

**Identification and morpho-molecuclar characterization of
genotypes of rice (*Oryza sativa* L.) showing resistance to
sheath blight disease**

Saba Mir
(2013-A-980-M)



Division of Biotechnology

Faculty of Horticulture

**Sher-e-Kashmir University of Agricultural Sciences &
Technology of Kashmir**

2015

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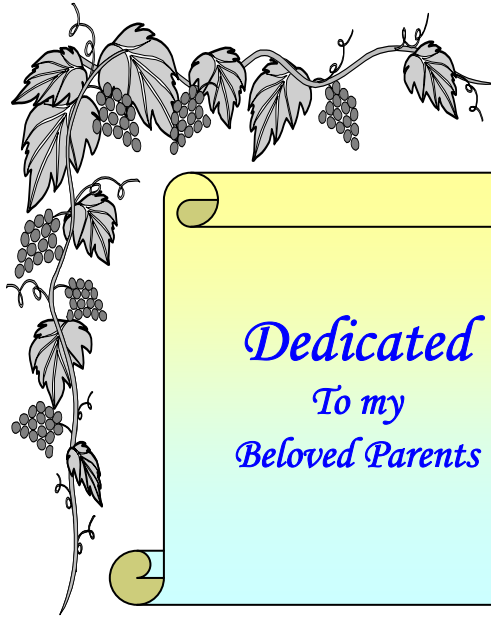
Thesis

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MASTER OF SCIENCE IN BIOTECHNOLOGY**

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Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Horticulture, Centre for Plant Biotechnology

Certificate – I

This is to certify that the thesis entitled, “**Identification and morpho-molecular characterization of genotypes of rice (*Oryza sativa* L.) showing resistance to sheath blight disease**” submitted in partial fulfillment of the requirements for the award of the degree of **Master of Science in Biotechnology**, to the **Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Saba Mir (Regd. No. 2013-A-980-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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Sheath Blight Disease”**

ABSTRACT

The present investigation entitled “Identification and Morpho-molecular characterization of genotypes of rice (*Oryza sativa* L.) showing resistance to sheath blight disease” was undertaken to obtain information on the variability and genetic divergence of the genotypes studied. Assessment of genetic diversity in crop germplasm is vital for identification of the genotypes to be used in the future breeding programme. Nature and magnitude of genetic variability, genetic diversity (D^2 statistics) at the phenotypic level was estimated in 102 rice genotypes and genetic distance at molecular level (using SSR primers) was assessed in 32 genotypes.

Screening of the genotypes for resistance/tolerance to rice sheath blight disease revealed that among all the genotypes the highest disease incidence (100%) and intensity (91.34%) was recorded in PUSA-3 followed by the genotype PUSA-5 (100% and 90.12% respectively) while the lowest disease incidence (10%) and intensity (1.11) was recorded in Niver Zag. Thus, about 40.19 per cent genotypes showed resistance. Of the remaining 61 genotypes 8 were moderately susceptible, 36 were susceptible and 17 were highly susceptible.

Morphological traits *vis-a-vis*, flag leaf angle, panicle type, awing, panicle threshability and seed coat colour was characterized using standard procedure of IRRRI on the basis of scale (scores 0-9). Flag leaf angle was erect/intermediate in nearly 69.33 per cent genotypes. Panicle type was compact/intermediate in nearly 80.43 genotypes with its panicle threshability as intermediate to easy in nearly 38.37 genotypes seed coat colour was white/brown in nearly 73.43 genotypes. Awning was absent in 84.31 genotypes.

Analysis of variance revealed significant differences among genotypes for all the traits studied. The highest phenotypic and genotypic coefficients of variability were observed for leaf width (cm) followed by tillering ability, grain yield ha^{-1} (q), plant height (cm), leaf length (cm) and panicle length (cm). In general the phenotypic coefficients of variation were higher than genotypic coefficients of variation which indicates the role of environment in the expression of traits under observation. The estimates of heritability in broad sense was high for all the characters. The present investigation indicates a great scope in the improvement of these traits as these characters in general possessed high estimates of heritability coupled with high genetic advancement except for days to maturity, grain length (mm) and days to 50% flowering (high heritability but moderate genetic gain) indicating the preponderance of additive gene action for control of these traits. Estimates of divergence among 102 rice genotypes revealed that significant divergence existed among them. The genotypes under study were grouped into 8 clusters as per Mahalanobis D^2 (1928) analysis employing Tochers method; with maximum number of genotypes in cluster IV (40) followed by cluster II (23), cluster VII (16), cluster VI (14) and cluster VIII (3). Maximum intercluster distance was observed between cluster VII and VIII (1146.52) while maximum intracluster distance was observed in cluster V (1271.69). The per cent contribution towards the total genetic divergence revealed that plant height (cm), days to 50% flowering, grain yield ha^{-1} (q), days to maturity, tillering ability and leaf width (cm) were the main contributing characters towards total genetic divergence.

Molecular characterization of selected 32 genotypes from amongst the 102 genotypes was carried out using SSR markers. Eight SSR primers were used, which identified a total of 22 alleles across these genotypes with a range of 2-5 alleles per locus and a mean of 2.87 per locus. The PIC values, measuring allelic diversity and polymorphism ranged from 0.22 (RM 245) to 0.67 (RM 202), with a mean PIC value of 0.42 per locus. Highest Effective Multiplex Ratio (1.58) was observed in primers RM 530 and RM 555 and lowest (0.14) in primer RM 245. Highest percentage of polymorphism (22.58%) was obtained in primer RM555 and RM 530 and lowest (7.14) was observed in primer RM 145. Similarity coefficients (measuring relatedness) were used to construct the dendrogram and clustering pattern of the genotypes. Pair wise similarity coefficients ranged from 0.10-0.93. Similarity coefficient value revealed presence of good genetic diversity among these genotypes at the molecular level. All the 32 genotypes got clustered into four clusters with maximum number of genotypes (13) in cluster III followed by cluster I.

Key words: Rice, *Oryza sativa* L., Sheath Blight, Microsatellite Marker, SSR

Signature of Student

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Signature of Major Advisor

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All praises are to Allah, the Creator and Sustainer of the World and endless blessings of Allah be upon Mohammad^{SAW} the great benefactor of mankind

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Chapter - 1

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important cereal crop and is a major food grain contributor to the total world food grain basket. It belongs to the genus *Oryza* family *Gramineae* (*Poaceae*) and tribe *Oryzaceae*. The genus *Oryza* consists of 23 wild and two cultivated species, viz., the Asian *O. sativa* and the African *O. glaberrima*. *O. sativa* earlier domesticated in Asia has now spread to almost all the rice growing areas of the world, while *O. glaberrima*, domesticated in Western tropical Africa is confined to that part of the world alone. The basic chromosome number of the genus is $n=12$. The species are either diploid with $2n=24$ chromosomes or tetraploids with $2n=48$ chromosomes. Because of long history of cultivation and selection under diverse environments, rice has acquired a broad range of adaptability and tolerance to abiotic stresses, thereby making its cultivation possible over a wide range of water, soil and climatic conditions that include areas from even below the mean sea level to about 2100 m a.m.s.l.

Most of the world's rice is cultivated and consumed in Asia, which constitutes more than half of the global population. In India, rice is grown under diverse ecosystems as rainfed uplands, rainfed shallow, semideep and deepwater lowlands, irrigated lands and hills. These are the major rice ecosystems found world over. No other plant species can grow under such wide range of conditions and produce grains to feed the population in Asia. Rice is life for Asians in general and Indians in particular. The crop constitutes the staple food of more than 65 per cent of the total population in India, 43 per cent of the total food grain production and 47 per cent of the total cereal production. Asia cultivates 137 million ha of rice of which India shares 42.56 million ha. Its annual production and productivity is 95.33 million tonnes and 2.22 t ha^{-1} , respectively (Directorate of Economics and Statistics, 2013).

Rice is the staple diet of the majority of people in Jammu and Kashmir. Rice crop ranks 2nd, both in area under cultivation and production. At present (2013-14) rice in the state is cultivated on an area of 274 thousand hectares, its production in 2013-14 was 9044 thousand quintals is 0.55 mt and productivity is 3.238 t ha⁻¹ (D.O.E.S., 2013-14). In the states of Jammu and Kashmir, it is cultivated within an altitude of 1560-2200 masl, expanding 30.25° to 35.2° N latitude, and 74 to 75.25°E longitude. The entire area is irrigated and the source of irrigation is melting snow at the higher ranges adversely affect the establishment of rice nurseries. Further, the mean temperature during growing period ranges from 13° to 26°C. The growing period is limited to 120 to 135 days. During the growing season, the temperatures are rather low. The days are long, nights are cool and wider differences exist between daily maximum and minimum temperatures (Sanghera *et al.*, 2011).

Despite the continuous increase in global demand of rice, it is subjected to various diseases that often place major constraints on its production. The situation has become worse during last few years with the introduction and wide spread cultivation of high yielding fertilizer responsive varieties producing maximum number of productive tillers per unit area of field, thus creating increase in relative humidity and prolonging leaf wetness, making the crop more vulnerable to infection by pathogens. Among the various fungal diseases of rice viz., Blast caused by *Pyricularia oryzae*, Rice sheath blight caused by *Rhizoctonia solani*, Brown Spot caused by *Helminthosporium oryzae*, Narrow brown leaf spot caused by *Cercospora janseana* are the major diseases of rice. Rice sheath blight (RSB) is one of the most devastating fungal diseases of rice worldwide (Stodart *et al.*, 2007; Bernardes-de-Assis *et al.*, 2009). It is a major disease of rice, second only to rice blast in reducing both grain yield and quality. It is becoming a major constraint to rice production, especially in the intensified cultivation system (Jayaprakashvel and Mathivanan, 2012). Each year, the blight causes up to a 50% decrease in the rice yield under favourable conditions around the world (Zheng *et*

al., 2013). Sheath blight of rice (*Rhizoctonia solani*) was reported for first time in India by Paracer and Chahal (1963) from Punjab, while in Jammu and Kashmir, the disease was recorded in 1972-73 from Anantnag (Mir, 1986). The reduction in the grain yield has been reported up to 10-30% (Xie *et al.*, 2008) and may reach up to 50% during prevalent years (Meng *et al.*, 2001). Its causal agent is the necrotrophic fungus *Rhizoctonia solani* Kuhn (teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk) anastomosis group (AG) 1, intraspecific group IA Webster *et al.* (1992). *Rhizoctonia solani* Kuhn is a worldwide, ecologically diverse soil-borne fungus belonging to Order Ceratobasidiales of the Basidiomycota and is the mycelial or imperfect state of *Thanatephorus cucumeris* (A.B. Frank) Donk. Relationships within the species *Rhizoctonia solani* are very complex and confusing. Identification is based on anastomoses groups (AG) among isolates and cultural characteristics. Based on hyphal anastomosis reactions, isolates within this species complex have been divided into several anastomosis groups (AGs). Currently, the *R. solani* species complex is divided into 14 AGs denoted as AG 1 to AG 13 and AG BI (Carling *et al.*, 2002). Isolates of AG-1 have been further divided into three subgroups, i.e. IA, IB and IC (Ogoshi 1987). Among them, isolates of *R. solani* of AG-1 IA have been associated with sheath blight in rice (Wang *et al.*, 2013).

R. solani survives in the absence of a host as asexually produced sclerotia. Sclerotia can survive in the soil for 2 years, accumulate over time, and are spread quickly during field preparation and flood application (Webster *et al.*, 1992). Mature sclerotia are buoyant and collect around rice plant culms with the application of permanent flood. Initial infections occur at the waterline as water soaked lesions on leaf sheaths. Subsequent infections are caused by runner hyphae that move upward to aerial portions of the plant, produce additional lesions, and complete the disease cycle by producing sclerotia on the surfaces of leaves and leaf sheaths (Webster *et al.*, 1992). The disease develops rapidly at flowering when the canopy is most dense, creating a microclimate favourable to fungal

growth and proliferation. High yielding, susceptible, semi-dwarf cultivars are particularly vulnerable to sheath blight as short stature, high tillering, dense planting, and high nitrogen input create conditions suitable for disease epidemics (Pan *et al.*, 1999). The ShB pathogen has a broad host range and no complete resistance has been identified in rice germplasm (Mew *et al.*, 2004); (Pinson *et al.*, 2005). However, substantial differences in the levels of susceptibility to the ShB pathogen among rice cultivars have been observed under field conditions (Jai *et al.*, 2007) ; (Marchetti *et al.*, 1998); Marchetti *et al.*,1991).

