | 43.50 | 67.00 | 100.00 | 5.50 | 40.03 | 40.58 | 6.68 | 3.68 |
| 41 | 3.00 | 3.00 | 11.36 | 31.00 | 52.50 | 93.98 | 6.75 | 35.33 | 38.58 | 6.20 | 3.15 |
| 42 | 3.00 | 3.00 | 16.34 | 50.50 | 70.50 | 89.60 | 6.25 | 38.33 | 47.00 | 6.15 | 3.23 |
| 43 | 3.00 | 3.50 | 11.65 | 35.00 | 73.50 | 90.67 | 5.25 | 41.45 | 43.90 | 5.73 | 2.90 |
| 44 | 3.00 | 3.00 | 8.70 | 41.00 | 58.00 | 88.88 | 4.25 | 35.63 | 40.50 | 5.45 | 2.95 |
| 45 | 3.00 | 3.00 | 15.72 | 46.00 | 72.00 | 88.06 | 5.25 | 41.88 | 42.63 | 5.70 | 3.25 |
| 46 | 3.00 | 3.00 | 11.74 | 39.50 | 56.50 | 96.06 | 7.50 | 40.38 | 41.00 | 6.40 | 3.08 |

**R_80:** Rust score at 80 DAS; **LLS_80:** LLS score at 80 DAS; **PYPP:** Pod yield per plant (gm); **TW:** Test weight (gm); **SP:** Shelling percentage; **SMKP:** Sound mature kernel percentage; **NPB**: Number of primary branch; **PH:** Plant height (cm); **HLPB:** height of longest primary branch (cm); **LL:** Leaf length (cm); **LW:** Leaf width (cm)

# VALIDATION OF LATE LEAF SPOT AND RUST RESISTANCE-LINKED MARKERS AND TRANSFER OF ASSOCIATED QTL TO JL 24 IN GROUNDNUT (*Arachis hypogaea* L.)

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**Major Advisor**

**ABSTRACT**

This study was aimed at validating the markers linked to resistance for late leaf spot (LLS) and rust diseases of groundnut using Heterogeneous Inbred Family (HIF)-derived Near-Isogenic Lines (NILs), and to develop backcross lines in JL 24 using marker assisted backcross breeding (MABC) to improve foliar disease resistance. The NILs from TAG 24 × GPBD 4 and TG 26 × GPBD 4 could validate IPAHM103, GM2301 and GM1536 markers linked to LLS and rust resistance QTL. Similarly single marker analysis among Recombinant Inbreed lines (RILs) of VL 1 × 110 showed significant association of GM1009, GM2009, IPAHM103 and *AhMITE1*-PCR with LLS resistance at 70 DAS, but not with rust resistance.

In the MABC programme, JL 24 was crossed to LLS and rust resistant variety, GPDB 4 and two interspecific derivatives, ICGV 86699 and ICGV 99005. Backcrossing and selfing resulted in the development of $BC_1F_4$, $BC_2F_3$ and $BC_3F_3$. Evaluation of these backcross generations resulted in the identification of six lines (JG4_81, JG4_43, J8-4_10, J8-4_24, J9-4_19 and J9-4_20) in $BC_1F_4$ and four lines (J8-2-3_5, J8-2-3_41, JG2-3_14 and J8-2-3_5) in $BC_2F_3$. They were resistant to LLS and rust, and showed significantly superior productivity traits over JL 24. Ten homozygous lines were identified in $BC_3F_2$. They carried resistance allele at IPAHM103, GM2301 and pPGPseq8D09 marker loci linked to LLS and rust resistance. Background selection for JG-18 from JL 24 × GPBD 4 showed 87% genome similarity to JL 24 when analysed with 30 AhTE markers that were polymorphic between JL 24 and GPBD 4. All the ten homozygous lines were comparable to JL 24 for pod and kernel features. Further, line J9-08, J8-08 and J8-11 exhibited significant superiority for productivity traits over JL 24. These backcross lines make up a useful genetic resource for the development of LLS and rust resistant lines from JL 24.