Breeding and utilization of resistant varieties are still considered the most economic and effective ways for controlling rice sheath blight. RSB is difficult to control due to the low level of inherent resistance of rice against this disease (Khodayari *et al.*, 2009). Despite extensive screening for complete resistance or immunity, no complete sheath blight resistance has been identified to date and only moderate or partial resistance is available (Rao, 1995). Nevertheless, there are several lines of evidence that partial resistance can offer adequate protection against the pathogen under field conditions (Li *et al.*, 1995). RSB is still not efficiently controlled by resistance breeding (Wu *et al.*, 2003). The major management strategies include agricultural preventive treatments and the use of the antifungal antibiotics jinggangmycin or validamycin A, neither of which is highly effective (Jiang *et al.*, 2012). Moreover, the application of fungicides results in environmental concerns (González *et al.*, 2012). Hence, development of resistant varieties as a means to control RSB is desirable, but greatly dependent on the identification of resistant genotypes and population structure of the pathogen.

Rice resistance to SB is controlled by polygenes belonging to a typical quantitative trait (Srinivasachary *et al.*, 2011; Taguchi-shiobara *et al.*, 2013; Zuo *et al.*, 2013). The introduction of RFLP (restriction fragment length polymorphism) markers for QTL mapping of sheath blight (Li *et al.*, 1995), some major effective QTLs have been identified from resistant varieties such as Teqing, Jasmine 85 and Tetep. Using a saturated SSR linkage map, the main-

effect QTL, epistatic QTL and QE interaction of seven sheath blight resistance-related traits were investigated (Liu *et al.*, 2014). Morphological traits such as plant height, plant compaction, tiller angle, and leaf size are found affecting rice SB resistance by changing plant micro-climate (Srinivasachary *et al.*, 2011). The heading date is also correlated with SB resistance, and the varieties with later maturing are generally more resistant to SB (Srinivasachary *et al.*, 2011).

Morphological characterization is the first step in the classification and evaluation of germplasm (Smith *et al.*, 1991). Besides, DNA markers also offers opportunities to delineate the genetic differences at the molecular level in the available materials. A range of biochemical and molecular approaches are used to study the diversity in conserved germplasm. PCR based markers like Randomly Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs) and Amplified Fragment Length polymorphism (AFLP) are frequently used for such purposes. SSRs are subsets of tandemly repeated DNA families represented by extremely short nucleotide sequence repeat of 1-5 base pair that are abundantly present in the eukaryotic genome, are highly polymorphic and co-dominant in nature with Mendelian inheritance (Beckmann and Soller, 1990). SSR markers show potential for large scale DNA fingerprinting of rice due to high level of polymorphism detected, their analysis by automated systems as well as high accuracy and repeatability.

Incorporation of important traits available in the local landraces and cultivars into economically important varieties assumes significance particularly when their genetic base has considerably narrowed down, and facilitates improvement of rice varieties suitable for temperate regions of the state. The first step in this direction is to identify promising genetic resources through use of robust, reliable and cost effective methods for determination of variation and diversity, both through conventional and molecular means. Though a few studies have been made at national and state level but no work till date has been undertaken in Jammu and Kashmir for identification of promising genotypes for

Sheath Blight resistance. Keeping this fact in view, the present investigation was carried out with the following objectives:-

- Screening of rice genotypes for sheath blight disease resistance.
- Morpho-molecular characterization of genotypes of rice showing resistance to sheath blight disease.

Chapter - 2

REVIEW OF LITERATURE

Characterization of rice germplasm is essential for identification of disease resistant genotypes. Breeding and utilization of resistant varieties are considered the most economic and effective ways for controlling rice sheath blight. Hence, development of resistant varieties as a means to control RSB is desirable. The study was designed to identify and characterize the rice genotypes for sheath blight disease. No or scanty work on the proposed problem has been done in Kashmir. However work done on rice sheath blight in India and abroad in relation to different aspects is available. So an effort has been made to evaluate and summarize the literature regarding the topic as follows:

Zou *et al.* (2000) conducted a study to evaluate an F₂ clonal population of Jasmine 85/Lemont. The F₂ population, including 128 clonal families, was inoculated by short toothpicks incubated with a strain, RH-9 of the fungus. Based on field disease evaluations in 2 years and a genetic map with 118 evenly distributed molecular markers, six quantitative trait loci (QTLs) contributing to sheath blight resistance were identified. These QTLs, qSB-2, qSB-3, qSB-7, qSB-9-1, qSB-9-2 and qSB-11, were located on chromosomes 2, 3, 7, 9 and 11, respectively. The respective alleles of qSB-2, qSB-3, qSB-7, and qSB-9-2 from Jasmine 85 could explain 21.2, 26.5, 22.2 and 10.1% of the total phenotypic variation, respectively; while the alleles of qSB-9-1 and qSB-11 from Lemont could explain 9.8% and 31.2% of the total phenotypic variation. Of these qSB-2 and qSB-11 could be detected in both years, while remaining loci were detected only in a single year. Furthermore, four QTLs (qHD-2, qHD-3, qHD-5 and qHD-7) controlling heading date and three QTLs (qPH-3, qPH-4 and qPH-11) controlling plant height were also identified. Though rice sheath blight resistance may be influenced by morphological traits, such as heading date and plant height, in this study most detected resistance loci were not linked to the loci for heading date or plant height.

Eizenga *et al.* (2002) screened *Oryza* species plants for rice sheath blight Resistance. Rice wild relatives, *Oryza* species, are one possible source of sheath blight (*Rhizoctonia solani*) resistance genes. However, *Oryza* spp. cannot be screened in the field as is done for cultivated rice (*O. sativa*) because the plant canopy does not favour disease development and many plants drop mature seed. Thus, a growth chamber - greenhouse method of screening *Oryza* spp. and their early generation progeny is needed. Primary-secondary and ratoon tillers of rice cultivars- germplasm which ranged from moderately resistant to very susceptible were evaluated first for sheath blight susceptibility. Plants were inoculated by placing *R. solani* - colonized toothpicks at the leaf collar, then incubating plants in a growth chamber. After 7 days, plants were visually rated for sheath blight severity, and the lesion length of each leaf was measured. Ranking of cultivar germplasm susceptibility by visual rating of primary-secondary tillers corresponded to the ranking from field ratings. Visual ratings correlated best with combined lesion length of the second and third leaves. For ratoon tillers, visual ratings correlated best with second-leaf lesion length. Next, this method was used with ratoon tillers to evaluate sheath blight susceptibility of 21 *Oryza* spp. accessions and F₁ progeny from crosses between 17 accessions and cultivated rice. This method proved useful on a limited scale for screening germplasm that could not be evaluated under field conditions.

Peng *et al.* (2003) investigated the relationship between morphological traits and sheath blight resistance by using a sergeant population consisting of 240 inbred recombinant lines, derived from an elite combination of Zhenshan 97 × Minghui 63. Partial correlation analysis between phenotypic data (morphological characteristics and heading date (HD) and sheath blight rating (SBR) showed that only one trait, plant compactness, was significantly correlated with resistance to sheath blight, eight traits such as, plant height, heading date, penultimate leaf angle, were significantly correlated with SBR and the remaining 10 morphological traits were not consistently associated with SBR. A major

quantitative trait loci (QTL) controlling plant compactness was found to be in the site on chromosome 9 adjacent to the region responsible for qSB9, a major QTLs conferring sheath blight resistance. One out of the three QTLs contributing to penultimate leaf angle was mapped approximately on same region as another sheath blight resistance QTL, qSB5, located on chromosome 5. Whereas no QTLs underlying most other traits was detected on chromosome region correlated with sheath blight resistance QTL.

Le *et al.* (2004) studied the genetic control of resistance to sheath blight of rice. Based on disease evaluation and a genetic map with 148 molecular makers, 15 M-QTLs detected for lesion height (LH) and actual lesion length (ALL) over assessment times were mapped on seven chromosomes (1, 2, 3, 4, 5, 9, and 12), explaining 35.8%-93.8% of the phenotypic variation. The QTLs with high additive effects for most resistance traits were found at the markers RM341 (on chromosome 2), RM156 (on chromosome 3), and RM280 (on chromosome 4). All the QTLs had the positive alleles from parent Teqing; Lemont did not contribute any alleles associated with higher disease resistance. Despite different assessment methods for host plant resistance, most QTLs controlling lesion height and actual lesion length in each assessment time were approximately mapped at the same chromosomal regions. Additive effects appeared to be more important than digenic interactions. Rice sheath blight resistance was found influenced by some morphological characters such as heading date but had no correlation with plant height. However, it cannot be ascertained if the association between sheath blight resistance and morphological traits is due to pleiotropic effect or to close linkage. Four QTLs, namely, QSbr1a, QSbr2a, QSbr4c, and QSbr9b that were found not associated with plant morphology or heading date are potentially useful in breeding programs for sheath blight resistance.

Brooks (2007) developed a method to isolate and utilize a phytotoxin from *Rhizoctonia solani* to investigate the genetics of sheath blight susceptibility. Infiltration of the toxin preparation into plant leaves induced necrosis in rice,

maize, and tomato. Using 17 rice cultivars known to vary in sheath blight resistance, genotypes were identified that were sensitive (tox-S) and insensitive (tox-I) to the toxin, and a correlation ($r = 0.66$) between toxin sensitivity and disease susceptibility was observed. Given the broad host range of *R. solani*, genotypes of host species may be both tox-S and tox-I. A total of 154 F2 progeny from a cross between Cypress (tox-S) and Jasmine 85 (tox-I) segregated in a 9:7 ratio for tox-S/tox-I, indicating an epistatic interaction between two genes controls sensitivity to the toxin in rice. This work provides the means to genetically map toxin sensitivity genes and eliminate susceptible genotypes when developing sheath blight-resistant rice cultivars.

Jia *et al.* (2007) developed a standardized laboratory micro-chamber screening method to quantify resistance to *R. solani* in rice, five rice cultivars, representing a wide range of observed disease reactions under field conditions, were examined in a blind inoculation test at three locations (Arkansas, Texas, and Colombia). Rice seedlings were inoculated at the three- to four-leaf stage with potato dextrose agar plugs containing mycelium and then covered with a 2- or 3-liter transparent plastic bottle for maintaining high humidity after inoculation. Two cultivars, Jasmine 85 and Lemont, that consistently have shown the highest and lowest levels of resistance, respectively, in previous field and greenhouse studies, were used as standards. Concurrent field experiments in Arkansas and Texas also were performed to compare the greenhouse disease ratings with those observed under field conditions. Overall, the relative disease ratings of the seven test cultivars were consistent between test locations and with field evaluations. Thus, the micro-chamber screening method can be used as an effective approach to accurately quantify resistance to the sheath blight pathogen under controlled greenhouse conditions and should help expedite the selection process to improve resistance to this important pathogen.

Venu *et al.* (2007) aimed to elucidated the molecular basis of rice defense to the pathogen, RNA isolated from *R. Solani* infected leaves of Jasmine 85 was

used for both RL-SAGE library construction and microarray hybridization. Robust LongSage (RL-SAGE) sequence analysis identified 20,233 and 24,049 distinct tags from the control and inoculated libraries, respectively. Nearly half of the significant tags (≥ 2 copies) from both libraries matched TIGR annotated genes and KOME full-length cDNAs. Among them, 42% represented sense and 7% antisense transcripts, respectively. Interestingly, 60% of the library-specific (≥ 10 copies) and differentially expressed (>4.0 -fold change) tags were novel transcripts matching genomic sequence but not annotated genes. About 70% of the genes identified in the SAGE libraries showed similar expression patterns (up or down-regulated) in the microarray data obtained from three biological replications. Some candidate RL-SAGE tags and microarray genes were located in known sheath blight QTL regions. The expression of ten differentially expressed RL-SAGE tags was confirmed with RT-PCR. The defense genes associated with resistance to *R. solani* identified in this study are useful genomic materials for further elucidation of the molecular basis of the defense response to *R. solani* and fine mapping of target sheath blight QTLs.

Xie *et al.* (2008) aimed to detect quantitative trait loci (QTLs) for resistance to rice sheath blight (SB) that expressed stably in different genetic backgrounds and environments, the reciprocal introgression line populations derived from the cross of Lemont and Teqing (LT-ILs and TQ-ILs) were planted in the field in 2006-2007 and inoculated with isolate RH-9 of *Rhizoctonia solani*. Lemont was highly susceptible, whereas Teqing was moderately resistant to RH-9. The relative lesion height (a ratio of lesion height to plant height) of TQ-ILs was normally distributed, whereas that of LT-ILs was apparently inclined to the susceptible parent Lemont. A total of 10 main-effect QTLs (M-QTLs) and 13 epistatic QTLs (E-QTLs) conferring sheath blight resistance (SBR) were mapped using data obtained from different years and genetic backgrounds. Among them, 6 M-QTLs detected in 2006 were verified in 2007, suggesting that these M-QTLs had reliable performance across years. *QRlh4* was the only M-QTL expressed

under the reciprocal backgrounds. On chromosome 10, *QRlh10a* between *RM216* and *RM311* detected in TQ-ILs and *QRlh10b* between *RM222* and *RM216* detected in LT-ILs were regarded as different genes because their directions of additive effect were opposite. Most QTLs identified in TQ-ILs were not expressed in LT-ILs, indicating the presence of a significant effect of genetic background. By comparative mapping, 8 M-QTLs detected in this study were located in the same or near regions that were associated with SBR identified in the previous studies. These M-QTLs have great potential to be applied in rice breeding for SBR by marker-assisted selection (MAS), and M-QTLs expressed stably in different backgrounds are favorable for gene pyramiding in SBR improvement in rice.

Prasad and Eizenga (2008) evaluated 73 *Oryza* genotypes with three different methods conducted in the greenhouse, growth chamber, or laboratory because there are significant limitations to screening wild *Oryza* spp. under field conditions, to identify resistant sources for sheath blight disease. For the microchamber method, 4-week-old seedlings were inoculated with a potato dextrose agar plug containing mycelia, covered with a 2-liter soft drink bottle, and rated 1 week after inoculation. A detached-leaf method involved placing a potato dextrose agar plug containing mycelia on the abaxial surface of a leaf section that was cut from a 5-week-old plant and placed on moist filter paper in a petri dish under constant light, then evaluated after 72 h. For the toothpick inoculation method, toothpicks colonized with mycelia were placed in the leaf collar region of plants at the panicle initiation stage, plants were placed in a growth chamber, and disease symptoms were evaluated after 7 days. The microchamber method gave a more uniform, reproducible response, and was easier to use under greenhouse conditions. Seven *Oryza* spp. accessions were identified as moderately resistant with three accessions classified as *O. nivara* (IRGC104705, IRGC100898, and IRGC104443) and one each as *O. Barthii* (IRGC100223), *O. meridionalis*

(IRGC105306), *O. nivara*/*O. sativa* (IRGC100943), and *O. officinalis* (IRGC105979).

Park *et al.* (2008) developed new methods for effective and uniform infection and accurate evaluation of the disease to facilitate detailed analysis of sheath blight resistance at genetic, molecular, biochemical, and functional genomic levels. The efficiency of *R. solani* infection was tested on two resistant (Tetep and Jasmine 85) and two susceptible (Chucheongbyeo, Junambyeo) cultivars using three different inoculum types (agar block, liquid cultured mycelia ball, and mycelia suspension). By covering the inoculated sheaths with aluminium foil to maintain humidity, 100% infection rate was achieved in this study. Liquid cultured mycelia balls caused significantly longer lesions (5.4 cm) than other types of inoculum, including agar block (2.4 cm) and mycelia suspension (1.6 cm). An improved method for evaluating sheath blight disease was selected by comparing two methods for evaluating disease severity among three partially resistant cultivars and five susceptible cultivars inoculated with liquid cultured mycelia balls. In addition, a new formula was developed to calculate the disease susceptibility index. Lesion length and the susceptibility index generally were correlated in each leaf, but there were discrepancies between the two evaluation methods due to differences in plant architecture among the cultivars. The susceptibility index calculated using the new formula was the most accurate method for evaluating sheath blight disease across all cultivars. The effect of heading date and panicle number also was evaluated in relation to sheath blight resistance. Cultivars with late heading dates generally were more resistant to sheath blight than those with early heading dates.

Zuo *et al.* (2008) investigated the prospect of QTL-qSB-9Tq utilized in molecular breeding program of *japonica* rice for sheath blight resistance. Most of the *japonica* rice cultivars showed lower level of sheath blight resistance than the *indica* rice cultivars. At the corresponding site of qSB-9Tq, nine typical *japonica* rice cultivars from different ecological

regions or countries proved to possess the susceptible allele(s). Introgression of qSB-9Tq into these cultivars enhanced their resistance level by decreasing sheath blight score of 1.0 (0.5-1.3), which indicated that qSB-9Tq had a large potential in strengthening the resistance of *japonica* rice to sheath blight. The use of the three molecular markers, which were polymorphic between Teqing and many *japonica* rice cultivars, promotes the application of qSB-9Tq in a concrete molecular breeding program.

Sharma *et al.* (2009) analysed a population of 279 F2:3 progeny rows derived from a cross between two tropical *japonica* U.S. rice cultivars, Rosemont (semi-dwarf, SB susceptible) and Pecos (tall, SB resistant), to map SB resistance. Progeny families were evaluated for disease reactions, plant height (PH), and heading date (HD) in replicated field trials for 2 year and genotyped with 149 simple sequence repeat markers. Correlation analysis between SB ratings with PH and HD showed that both agronomic traits were significantly correlated with SB resistance. Four significant (logarithm of odds ratio ≥ 3.6) quantitative trait loci (QTLs) were identified for SB resistance, with individual effects explaining 5.6 to 33.4% of the total phenotypic variation. Plant height appeared to have a direct influence on SB resistance, with QTLs for these traits collocated on chromosome 1. Consistent results across years indicated the stability of the identified QTLs and their potential for improving rice SB resistance using marker-assisted selection.

Liu *et al.* (2009) determined the ability of controlled environment inoculation assays to detect ShB resistance quantitative trait loci (QTLs) in a cross derived from the susceptible cv. Lemont and the resistant cv. Jasmine 85. The disease reactions of 250 F5 recombinant inbred lines (RILs) were measured on the seedlings inoculated using micro-chamber and mist-chamber assays under greenhouse conditions. In total, 10 ShB- QTLs were identified on chromosomes 1, 2, 3, 5, 6, and 9 using these two methods. The micro-chamber method identified four of five new ShB-QTLs, one on each of chromosomes 1, 3, 5, and 6. Both micro-chamber and mist-chamber methods identified two ShB-QTLs, qShB1 and

qShB9- 2. Four of the ShB-QTLs or ShB-QTL regions identified on chromosomes 2, 3, and 9 were previously reported in the literature. The major ShB-QTL qShB9-2, which cosegregated with simple sequence repeat (SSR) marker RM245 on chromosome 9, contributed to 24.3 and 27.2% of total phenotypic variation in ShB using microchamber and mist- chamber assays, respectively. qShB9-2, a plant-stage-independent QTL, was also verified in nine haplotypes of 10 resistant Lemont/Jasmine 85 RILs using haplotype analysis. These results suggest that multiple ShB- QTLs are involved in ShB resistance and that microchamber and mist- chamber methods are effective for detecting plant-stage-independent QTLs. Furthermore, two SSR markers, RM215 and RM245, are robust markers and can be used in marker-assisted breeding programs to improve ShB resistance.

Yin *et al.* (2009) investigated the accurate resistance effect of qSB-9Tq, a major QTL that derived from the indica cultivar Teqing, by using a backcross population of Teqing and Lemont (the latter as the recurrent parent) and a set of near-isogenic lines (NIL) developed in their study. A strong pathogenic *Rhizoctonia solani* line, RH-9, was used for inoculation. Two different experimental designs were adopted. One was a completely randomized experiment in which plants that exhibited three different genotypes at qSB-9Tq locus were distinguished by detecting the marker genotypes flanking qSB-9Tq in the BC6F2 segregation population. The other was a randomized block design experiment with three replicates using the three NILs of qSB-9Tq. The combined results of the two experiments suggested that qSB-9Tq was a dominant resistance QTL and could reduce disease rating by approximately 1.0 using a 0_9 disease rating scale. The results of the study provide a strong foundation for the fine mapping of qSB-9Tq and its utilization in breeding.

Zuo *et al.* (2011) evaluated the effect of QTL qSB-11LE, located on chromosome 11 of an SB-susceptible *Oryza japonica* rice cultivar Lemont, on SBresistance and its potential in breeding programs by using a set of near-isogenic lines (NILs) that exhibit three different genotypes at the qSB-11LE locus and

seven backcross populations. Results of field disease evaluation under artificial inoculation showed that the inheritance of resistance of qSB-11LE to SB is controlled by additive gene action and that the genes have a dosage effect on SBresistance. In greenhouse evaluations, the resistance effect of qSB-11LE was expressed at 11 and 14 d after inoculation at the tillering stage. Analysis of field resistance of six BC1F1 populations and one BC2F1 population, developed from the backcrosses between Lemont as the donor parent and six commercial O. indica rice cultivars as recurrent parents, indicated that qSB-11LE could be effectively used to enhance these cultivars' SBresistance. These observations suggest that the qSB-11LE has considerable potential in rice breeding for resistance to SB, and that its flanking molecular markers could be employed in practical breeding programs for marker-assisted selection.

Attaullah *et al.* (2012) studied the effect of surface disinfection, temperatures, nutrients and chemicals on sclerotial germination of *Rhizoctonia solani* from potato tubers on potato dextrose agar (PDA). Germination occurred from tuber pieces bearing sclerotia pre-treated for one min with 0.1% of mercuric chloride. Tuber pieces pretreated with 0.5 and 1% of mercuric chloride for various lengths of times, including 15 sec, 30 sec and one min showed no sclerotial germination on PDA. Similarly sclerotial germination did not occur after pretreated with sodium hypochlorite for same concentrations and times. Sclerotial germination was optimal at a temperature of 25°C on PDA. Germination of sclerotia was best on PDA followed by cornmeal agar (CMA). Minimum mycelial growth occurred on water agar (WA) while sclerotial germination was minimal on malt extract agar (MEA) after one week of inoculation. Tuber pieces bearing sclerotia were also treated with 0.1% of mercuric chloride, sodium hypochlorite, potassium hydroxide and ethanol for one min before inoculation but no sclerotial germination occurred on PDA except from pieces treated with mercuric chloride.

Lal *et al.* (2012) aimed to determine the virulence diversity of isolates, total six cultivars of rice were artificially inoculated with 25 isolates of *R. solani*

in glass house. Three susceptible (pusa basmati-1, annapurna, swarna) and three tolerant (pankaj, manasarovar, swarnadhan) cultivars were tested against all isolates. All isolates were pathogenic on six cultivars except isolate rs-25 pathogenic, which was pathogenic on four cultivars and non-pathogenic on two cultivars namely, manasarovar and swarnadhan. On the basis of virulence reaction on different cultivars, the isolates were categorized into four groups; highly virulent, moderately virulent, less virulent and avirulent. maximum relative lesion size on cultivars pb-1, annapurna, swarna, pankaj, manasarovar and swarnadhan was observed as 69.66 with rs-21, 72.74 with rs-5, 65.09 with rs-16, 65.43 with rs-17, 59.09 with rs-18 and 61.87 with rs-6 respectively. Maximum virulence index of 7.33 was at par in rs-16 and rs-18 and maximum susceptibility index was found on cv. annapurna (7.76). Four isolates viz, rs-16, rs-18, rs-20 and rs-21 can be used in future for screening for resistance against sheath blight of rice.

Nelson *et al.* (2012) evaluated 197 doubled-haploid lines from a cross between MCR10277 (resistant) and Cocodrie (susceptible) in field and greenhouse assays with U. S. and Colombian pathogen isolates and genotyped at 111 microsatellite marker loci. Four SBR QTLs from MCR10277 were identified, together accounting for 47% of field genetic variation. In all trials the strongest effect was provided by a chromosome- 9 QTL, qsbr-9.1, but some QTLs differed for U. S. and Colombian *R. solani* isolates. SBR QTLs coincided with only two of several height or heading time QTLs, suggesting that the relationship between these developmental traits and SBR is not simple. For the U. S. isolates, a microchamber greenhouse assay revealed the same QTLs as did field inoculation.

Jia *et al.* (2012) aimed to identify ShB QTLs via association mapping in rice using 217 sub-core entries from the USDA rice core collection, which were phenotyped with a micro-chamber screening method and genotyped with 155 genome-wide markers. Structure analysis divided the mapping panel into five groups, and model comparison revealed that PCA5 with genomic control was the best model for association mapping of ShB. Ten marker loci on seven

chromosomes were significantly associated with response to the ShB pathogen. Among multiple alleles in each identified loci, the allele contributing the greatest effect to ShB resistance was named the putative resistant allele. Among 217 entries, entry GSOR 310389 contained the most putative resistant alleles, eight out of ten. The number of putative resistant alleles presented in an entry was highly and significantly correlated with the decrease of ShB rating ($r=20.535$) or the increase of ShB resistance. Majority of the resistant entries that contained a large number of the putative resistant alleles belonged to indica, which is consistent with a general observation that most ShB resistant accessions are of indica origin. These findings demonstrate the potential to improve breeding efficiency by using marker-assisted selection to pyramid putative resistant alleles from various loci in a cultivar for enhanced ShB resistance in rice.

Adhipathi *et al.* (2013) analysed resistant genotypes of rice against sheath blight disease and virulence diversity among the isolates of pathogen. They collected 12 *R. solani* isolates and characterized them based on morphological and sclerotial characters and virulence diversity on 10 different rice varieties. The disease progress and severities were analysed using area under disease progress curve (AUDPC) value on the basis of lesion length recorded on 4, 8, 12 and 16 DAI. Evaluation on the basis of AUDPC value, out of 10 varieties of rice, Sarju-52 depicted highly resistant (19.91) while Jaya (21.87), UPR-2005-38 (23.97) and IET-15182 (23.16) showed moderately resistant disease reaction with most of the isolates tested; whereas the variety Pusa Basmati-1 (46.57) depicted highly susceptible disease reaction. Among these some isolates were highly and moderately virulent whereas most of the isolates were less virulent. The identified resistant (Sarju-52) and susceptible (Pusa Basumati-1) is the potential source for the breeding programmes for further development of resistant varieties in rice.

Kutubuddin *et al.* (2013) reported the generation of transgenic rice lines overexpressing the rice *oxalate oxidase 4* (*Osoxo4*) gene in a green tissue-specific manner which breaks down oxalic acid (OA), the pathogenesis factor secreted by

R. Solani, in order to examine sheath blight resistance. Transgenic plants showed higher enzyme activity of oxalate oxidase (OxO) than nontransgenic control plants, which was visualized by histochemical assays and sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDSPAGE). Transgenic rice leaves were more tolerant than control rice leaves to exogenous OA. Transgenic plants showed a higher level of expression of other defence-related genes in response to pathogen infection. More importantly, transgenic plants exhibited significantly enhanced durable resistance to *R. solani*. The overexpression of *Osoxo4* in rice did not show any detrimental phenotypic or agronomic effect. Findings indicated that rice OxO can be utilized effectively in plant genetic manipulation for sheath blight resistance, and possibly for resistance to other diseases caused by necrotrophic fungi, especially those that secrete OA. This is the first report of the expression of defence genes in rice in a green tissue-specific manner for sheath blight resistance.

Liu *et al.* (2013) evaluated ShB resistance using 216 LJRILs under field conditions in Arkansas, Texas, and Louisiana during 2008 and 2009. They confirmed the presence of the major ShB-QTL *qShB9-2* based on the field data and also identified one new ShB-QTL between markers RM221 and RM112 on chromosome 2 across all three locations. Based on the field verification of ShB evaluations, the microchamber and mist-chamber assays were simple, effective, and reliable methods to identify major ShB-QTLs like *qShB9-2* in the greenhouse at early vegetative stages. The markers RM215 and RM245 were found to be closely linked to *qShB9-2* in greenhouse and field assays, indicating that they will be useful for improving ShB resistance in rice breeding programs using MAS.

Zuo *et al.* (2013) reported the precise location of qSB-11LE. They developed a near isogenic line, NIL-qSB11TQ, by marker-assisted selection that contains susceptible allele(s) from Teqing (TQ) at the qSB-11 locus in the LE genetic background. NIL-qSB11TQ showed higher susceptibility to SB than LE in both field and greenhouse tests, suggesting that this region of LE contains a

QTL contributing to SB resistance. In order to eliminate the genetic background effects and increase the accuracy of phenotypic evaluation, a total of 112 chromosome segment substitution lines (CSSLs) with the substituted segment specific to the qSB-11LE region were produced as the fine mapping population. The genetic backgrounds and morphological characteristics of these CSSLs are similar to those of the recurrent parent LE. The donor TQ chromosomal segments in these CSSL lines contiguously overlap to bridge the qSB-11LE region. Through artificial inoculation, all CSSLs were evaluated for resistance to SB. For the recombinant lines, their phenotypes were evaluated in the field and also evaluated in a controlled greenhouse environment, showing a consistent phenotype in SB resistance across years and conditions. After comparing the genotypic profile of each CSSL with its phenotype, they were able to localize qSB-11LE to the region defined by two cleaved-amplified polymorphic sequence markers, Z22- 27C and Z23-33C covering 78.871 kb, based on the rice reference genome. Eleven putative genes were annotated within this region and three of them were considered the most likely candidates. The results of the study greatly facilitated the cloning of the genes responsible for qSB-11LE and marker-assisted breeding to incorporate qSB-11LE into other rice cultivars.

Shiobara *et al.* (2013) identified three landraces from the Himalayas—Jarjan, Nepal 555 and Nepal 8—with resistance to sheath blight in 3 years' field testing, among 33 rice accessions, mainly from National Institute of Agrobiological Sciences (NIAS) Core Collection. Backcrossed inbred lines (BILs) derived from a cross between Jarjan and the leading Japanese cultivar Koshihikari were used in QTL analyses. Since later-heading lines show fewer lesions, they used only earlier-heading BILs to avoid association with heading date. They detected eight QTLs; the Jarjan allele of three of these increased resistance. Only one QTL, on chromosome 9 (between markers Nag 08KK18184 and Nag 08KK18871), was detected in all 3 years. Chromosome segment substitution lines (CSSLs) carrying it showed resistance in field tests. Thirty F2

lines derived from a cross between Koshihikari and one CSSL supported the QTL.

Kamal *et al.* (2014) screened six moderately resistant cultivars, namely 'Teqing', 'Jasmine85', 'Tetep', 'Pecos', 'Azucena' and 'Taducan', one susceptible local cultivar, 'MR 219', and two new advanced breeding lines, 'UKMRC 2' and 'UKMRC 9', by using micro-chamber and mist-chamber methods. The fungal isolate was confirmed as *R. solani* using ITS-rDNA sequencing. Severe sheath blight was recorded following inoculation with *R. solani* under micro-chamber conditions. The most resistant cultivar was 'Tetep', followed by 'Teqing'. In mist-chamber screening, 'UKMRC 2' showed the highest level of susceptibility with a disease severity index (DSI) of 6.67, while 'MR 219' produced the highest DSI of 7.22 in the microchamber. Significant correlation of plant height and disease was obtained with relative lesion height (RLH) indices. Significant correlations were also observed among diseased plant affected area (DPAA), VRT (visual rating) and RLH, with VRT being the most accurate. On the basis of the disease reactions, 'Tetep' and 'Teqing' were identified as suitable donors to improve resistance in 'UKMRC 2' and 'MR 219'. Mist-chamber screening method was more reliable to evaluate sheath blight under greenhouse conditions than the micro-chamber method.

Shu *et al.* (2014) observed the genetic diversity and population structure among 72 rice-infecting isolates of *Rhizoctonia solani* AG-1 IA, collected from 12 counties in south China, and were investigated using nine inter-simple sequence repeat (ISSR) markers. A total of 116 bands were amplified, with a majority of amplified fragments ranging from 500 bp to 2500 bp in size, of which 110 (94.8%) were polymorphic. Seventy-two isolates were grouped into six major clusters at 73% genetic similarity coefficient by the unweighed pair group method with arithmetic mean (UPGMA) with Dice's distance matrices. The genetic diversity was high [percentage of polymorphic bands (P %) = 94.83%; Shanon's diversity index (I) = 0.3175; Nei's diversity (h) =0.2034] at the population level, but low within populations [P % = 53.38%; Shanon's diversity index (I) =0.2734;

Nei's diversity (h) = 0.1811]. The mean coefficient of gene differentiation (G_{st}) was 0.165, indicating that 83.5% of the genetic diversity resided within the population. The genetic similarity values among 12 subpopulations ranged from 0.9672 to a minimum of 0.8641, and genetic distance values ranged from 0.1461 to 0.0333. Total gene flow (N_m) of 5.5810 indicated that there was significant gene flow among the sampled populations. Analysis of molecular variance (AMOVA) demonstrated that there was a relatively high level (81.93%) of genetic variation within subpopulations, with the gene differentiation coefficient (Φ_{ST}) being 0.181. A Mantel test suggested that there was no significant correlation between genetic differentiation and geographical distance.

Liu *et al.* (2014) analysed recombinant inbred line (RIL) population from a cross between 'H H1B' and 'R SB02' (a deep-water rice variety with resistance to sheath blight) planted in two locations for four different growing seasons. Seven traits were used to evaluate the disease severity, namely disease rating (DR), lesion length (LL), lesion height (LH), relative lesion length (RLL), relative lesion height (RLH), plant height (PH) and heading date (HD). Based on a linkage map of 163 simple sequence repeat (SSR) markers, a total of 37 QTLs were mapped on nine chromosomes. Additionally, 32 epistatic QTLs were identified, distributed on all the 12 chromosomes. The contribution of a single QTL's additive and epistatic effect was of low magnitude for most cases (from 0.39% to 24.62%). Among QTL x environment interaction test, 28 additive QTLs and six pairs of epistatic interactions were involved. Correlation analysis showed that DR had significant positive correlations with LL, RLL and RLH, but had a negative correlation with PH, two of six QTLs controlling DR were mapped in the same chromosome regions as the QTLs controlling PH. The alleles which can enhance disease resistance and increase PH are from the resistant parent 'R SB02', indicating that PH has certain effect on sheath blight resistance in the present study.

Zuo *et al.* (2014) employed *qSB-9TQ* and *TACITQ*, which control SB

resistance and tiller angle (TA), respectively, to test whether the combination of the two genes will further improve rice SB resistance and reduce yield loss rather than only one of them or neither. Using two pairs of near isogenic lines (NILs), *TACITQ* was confirmed to contribute to SB resistance. However, its effect was less considerable than that of *qSB-9TQ*. Subsequently, the two genes were introduced into two commercial rice varieties to develop a series of NILs. The NILs carrying both *TACITQ* and *qSB-9TQ* showed more resistance than the NILs containing only one of them. Comparing the grain yield per plant (GYP) under the three different SB disease conditions, namely slight, moderate and severe conditions, NILs carrying both genes apparently lost lower GYP than the NILs without the two genes and the NILs carrying only *TACITQ*. Under slight disease condition, no significant differences were found on morphology, development and GYP associated traits except for TA between the NILs carrying both genes and either of them, indicating that the two genes have no inferior effect on rice agronomic traits. Results indicated that *TACITQ* and *qSB-9TQ* have high breeding potential, and pyramiding SB resistance QTL and morphological trait QTL is a potential approach in improving rice SB resistance.

Nadarajah *et al.* (2014) isolated two field isolates of *Rhizoctonia solani* from infected paddy plants in Malaysia. These isolates were verified via ITSrDNA analysis that yielded 720 bp products of the ITS1-5.8S-ITS4 region, respectively. The sequenced products showed insertion and substitution incidences which may result in strain diversity and possible variation in disease severity. These strains showed some regional and host-specific relatedness via maximum likelihood and further phylogenetic analysis via Maximum Parsimony showed that these strains were closely related to *R. solani* AG1-1A (with 99-100% identity). Subsequent to strain verification and analysis, these isolates were used in the screening of twenty rice varieties for tolerance or resistance to sheath blight via mycelial plug method where both isolates (1801 and 1802) showed resistance or moderate resistance to Teqing, TETEP, and Jasmine 85. Isolate 1802 was more virulent based on the

disease severity index values. This study also showed that the mycelial plug techniques were efficient in providing uniform inoculum and humidity for screening. In addition this study shows that the disease severity index is a better mode of scoring for resistance compared to lesion length. These findings will provide a solid basis for our future breeding and screening activities at the institution.

Chapter - 3

MATERIALS AND METHODS

The present investigation entitled “Identification and morpho-molecular characterization of genotypes of rice (*Oryza sativa* L.) showing resistance to sheath blight disease” was carried out at Experimental Fields and Biotechnology Laboratory, centre for Plant Biotechnology, SKUAST-Kashmir, Shalimar during years 2014-2015. The experimental field was located at the main campus, Shalimar, Srinagar which is 15 km away from Srinagar city on the foot hills of Mahadev. The altitude of the location is 1685 meter above mean sea level and situated 34° N of latitude and 74.89° E of longitude. The climate is temperate characterized by mild summers. June and July are the hottest months while January and February are the coldest. The maximum rain fall is received during March to April.

3.1 Experimental material

A total of 102 rice genotypes were used for screening under field conditions for sheath blight resistance. The name of the varieties/genotypes along with their source are given in the Table-1. The experimental fields were well prepared and standard cultural, manural and plant protection practices were followed to ensure a healthy crop growth.

3.2 Isolation, identification of pathogen and screening of rice cultivars

3.2.1 Isolation

Sheaths of the rice plants showing typical sheath blight symptoms, were used for isolation of the pathogen. The rice sheath bits (3-4 mm) containing diseased and healthy sheath portions, were cut with sterilized scissors, surface sterilized in 0.1 per cent sodium hypochlorite solution for 1 minute, rinsed 2-3 times in sterilized distilled water, blotted dry and placed aseptically on potato dextrose agar (PDA) medium. Four such bits were placed on PDA in each Petri dish and incubated for two days at 28±2°C.

Table 1: List of the genotypes used in the present study

| S. No. | Name of the genotype | S. No. | Name of the genotype |
|---|-----------------------------|---------------|-----------------------------|
| Local landraces | | | |
| 1 | Gulbara | 6 | Mushkbudji |
| 2 | Tumlahaal | 7 | Niver Zag |
| 3 | Gurah | 8 | Local Budgam 1 |
| 4 | Baber | 9 | Local Budgam 2 |
| 5 | Wazul krea | 10 | Local Budgam 3 |
| Released Varieties/Genotypes of SKUAST | | | |
| S. No. | Name of the genotype | S. No. | Name of the genotype |
| 1 | SKAU-429 | 20 | SKAU-403 |
| 2 | SK-356 | 21 | Chenab |
| 3 | Shalimar Rice-1 | 22 | SKAU-339 |
| 4 | KHD/GP/120 | 23 | China-1039 |
| 5 | KHD/GP/27 | 24 | SKAU-292 |
| 6 | Shalimar Rice-3 | 25 | China-988 |
| 7 | China-1031 | 26 | SK-338 |
| 8 | SKAU-336 | 27 | SKAU-39 |
| 9 | SKAU-2 | 28 | China-1007 |
| 10 | SKAU-337 | 29 | Pusa sugand-5 |
| 11 | Pusa Sugand-3 | 30 | SKAU-46 |
| 12 | SKAU-408 | 31 | SKAU-402 |
| 13 | SKAU-389 | 32 | SKAU-404 |
| 14 | Shalimar Rice -2 | 33 | SKAU-3 |
| 15 | KHD/GP/150 | 34 | SKAU-98 |
| 16 | Shalkew | 35 | SKAU-405 |
| 17 | K-332 | 36 | China-972 |
| 18 | K-116 | 37 | Kamad |
| 19 | Jehlum | | |

| Other genotypes from IRRI etc. | | | |
|---------------------------------------|-----------------------------|---------------|-----------------------------|
| S. No. | Name of the genotype | S. No. | Name of the genotype |
| 1 | Calaro | 29 | T2 (MAS 71) |
| 2 | C101A51 | 30 | NP 125 |
| 3 | C101PKT | 31 | IRBN 2008 V-87 |
| 4 | IR61728-413-2-1-1 | 32 | Dular |
| 5 | Shai Tai Tsau | 33 | CO-39 |
| 6 | C101A51/ARBN 152 | 34 | BL-122/ARBN 141 |
| 7 | BL-122 | 35 | BL-142/ARBN 142 |
| 8 | Rasi | 36 | IRBL Z-FU/EC 565188 |
| 9 | IR64 | 37 | KHD/GP/208 |
| 10 | IR68333-RR-B-19 | 38 | T1 (MAS 70) |
| 11 | Kanto-51 | 39 | B-26 |
| 12 | RIL-10 | 40 | B-10 |
| 13 | IRBL1-CL/EC 565160 | 41 | B-13 |
| 14 | IRBL 5M/EC 565166 | 42 | B-5 |
| 15 | A57-115-4/ARBN 148 | 43 | B-3 |
| 16 | B-4 | 44 | B-19 |
| 17 | IRBN KS-S/EC 565178 | 45 | B-1509 |
| 18 | C104PKT | 46 | B-28 |
| 19 | A-57 | 47 | B-32 |
| 20 | B-9 | 48 | B-8 |
| 21 | B-14 | 49 | B-11 |
| 22 | RIL-29 | 50 | B-24 |
| 23 | Usen | 51 | YUNLEN-19 |
| 24 | Raminad Str 3 | 52 | A3(IRRI LINE) |
| 25 | BL 245 | 53 | C105TTP.4L23 |
| 26 | BL-42 | 54 | IR63347-AL-201-1 |
| 27 | SAFED BREZ | 55 | K-225 |
| 28 | KHD/GP/154 | | |

3.2.2 Purification and identification

The hyphal tips emanating from the tissue bits were cut off and transferred to PDA in Petri plates for purification and further growth of the pathogen. For identification, morphological characteristics such as shape, size and colour of sclerotia, branching type, septation and colour of mycelium, presence/absence of moniloid cells, growth rate etc., were observed under compound microscope and compared with standard description given by Parmeter and Whitney (1970).

3.2.3 Screening of rice cultivars under natural conditions

The screening of the cultivars was done under field conditions for which the material was planted in the experimental field of the SKUAST-K, Shalimar according to Randomized Complete Block Design (RCBD) with the replicates during kharief 2014. Healthy and stout paddy seeds free from the diseases were kept into fresh cotton bags and soaked in fresh water for 24 hours. After 24 hours the seed bags were brought out and kept under the temperature of 20 -30°C. After 4-6 days the sprouts emerged and were sown in nursery. The transplanting of the seedlings was done after 35 days of sowing. The *R. Solani* inoculum was prepared based on the following procedure. Dry sweet corn (40 g) and dry rye grass grain (30 g) were mixed in 1 liter of water and autoclaved for 45 min twice. Mycelial plugs were incubated in the mixture for 4 to 5 days, and then transferred to a paper bag for producing sclerotia, which were ground and used as inoculum. *R. solani* was allowed to grow throughout the mixture for 3 days before it was sprinkled by hand over all field plants.

3.2.3 Screening under polyhouse

Three seedlings of similar germination rate were selected and planted at least 3 cm apart in a 15 cm diameter plastic pot filled with soil. Soil was first sterilized to ensure that the study was not confounded by the presence of soilborne *R. solani* inoculum. Each cultivar was represented by three replicate plants per



Plate-1 : Symptoms of sheath blight (*Rhizoctonia solani*) of rice disease



Plate-2 : Mycelia of seven day cultures of *Rhizoctonia solani*



Plate-3 : Sheath blight disease screening in greenhouse

replication. Each seedling was inoculated with a round mycelial disk placed and pressed up to the base of the stem, assuring that mycelium was in contact with the plant.

The genotypes were scored as per the standard SES scale:

| Score | Description |
|--------------|--|
| 0 | No infection |
| 1 | Vertical spread of the lesions upto 20% of plant height |
| 3 | Vertical spread of the lesions upto 21-30% of plant height |
| 5 | Vertical spread of the lesions upto 31-45% of plant height |
| 7 | Vertical spread of the lesions upto 46-65% of plant height |
| 9 | Vertical spread of the lesions more than 66% of plant height |

3.3 Morphological characterization

3.3.1 Morphological, maturity and yield component traits

Observations were recorded at the appropriate developmental stages of plant growth as per the descriptors for rice formulated by IBPGR-IRRI Advisory committee (IRRI, 1980). The characters included for the study are:

1. Plant height (cm)

Height of the tagged plants was measured as the distance from ground level to the tip of the tallest panicle excluding awns at maturity as suggested by Gomez (1972).

2. Days to 50% flowering

Recorded as number of days taken from seed sowing to the emergence of flowers in 50 per cent plants in each experimental plot.

3. Panicle length (cm)

Panicle length of the main tiller of the tagged plants was measured from the panicle base to the tip of the panicle at the dough stage.

4. Days to maturity

Recorded as number of days taken from seed sowing to grain ripening (physiological maturity) on whole plot basis.

5. Grain yield ha⁻¹ (q)

The grains obtained from the net plot were dried for two to three days, cleaned and then weighed.

3.3.2 Diagnostic plant characteristics

Diagnostic characteristics were recorded for each genotype. The data was recorded as per the Standard method suggested by Standard Evaluation System for Rice, IRRI, Philippines (1996).

1. Leaf length (cm)

Leaf length was measured on the leaf just below the flag leaf at the growth stage 5-6.

2. Leaf width (cm)

Leaf width was measured on the leaf just below the flag leaf at the stage 5-6.

3. Flag leaf angle

Leaf angle was measured at growth stage 4-5 with the code as:

- 1 = Erect
- 2 = Intermediate
- 3 = Horizontal
- 4 = Descending

4. Panicle type

The type of panicle (a determinate inflorescence on the terminal shoot) was recorded at the growth stage-8 with following code:

- 1 = Compact
- 3 = Intermediate
- 5 = Open

5. Grain length (mm)

It was recorded from the base of the lowermost sterile lemma to the tip apiculus of the fertile lemma or palea at growth stage-9. In case of awned varieties, the grain was measured to a point comparable to the tip of the apiculus.

6. Grain width (mm)

It was recorded as the distance across the fertile lemma and the plea at the widest point at growth stage-9.

7. Awning

Awn, a filiform extension of different lengths arising from the keel (middle nerve) of the lemma, was recorded at the growth stage 7-9 with the following codes:

- 0 = Absent
- 1 = Short and partly awned
- 5 = Short and fully awned
- 7 = Long and partially awnwd
- 9 = Long and fully awned

8. Tillering ability

Tillering stage followed the seedling stage with the appearance of the first tiller from the axillary bud in one of the lower most nodes. Tillering ability

was recorded at the growth stages 2-6 by applying the following scale.

| | | |
|---|---|---------------------------------------|
| 1 | = | Very prolific |
| 3 | = | Good (20-25 tiller/plant) |
| 5 | = | Medium (10-19) |
| 7 | = | Poor (5-9) |
| 9 | = | Very poor (less than 5 tillers/plant) |

9. Panicle threshability

The panicle was firmly grasped and hand pulled over the panicle to estimate percentage of shattered grains. The observation were recorded at growth stage-9 by applying the following scale:

| | | |
|---|---|--|
| 1 | = | Difficult (Less than 1% shattering) |
| 3 | = | Moderately difficult (1-5% shattering) |
| 5 | = | Intermediate (6-25% shattering) |
| 7 | = | Loose (26-50% shattering) |
| 9 | = | Easy (51-100% shattering) |

3.4 Quality traits

1. 1000-seed weight (g)

From the bulk sample of grains obtained from the panicles of tagged plants, 1000 well filled grains were counted and weighed in g.

2. Seed coat (bran) colour

The observation was recorded at the growth stage-9 with the following code:

| | | | | | |
|---|---|----------------|---|---|-----------------|
| 1 | = | White | 5 | = | Red |
| 2 | = | Light brown | 6 | = | Variable purple |
| 3 | = | Speckled brown | 7 | = | Purple |
| 4 | = | Brown | | | |

3.5 Statistical and biometrical analysis

The data recorded during the present investigation was subjected to the following statistical and biometrical analysis.

- Analysis of variance and estimation of the components of variability,
- Estimation of heritability and expected genetic gain,
- Estimation of phenotypic and genotypic correlation coefficient,
- Divergence analysis

3.5.1 Analysis of variance and estimation of the components of variance

3.5.1.1 Analysis of variance

Analysis of variance for all the characters in simple square lattice design was carried out for testing variation among the genotypes. The characteristic of this design is that the number of treatments is a perfect square and the block size is the square root of the number of treatments.

The analysis of variance table was set up as under:

| Source of variation | d.f | SS | MSS | Fcal |
|---------------------|-------------|------|-----------------------|---------|
| Replication | r-1 | SSQr | MSR= SSQr/r-1 | |
| Treatments | T-1 | SSQt | MST=SSQt/T-1 | MST/MSE |
| Error | (r-1) (T-1) | SSQe | MSE= SSQe/(r-1) (T-1) | |
| Total | rt-1 | | | |

Where,

R = number of replications

K^2 = number of treatments

K = number of blocks

SS = Sum of squares

- MSS = Mean sum of squares
- SSQr = sum of squares due to replication
- MSR = Mean sum of squares due to replication
- MSE = Mean sum of squares due to intra-block error

The significance of varietal differences was tested by F-test comparing calculated F-value at 5 and 1 per cent level of significance at treatment (t-1) and error (t-1) (r-1) degrees of freedom. If the F-value was found to be significant, comparison was further extended to compare each treatment mean with every other treatment utilizing critical difference (C.D.), which was calculated by the following formula.

$$\text{Critical difference (C.D.)} = \frac{\text{Standard error of difference SE (d) x t (0.05) at error d.f.}}{= (2\text{MSe}/r)^{1/2} \text{ x t (0.05) at error d.f.}}$$

Where,

MSe= Error mean square

r = Number of replications

3.5.1.2 Genotypic variance

Genotypic variance was calculated using the method suggested by Johnson *et al.* (1955).

$$\hat{\sigma}^2_g = \frac{\text{MSG} - \text{MSE}}{r}$$

Where,

$\hat{\sigma}^2_g$ = Genotypic variance,

MSG = mean sum of squares due to genotypes,

MSE = mean sum of squares due to error, and

R = number of replications

3.5.1.3 Phenotypic variance

Phenotypic variance was calculated as per the procedure given by Johnson *et al.* (1955).

$$\hat{\sigma}^2 p = \hat{\sigma}^2 g + \hat{\sigma}^2 e$$

Where,

$\hat{\sigma}^2 p$ = Phenotypic variance

$\hat{\sigma}^2 g$ = genotypic variance, and

$\hat{\sigma}^2 e$ = error variance

3.5.1.5 Phenotypic and genotypic co-efficient of variation

The magnitude of phenotypic co-efficient of variation (PCV) and genotypic co-efficient of variation (GCV) existing in a trait was worked out by the formula given by Burton (1952) :

$$\text{PCV} = \frac{\sqrt{\hat{\sigma}^2 p}}{\bar{x}} \times 100$$

Where,

$\hat{\sigma}^2 p$ = Phenotypic variance, and

\bar{x} = grand mean of the trait studied

$$\text{GCV} = \frac{\sqrt{\hat{\sigma}^2 g}}{\bar{x}} \times 100$$

Where,

$\hat{\sigma}^2 g$ = Genotypic variance, and

\bar{x} = grand mean of the trait studied

3.5.2 Estimation of heritability genetic advance and expected genetic gain

3.5.2.1 Heritability (broad sense)

It was estimated as per the procedure presented by Burton and Devane

(1953), Johnson *et al.* (1955) and Hanson *et al.* (1956).

$$h^2 = \frac{\sigma^2_g}{\sigma^2_p}$$

Where,

h^2 = Estimate of heritability in broad sense,

σ^2_g = Genotypic variance, and

σ^2_p = Phenotypic variance

3.5.2.2 Genetic advance

Genetic advance at 5 per cent selection intensity was worked out by using the formula given by Lush (1949) and Johnson *et al.* (1955).

$$GA = \frac{\sigma^2_g}{\sigma^2_p} \times (\sigma^2_p)^{0.5} \times K$$

Where,

GA = Genetic advance of the trait,

σ^2_g = genotypic variance of the trait,

σ^2_p = phenotypic variance of the trait, and

K = selection differential; (K= 2.06 at 5 per cent selection intensity)

3.5.2.3 Expected genetic gain (genetic advance as per cent of mean)

It was estimated as per the method suggested by Johnson *et al.* (1955).

$$\text{Genetic gain} = \frac{GA}{\bar{X}} \times 100$$

Where,

G.A. = Genetic advance of the trait, and

\bar{X} = mean of the trait

3.5.3 Estimation of genotypic and phenotypic covariances and correlation coefficients

Covariance analysis followed the same pattern as the variance analysis. The genotypic and phenotypic covariances between two characters were obtained in the same fashion as corresponding variances. Estimate of genotypic and

phenotypic variances and covariances were substituted in the following formula suggested by Panse and Sukatme (1985) to calculate correlation co-efficient between all possible pairs of characters.

3.5.1 Genotypic correlation co-efficient

$$r_{xy} (g) = \frac{\hat{\sigma}^2_{xy} (g)}{\sqrt{\hat{\sigma}^2_x (g) \hat{\sigma}^2_y (g)}}$$

3.5.2 Phenotypic correlation coefficient

$$r_{xy} (p) = \frac{\hat{\sigma}^2_{xy} (p)}{\sqrt{\hat{\sigma}^2_x (p) \hat{\sigma}^2_y (p)}}$$

Where,

- $r_{xy} (g), r_{xy} (p)$ = Genotypic and phenotypic correlation coefficients, respectively, between a pair of characters x and y
- $\hat{\sigma}^2_{xy} (g), \hat{\sigma}^2_{xy} (p)$ = Genotypic and phenotypic covariances, respectively, for a pair of characters x and y
- $\hat{\sigma}^2_x (g), \hat{\sigma}^2_y (g)$ = Genotypic variance for characters x and y, respectively, and
- $\hat{\sigma}^2_x (p), \hat{\sigma}^2_y (p)$ = Phenotypic variance for character x and y, respectively.

3.5.3 Test of significance

The significance of a correlation co-efficient was tested by the following formula :

$$t = \frac{r (n - 2)^{0.5}}{(1 - r^2)^{0.5}}$$

Where,

- R = Correlation coefficient and
- N = number of observations

Any value (\pm) exceeding the table value of t at n-2 d.f is significant.

3.6 Estimates of genetic divergence

The genetic divergence was computed using the procedure as described by Rao (1952) and Singh and Choudhary (1985). The details of analysis are described under the following heads:

- 1) Test of Wilk's criterion,
- 2) Transformation of correlated variables,
- 3) Computation of D^2 values,
- 4) Relative contribution of individual characters towards total divergence, and
- 5) Group constellation

3.6.1 Test of Wilk's criterion

Variances and covariances were obtained from analysis of variance and covariance tables and the following analysis of dispersion table was constructed:

Analysis of dispersion

| Dispersion due to | d.f. | Matrix due to | | | |
|------------------------|----------------|----------------|----------------|-----------------|------------------|
| | | Sum of squares | | Sum of products | |
| | | X_1^2 | X_2^2, \dots | $X_1 X_2$ | $X_1 X_3, \dots$ |
| Replications | r-1 | A | b | C | d..... |
| Between treatments (Q) | Q | a' | b' | c' | d'..... |
| Within treatments (W) | By subtraction | A- (a+a') | B- (b+b') | C- (c+c') | D- (d+d').... |
| Total | N | A | B | C | D..... |

The determination of error and error + variety variance-covariance matrix were calculated by pivotal condensation method of using 'V' statistics which, in turn, utilizes Wilk's criteria. A simultaneous test of differences between mean values of characters from all the genotypes in the present study was performed, as per the details given below:

The Wilk's test is :

$$V = - m \log e \lambda$$

Where,

$$\lambda = \frac{W}{W + Q}$$

$$= \frac{\text{Determinant of error matrix}}{\text{Determinant of error + variety matrix}}$$

and,

$$m = n - \frac{q + k + 1}{2}$$

Where,

N = Total number of observations minus one,

Q = number of variable minus one, and

K = number of characters under study

'V' Statistics so obtained was compared with the tabulated value of χ^2 for 2qk degrees of freedom.

3.6.2 Transformation of correlated variables

Plot means of the varieties corresponding to the characters studied were transformed to uncorrelated variables by Pivotal Condensation Method, which rendered the computation of D^2 values between any combinations of two varieties to simple summation of squares of differences in transformed values for various characters. The skeleton procedure of obtaining transformed variables by Pivotal

Condensation Method is described below:

Let dispersion matrix of original variables x_1, x_2, \dots, x_p be

$$\begin{matrix} \lambda_{11} & \lambda_{12} & \dots & \lambda_{1p} \\ \lambda_{21} & \lambda_{22} & \dots & \lambda_{2p} \\ \cdot & \cdot & \dots & \cdot \\ \cdot & \cdot & \dots & \cdot \\ \cdot & \cdot & \dots & \cdot \\ \cdot & \cdot & \dots & \cdot \\ \cdot & \cdot & \dots & \cdot \end{matrix}$$

$$\lambda_{p1} \quad \lambda_{p2} \quad \dots \quad \lambda_{pp}$$

and consider the extended matrix

$$\begin{matrix} \lambda_{11} & \lambda_{12} & \dots & \lambda_{1p \times 1} \\ \lambda_{21} & \lambda_{22} & \dots & \lambda_{2p \times 2} \\ \cdot & \cdot & \dots & \cdot \\ \cdot & \cdot & \dots & \cdot \\ \cdot & \cdot & \dots & \cdot \\ \cdot & \cdot & \dots & \cdot \\ \cdot & \cdot & \dots & \cdot \end{matrix}$$

$$\lambda_{p1} \quad \lambda_{p2} \quad \dots \quad \lambda_{pp \times p}$$

taking λ_{11} as the first pivotal element, the first row is replaced by

$$1 \quad \frac{\lambda_{12}}{\lambda_{11}} \quad \dots \quad \frac{\lambda_{1p}}{\lambda_{11}} \quad \frac{x_1}{\lambda_{11}}$$

Sweeping out first column and using the first pivotal row, following reduced matrix is obtained

$$\left(\begin{matrix} \lambda_{22'} & \dots & \lambda_{2p'} \\ \cdot & & \cdot \\ \cdot & & \cdot \\ \cdot & & \cdot \\ \cdot & & \cdot \\ \lambda_{p2'} & \dots & \lambda_{pp'} \end{matrix} \right) \begin{matrix} (X_{2'}) \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ (X_{p'}) \end{matrix}$$

Where,

$$\lambda_{ij} = - \frac{\lambda_{ij}}{\lambda_{11}} \lambda_{ij} x_i$$

$$x_i = - \frac{\lambda_{i1}}{\lambda_{11}} X_1$$

$$\begin{aligned} \text{Now, } V_{(x_i)} &= V_{(x_i)} - \frac{2\lambda_{i1}}{\lambda_{11}} \text{Cov.}(x_i X_1) + \frac{2\lambda_{i1}}{\lambda_{11}} V(X_1) \\ &= \lambda_{ii} - \frac{\lambda_{i1}^2}{\lambda_{11}} \end{aligned}$$

$$\text{Now, } V_{(x_i')} = + \frac{\lambda_{i1}}{\lambda_{ii}} V(x_1)$$

$$\text{Similarly, Cov.}(x'_{i1} x'_j) = \lambda_{ij}'$$

$$\text{Similarly, Cov.}(x'_i x'_j) = \lambda_{ij}'$$

$$\begin{aligned} \text{also, cov.}(x_1 x'_i) &= \text{cov.}(x_1 x_i) - \frac{\lambda_{i1}}{\lambda_{11}} v(x_i) \\ &= \lambda_{i1} - \lambda_{i1} = 0 \end{aligned}$$

So the new variables are uncorrelated.

Considering the second pivotal row

$$\frac{\lambda_{23}}{\lambda_{22'}} \quad \frac{\lambda_{2p'}}{\lambda_{22'}} \quad \frac{x_2'}{22'}$$

the further reduced matrix is

$$\left| \begin{array}{ccc|ccc} \lambda_{33}'' & \dots & \lambda_{3p}'' & & & \lambda_{x3}'' \\ \cdot & & \cdot & & & \cdot \\ \cdot & & \cdot & & & \cdot \\ \cdot & & \cdot & & & \cdot \\ \lambda_{p3}'' & & \lambda_{pp}'' & & & x_p'' \end{array} \right|$$

resulting into variables

$$\begin{aligned} x_1' \ x_2' \ x_3'' \dots \dots \dots \text{ with variance} \\ x_{11}' \ x_{22}' \ \lambda_{33}'' \dots \dots \dots \end{aligned}$$

They are all mutually uncorrelated as shown above and further x'_2 , depends on x_1 and x_2' , and x_3 on x_1' , x_2 and x_3 only.

3.6.3 Computation of D^2 values

For each pair-wise combination of the varieties the differences in transformed values for various characters were computed and D^2 -values were calculated according to the following formula:

$$D^2 = \sum_{i=1}^p (\bar{Y}_{ij} - Y_{ik})^2$$

Where,

- P = number of characters studied, and
 Y_{ij} and Y_{ik} = are two transformed variables of the i^{th} character for two genotypes

3.6.4 Relative contribution of individual characters towards total divergences

The ranking of differences in uncorrelated means between all the characters for all pair-wise combinations of varieties was carried out, with first rank being assigned to the highest differences. Finally relative contribution of a character towards total divergence was estimated by calculating the percentage of first rank in that character.

3.6.5 Group constellation

Tocher's method was used for assigning various varieties to different clusters. The two varieties having smallest distance from each other were considered first to which a third variety having smallest average D^2 value from the first two varieties was added. Next come the nearest fourth variety and the process continued till the average D^2 value increased. The remaining varieties were then considered for the next cluster and the process was continued till all varieties were included in various clusters.

The spatial distances between clusters were arrived at by taking square root of average intra and inter cluster D^2 values.

For each combination (pair of genotypes) the mean deviation (d^2_i) i.e. $Y_1 - Y_1$ with $I = 1, 2, 3 \dots \dots \dots p$ was computed and D^2 values were calculated as sum of these deviations i.e. $(y_i^1 - y_i^2)$, where, y_i is the transformed variable from the original variable x_i . Accordingly D^2 values for all combinations were calculated. The D^2 values so obtained for each pair of population were treated as x^2 and were tested against the tabulated values of λ^2 for p degrees of freedom, where p is the number of traits considered.

In all combinations each character was ranked on the basis of $d_i = y_{ij} - y_{ik}$ values. Rank 1 was given to the highest mean difference and rank p to the lowest mean difference, where p is the total number of characters. In this manner contribution of each character to the total divergence was computed.

Tocher method for grouping of varieties into various clusters was adopted. This method is detailed in a simplified way by Rao (1952) and Singh and Choudhary (1985).

All the above computations were carried out using the software Indostat at Bio-informatics Laboratory of the Division of Plant Breeding and Genetics, SKUAST-Kashmir, Shalimar.

3.7 Molecular characterization

The plant material used for molecular characterization are given in the Table-2.

Table 2: List of genotypes used for molecular characterization

| S. No. | Name of the genotype |
|---------------|-----------------------------|
| 1 | Calaro |
| 2 | IRBN 1-CL/EC 565160 |
| 3 | SKAU 356 |
| 4 | IR61728-413-2-1-1 |
| 5 | RIL-10 |
| 6 | Shalimar Rice-1 |
| 7 | Pusa Sugand-3 |
| 8 | SKAU-389 |
| 9 | B-9 |
| 10 | Gureh |
| 11 | SKAU-408, |
| 12 | Rasi |
| 13 | C101PKT |
| 14 | B-14 |
| 15 | Shalimar Rice -2 |
| 16 | Tumlahaal |
| 17 | C101A51/ARBN 152 |
| 18 | A57-115-4/ARBN 148 |
| 19 | SK-336 |
| 20 | China-1031 |
| 21 | IR64 |
| 22 | Shalimar Rice -3 |
| 23 | IR68333-RR-B-19 |
| 24 | SKAU-337 |
| 25 | IRBL Z-FU/EC 565188 |
| 26 | B-4 |
| 27 | A-57, |
| 28 | Niver Zag |
| 29 | IRBN5M/EC 565166 |
| 30 | KHD/GP/27 |
| 31 | Baber |
| 32 | KHD/GP/150 |

3.7.1 Methods adopted

The present investigation was carried out under different headings.

3.7.1.1 Genomic DNA extraction

Plant DNA was isolated using CTAB (Cetyl Trimethyl Ammonium Bromide) method as modified by Saghai-Marroof *et al.* (1994). In this young leaves at 5 leaf stage were harvested (approximately 5-7g of fresh weight). Harvested leaves were placed in glassine bags transported in ice and stored in -20°C. The leaves were ground to fine powder using pre-chilled pestle and mortar after adding liquid nitrogen to make leaves brittle as well as to stop DNase activity. The powder was transferred immediately to a 50 ml autoclaved polypropylene centrifuge tube containing 15 ml of pre-warmed (65°C) 2X CTAB extraction buffer. The composition of extraction buffer is given in Table 3. The powder was suspended in the buffer by inverting and rotating the tubes properly. The tubes were incubated at 65°C for 30-40 minutes in a water bath. The samples were mixed occasionally while maintaining a temperature of 65 ±1°C. After incubation, 15ml of chloroform: isoamyl alcohol (24:1) was added and tubes were swirled, till it made a dark green emulsion. The tubes were placed on a rotary shaker for 30 minutes and then centrifuged at 10,000 rpm for 10 minutes at room temperature. The supernatant was transferred to a clean sterile 50 ml Falcon tube. 5µl RNase (10mg/ml) was added to each tube and incubated at 37°C in water bath for 1 hour. Chloroform: isoamyl alcohol extraction and centrifugation step was repeated after RNase treatment, if required.

Table-3 : Composition of 2X CTAB Extraction Buffer

| Components | Final concentration |
|-------------------|----------------------------|
| CTAB | 2% |
| Tris HCl | 200 mM |
| NaCl | 1.4 M |
| EDTA | 20 mM |
| Mercaptoethanol | 0.2% |

Following centrifugation, the upper aqueous phase was transferred to a clean sterile 50 ml Falcon tube. About 0.8 volume of chilled isopropyl alcohol was added and the tubes were inverted gently several times. The DNA formed white cotton like precipitate and good quality DNA floated atop. The floating DNA was hooked out using a sterile hooked Pasteur pipette. If the DNA was not hookable, it was pelleted by centrifugation. The hooked or pelleted DNA was transferred into a clean sterile 2.0 ml microfuge tubes and was rinsed with 70 per cent ethanol for five minutes so as to remove any residual salts followed by re-centrifugation. Pellet was collected and the leftover ethanol was dried up completely by turning down microfuge tubes on a blotting paper and was allowed to air dry (at room temperature) for one hour. Then added 500-800 μ l volume of 1X TE (Tris EDTA buffer-10mM Tris HCl, 1mM EDTA, pH 8.0). The tubes were left for few hours at room temperature to allow DNA to dissolve.

3.7.1.2 Assessment of quality and quantity of DNA

Quantity of DNA was checked by Agarose gel electrophoresis. In this 0.8 g of agarose was dissolved in 100 ml of 1X TAE electrophoresis buffer (Tris base-45 mM, Acetic acid- 45 mM and EDTA- 1mM). The mixture was heated till the agarose was dissolved completely i.e. when solution became transparent and clear. It was cooled down to 60°C with constant stirring. Ethidium bromide was added to a final concentration of 0.5 μ g/ml of buffer. The agarose solution was

poured into an already prepared gel mould with combs and was left for 20-30 min for solidification. DNA samples for loading were prepared by adding 2 μ l loading dye (6X) (0.25% w/v bromophenol blue, 50% glycerol in sterile water) to 8 μ l DNA. Sterile water was added to make the volume (100 μ l) to the DNA such that the final concentration of loading dye was 1X. The DNA samples were loaded into wells with the help of micropipette. Along with the DNA samples, marker of known concentration (uncut λ DNA of 50 ng/ μ l concentration) was also loaded. The gel was run for about 1-2 hours at voltage of 5 V/cm. The gel was then visualized under UV transilluminator. Using photo Gel - documentation system and the DNA samples were photographed. The intensity of fluorescence of each sample was compared with that of a standard marker and then DNA concentration of each sample was ascertained. The quality of DNA samples was judged based on whether DNA formed a single high molecular weight band (good quality) or a smear (degraded/poor quality).

3.7.1.3 SSR analysis

3.7.1.3.1 Selection of primers

The SSR markers used in the present study were selected from www.gramene.org. The selected microsatellite markers along with their chromosomal location are presented in the Table-4.

3.7.1.3.2 PCR amplification

In vitro amplification using polymerase chain reaction (PCR) was performed in a 96 well microtiter plate in a Bio-Rad or Eppendorf master cycler using 75 ng of genomic DNA of each genotype in a final volume of 20 μ l per reaction. The stock and final concentration of different components that was used in PCR is given in Table-5.

Table 4: List of selected primers along with their primer sequence and chromosome number

| SSR | Chr. No. | Forward 5'-3' | Reverse 3'-5' |
|-------|----------|----------------------------|------------------------|
| RM104 | 1 | GGAAGAGGAGAGAAAGATGTGTGTCG | TCAACAGACACACCGCCACCGC |
| RM202 | 11 | CAGATTGGAGATGAAGTCCTCC | CCAGCAAGCATGTCAATGTA |
| RM245 | 9 | ATGCCGCCAGTGAATAGC | CTGAGAATCCAATTATCTGGGG |
| RM125 | 7 | ATCAGCAGCCATGGCAGCGACC | AGGGGATCATGTGCCGAAGGCC |
| RM287 | 11 | TTCCCTGTTAAGAGAGAAATC | GTGTATTTGGTGAAAGCAAC |
| RM530 | 2 | GCACTGACCACGACTGTTTG | ACCGTAACCCGGATCTATCC |
| RM55 | 3 | CCGTCGCCGTAGTAGAGAAG | TCCCGGTTATTTAAGGCG |
| RM555 | 2 | TTGGATCAGCCAAAGGAGAC | CAGCATTGTGGCATGGATAC |

Table-5: Stock and final concentration of different components used in PCR

| Components | Stock Conc. | Volume (μ l) | Final Conc. |
|-------------------|---------------|-------------------|--------------|
| Water | - | 3.8 | - |
| PCR buffer | 10X* | 2.0 | 1X |
| MgCl ₂ | 25mM | 1.2 | 1.5mM |
| dNTPs | 1mM | 4.0 | 100 μ M |
| Primer Forward | 5 μ M | 1.0 | 0.25 μ M |
| Primer Reverse | 5 μ M | 1.0 | 0.25 μ M |
| Taq Polymerase | 5U/ μ l | 0.2 | 1Unit |
| DNA template | 15ng/ μ l | 5 | 75ng |
| Total | | 20 | |

*10X PCR buffer: 10mM TrisHCl, pH 8.3, 50mM KCl, 1.5mM MgCl₂, 0.01 % Gelatin

The reaction mixture was overlaid with a drop of low molecular weight mineral oil (SIGA) and placed in 96 well thermal cycler. Amplification was performed using temperature profile mentioned in the Table.

Table-6 : Temperature profile used in PCR

| Step | Temperature | Time (minutes) | No. of cycles |
|----------------------|-------------|----------------|---------------|
| Initial denaturation | 94 | 4 | 1 |
| Denaturation | 94 | 0:30 | 35 |
| Annealing | 45-60 | 1 | |
| Elongation | 72 | 1 | |
| Final Extension | 72 | 7 | 1 |
| Hold | 12 | | |

3.7.1.3.3 Scoring of SSR allele profile

The SSR allele sizes was determined by the position of bands relative to the DNA ladder. Total number of alleles was recorded for each microsatellite marker in all the genotypes under study by giving the number to amplified alleles as 1,2,3,4 and so on. Number 1 was given to the allele having highest molecular weight. The amplified bands were recorded as 1 (band present) and 0 (band absent) in a binary matrix. The accession that did not show any amplification were scored as missing values if amplification was not repeated and as null alleles if the amplification was repeated 2-3 times. If the band appeared in the negative control the whole PCR reaction experiment was repeated.

3.7.1.3.4 Polymorphism information content

The PIC values as described by Botstein *et al.* (1980) were used to refer to the relative value of each marker with respect to the amount of polymorphism exhibited. PIC values for each of the primers were estimated using formula given by Nei (1973).

$$PIC = 1 - \sum_{i=1}^N (P_{ij})^2$$

Where P_{ij} is the frequency of j^{th} allele in i^{th} primer and summation extends over 'n' patterns. PIC is synonymous with the term 'gene diversity' as described by Weir (1990). The PIC takes into account not only the number of alleles that are expressed but also the relative frequencies of those alleles (Smith *et al.*, 1997).

3.7.1.3.5 Cluster analysis

Cluster analysis of the germplasm, using binary data generated by microsatellite markers, was conducted using computer software programme Numerical Taxonomic and Multivariate Analysis System (NTSYS-pc) version 2.02e (Rohlf, 1997). Microsatellite marker amplification profile for all the genotypes under study was compared to each other and DNA fragments were scored as present (scored as 1) or absent (scored as 0). Similarity was estimated using SIMQUAL function of NTSYS, which computes a variety of similarity coefficient for qualitative data (nominal data). Similarity matrix value based on Nei and Li (1979) coefficient of similarity (D_{ij}) were calculated as

$$D_{ij} = 2a/(2a+b+c)$$

Where 'a' represents matched fragments, b and c are unmatched fragments. The $2a+b+c$ are the total number of fragments amplified in a particular set. The similarity matrix was thus generated and Dendrogram was constructed using UPGA (Unweighted pair Group Method using Arithmetic Averages) available in NTSYS.

3.7.1.3.6 Data analysis

The amplified products from SSR marker analysis were scored quantitatively for presence or absence for each marker allele-genotype combination. The data were entered into a binary matrix as discrete variables.

Polymorphism information content (PIC) value of a marker was

calculated according to the following formula of Anderson *et al.* (1993).

$$PIC = 1 - \sum_{j=1}^n p_{ij}^2$$

Where, p_{ij} in the frequency of j^{th} allele for the i^{th} marker, and summed over on alleles PIC value exceeding 0.5 indicated presence of polymorphism for the alleles.

Pair wise comparisons of the cultivars based on the proportion of alleles were used to measure the genetic similarity. Estimates of genetic similarity (measuring genetic distance) were calculated between all pairs of the genotypes according to Nei and Li (1979) based on the formula:

$$\text{Similarity}(F) = 2 \text{ Nab}/(\text{Na}+\text{Nb})$$

Where,

- Nab : Number of fragments shared by the genotypes a and b,
- Na : Total number of fragments detected in genotype a, and
- Nb : Total number of fragments detected in genotype b.

The resultant similarity matrix ($n \times n$), where n is the number of genotypes studied, was employed to construct a Dendrogram based on the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) using Sequential Agglomerative Hierarchical Nesting (SAHN) module. Genetic similarities were estimated from the matrix of binary data using jaccard similarity coefficient. The similarity coefficients (reverse genetic distance) were used to construct a Dendrogram. Based on the analysis of SSR marker data, the genotypes were classified into different clusters to assess the genetic diversity resulting at the molecular level.

Chapter - 4

EXPERIMENTAL FINDINGS

The present investigation entitled “Identification and morpho-molecular characterization of genotypes of rice (*Oryza sativa* L.) showing resistance to sheath blight disease” was undertaken to elicit information on resistance/tolerance of 102 rice genotypes to sheath blight disease caused by *Rhizoctonia solani*. The material for the study was used to record observations on various aspects, as per the methodology detailed in Chapter-3. The data collected was subjected to appropriate statistical and biometrical analysis. The inferences drawn as experimental findings are presented in this chapter.

4.1 Screening of genotypes for disease resistance/tolerance

The genotypes in the present study were screened for resistance/tolerance to rice sheath blight disease. Among all the genotypes screened the highest disease incidence (100%) and intensity (91.34%) was recorded in Pusa Sugand-3 followed by the genotype Pusa Sugand-5 (100% and 90.12%, respectively) while the lowest disease incidence (10%) and intensity (1.11) was recorded in Niver Zag. Perusal to the Table-7 revealed that none of the genotypes was immune, while 33 genotypes were resistant viz. Niver Zag, Shalimar Rice-1, IR68333-RR-B-19, KHD/GP/150, IRBL1-CL/EC 565160, IR64, Shalimar Rice-3, RIL-10, Gurah, A-57, SKAU-389, B-4, IRBL 5M/EC 565166, SKAU-429, SKAU-408, C101PKT, SKAU-356, Calaro, C101A51/ARBN 152, IR61728-413-2-1-1, TUMLIHAAL, Baber, B-14, SKAU-337, KHD/GP/27, China-1031, B-9, Shalimar Rice-2, A57-115-4/ARBN 148, Shai Tai Tsau, C104PKT, SKAU-336 and K-225 and 8 were moderately resistant viz. BL-122, Rasi, KHD/GP/120, NP 125, Usen, C101A51, Gulbara and T2 (MAS 71). Thus, about 40.19 per cent genotypes showed resistance. Of the remaining 61 genotypes 8 were moderately susceptible, 36 were susceptible and 17 were highly susceptible viz. Pusa Sugand-3, Pusa Sugand-5, Local Budgam-1, Wazul Kew, SKAU-39, SKAU-292, B-5, SKAU-98, Shalaken, BL-245, C105TTP.4L23, Chenab, KHD/GP/208, K-116, B-19, SKAU-338 and Yunlen-19.

Table-7 : Incidence and intensity of sheath blight disease in rice genotypes

| S. No. | Genotype | Disease | | | |
|---|-----------------|---------------|---------------|-------------|----------|
| | | Incidence (%) | Intensity (%) | Score (0-9) | Reaction |
| Local Landraces | | | | | |
| 1 | Gulbara | 50 | 26.66 | 3 | MR |
| 2 | Tumlahaal | 10 | 5.55 | 1 | R |
| 3 | Gurah | 10 | 3.3 | 1 | R |
| 4 | Baber | 10 | 6.34 | 1 | R |
| 5 | Wazul krea | 70 | 77.34 | 9 | HS |
| 6 | Mushkbudji | 60 | 50.00 | 7 | S |
| 7 | Niver Zag | 10 | 1.11 | 1 | R |
| 8 | Local Budgam 1 | 80 | 77.77 | 9 | HS |
| 9 | Local Budgam 2 | 80 | 59.67 | 7 | S |
| 10 | Local Budgam 3 | 80 | 63.65 | 9 | S |
| Released Varieties/Genotypes of SKUAST | | | | | |
| 1 | SKAU-429 | 10 | 4.67 | 1 | R |
| 2 | SKAU-356 | 10 | 5.50 | 1 | R |
| 3 | Shalimar Rice-1 | 10 | 2.22 | 1 | R |
| 4 | KHD/GP/120 | 50 | 22.22 | 3 | MR |
| 5 | KHD/GP/27 | 10 | 6.66 | 1 | R |
| 6 | Shalimar Rice-3 | 10 | 3.00 | 1 | R |
| 7 | China-1031 | 10 | 6.87 | 1 | R |
| 8 | SKAU-336 | 40 | 17.56 | 1 | R |
| 9 | SKAU-2 | 40 | 31.11 | 5 | MS |
| 10 | SKAU-337 | 10 | 6.66 | 1 | R |
| 11 | Pusa Sugand-3 | 100 | 91.34 | 9 | HS |
| 12 | SKAU-408 | 10 | 4.67 | 1 | R |
| 13 | SKAU-389 | 10 | 4.07 | 1 | R |

Contd...

Table-7 contd...

| S. No. | Genotype | Disease | | | |
|--------|------------------|---------------|---------------|-------------|----------|
| | | Incidence (%) | Intensity (%) | Score (0-9) | Reaction |
| 14 | Shalimar Rice -2 | 10 | 7.45 | 1 | R |
| 15 | KHD/GP/150 | 10 | 2.34 | 1 | R |
| 16 | Shalkew | 80 | 74.76 | 9 | HS |
| 17 | K-332 | 30 | 38.89 | 5 | MS |
| 18 | K-116 | 80 | 70.00 | 9 | HS |
| 19 | Jehlum | 70 | 61.34 | 7 | S |
| 20 | SKAU-403 | 60 | 55.56 | 7 | S |
| 21 | Chenab | 80 | 72.67 | 9 | HS |
| 22 | SKAU-339 | 60 | 58.34 | 7 | S |
| 23 | China-1039 | 70 | 64.36 | 7 | S |
| 24 | SKAU-292 | 80 | 76.55 | 9 | HS |
| 25 | China-988 | 70 | 63.99 | 7 | S |
| 26 | SKAU-338 | 80 | 70.00 | 9 | HS |
| 27 | SKAU-39 | 90 | 76.88 | 9 | HS |
| 28 | China-1007 | 50 | 53.67 | 7 | S |
| 29 | Pusa sugand-5 | 100 | 90.12 | 9 | HS |
| 30 | SKAU-46 | 60 | 57.45 | 7 | S |
| 31 | SKAU-402 | 70 | 62.99 | 7 | S |
| 32 | SKAU-404 | 60 | 51.34 | 7 | S |
| 33 | SKAU-3 | 60 | 54.23 | 7 | S |
| 34 | SKAU-98 | 80 | 75.22 | 9 | HS |
| 35 | SKAU-405 | 60 | 56.44 | 7 | S |
| 36 | China-972 | 60 | 47.32 | 7 | S |
| 37 | Kamad | 50 | 48.23 | 7 | S |

Contd...

Table-7 contd...

| S. No. | Genotype | Disease | | | |
|---------------------------------------|---------------------|---------------|---------------|-------------|----------|
| | | Incidence (%) | Intensity (%) | Score (0-9) | Reaction |
| Other genotypes from IRRI etc. | | | | | |
| 1 | Calaro | 10 | 5.55 | 1 | R |
| 2 | C101A51 | 50 | 24.67 | 3 | MR |
| 3 | C101PKT | 10 | 5.00 | 1 | R |
| 4 | IR61728-413-2-1-1 | 10 | 5.55 | 1 | R |
| 5 | Shai Tai Tsau | 30 | 16.66 | 1 | R |
| 6 | C101A51/ARBN 152 | 10 | 5.55 | 1 | R |
| 7 | BL-122` | 30 | 20.67 | 3 | MR |
| 8 | Rasi | 40 | 22.22 | 3 | MR |
| 9 | IR64 | 10 | 2.22 | 1 | R |
| 10 | IR68333-RR-B-19 | 10 | 2.22 | 1 | R |
| 11 | Kanto-51 | 60 | 50.00 | 7 | S |
| 12 | RIL-10 | 10 | 3.33 | 1 | R |
| 13 | IRBL1-CL/EC 565160 | 10 | 2.22 | 1 | R |
| 14 | IRBL 5M/EC 565166 | 10 | 4.44 | 1 | R |
| 15 | A57-115-4/ARBN 148 | 10 | 7.57 | 1 | R |
| 16 | B-4 | 10 | 4.67 | 1 | R |
| 17 | IRBN KS-S/EC 565178 | 50 | 38.88 | 5 | MS |
| 18 | C104PKT | 40 | 16.86 | 1 | R |
| 19 | A-57 | 10 | 3.67 | 1 | R |
| 20 | B-9 | 10 | 7.03 | 1 | R |
| 21 | B-14 | 10 | 4.34 | 1 | R |
| 22 | RIL-29 | 60 | 37.99 | 5 | MS |
| 23 | Usen | 40 | 24.67 | 3 | MR |
| 24 | Raminad Str 3 | 80 | 64.67 | 7 | S |
| 25 | BL 245 | 70 | 74.34 | 9 | HS |
| 26 | BL-42 | 30 | 34.78 | 5 | MS |
| 27 | SAFED BREZ | 60 | 46.88 | 7 | S |

Contd...

Table-7 contd..

| S. No. | Genotype | Disease | | | |
|--------|---------------------|---------------|---------------|-------------|----------|
| | | Incidence (%) | Intensity (%) | Score (0-9) | Reaction |
| 28 | KHD/GP/154 | 40 | 43.82 | 5 | MS |
| 29 | T2 (MAS 71) | 40 | 26.66 | 3 | MR |
| 30 | NP 125 | 30 | 23.33 | 3 | MR |
| 31 | IRBN 2008 V-87 | 70 | 53.67 | 7 | S |
| 32 | Dular | 70 | 45.45 | 5 | MS |
| 33 | CO-39 | 50 | 50.00 | 7 | S |
| 34 | BL-122/ARBN 141 | 60 | 55.78 | 7 | S |
| 35 | BL-142/ARBN 142 | 60 | 56.78 | 7 | S |
| 36 | IRBL Z-FU/EC 565188 | 70 | 64.11 | 7 | S |
| 37 | KHD/GP/208 | 80 | 72.34 | 9 | HS |
| 38 | T1 (MAS 70) | 30 | 34.78 | 5 | MS |
| 39 | B-26 | 80 | 64.67 | 7 | S |
| 40 | B-10 | 60 | 54.56 | 7 | S |
| 41 | B-13 | 80 | 62.23 | 7 | S |
| 42 | B-5 | 80 | 76.11 | 9 | HS |
| 43 | B-3 | 60 | 53.56 | 7 | S |
| 44 | B-19 | 80 | 70.00 | 9 | HS |
| 45 | B-1509 | 70 | 55.38 | 7 | S |
| 46 | B-28 | 60 | 53.33 | 7 | S |
| 47 | B-32 | 70 | 58.78 | 7 | S |
| 48 | B-8 | 50 | 51.11 | 7 | S |
| 49 | B-11 | 50 | 56.33 | 7 | S |
| 50 | B-24 | 60 | 46.78 | 7 | S |
| 51 | YUNLEN-19 | 80 | 67.34 | 9 | HS |
| 52 | A3(IRRI LINE) | 40 | 31.56 | 5 | MS |
| 53 | C105TTP.4L23 | 80 | 71.56 | 9 | HS |
| 54 | IR63347-AL-201-1 | 70 | 60.99 | 7 | S |
| 55 | K-225 | 40 | 18.88 | 1 | R |

4.2 Morphological characterization

One hundred two genotypes were evaluated for morphological characteristics as per the standard procedure of the International Rice Research Institute, Philippines. A general scale with index value 0-9 was adopted for most of the traits. This scale generally provides information on the economic worth of the genotypes. Trait expression with a score of three or less is considered to be desirable for use as a parent for hybridization/commercial variety. Such genotypes express nearly full biological potential for much traits under all levels of stresses (diseases, insect pests, soil disorders, climatic variables, etc.). Traits with a score of 4-6 could be acceptable under certain limitations, for commercial cultivars, but traits with such a score are not considered for genetic improvement through introgression. Traits exhibiting a score of 7-9 are considered undesirable.

The morphological description of the genotypes in the present study with respect to five characters vis-a-vis, flag leaf angle, panicle type, awing, panicle threshability and seed coat colour is presented in the Table-8.

Perusal of the results (Table-8) revealed that genotypes with erect flag leaf angle were 45 (44.11%) and intermediate 32 (31.37%). Similarly horizontal flag leaf angle was recorded in 25 (24.50%) genotypes. Panicle type was compact in 56 (54.90%) and intermediate in 32 (31.37%) genotypes. It was open in 14 (13.72%) genotypes. Awning pattern revealed that it was absent in 87 (85.29%), short and partly awned in 9 (8.82%), short and fully awned in 3 (2.94%) and long and partially awned in 1 (0.98%). Seed coat colour was white in 23 (22.54%), light brown in 47 (39.16%) speckled brown colour in 3 (2.94%) genotypes and brown in 27 (26.47%). Panicle threshability was difficult in 7 (6.86%) genotypes. It was moderately in 31 (30.39%), intermediate in 49 (48.03%) and loose in 15 (14.70%) genotypes.

Table 8: Morphological characters recorded in the rice genotypes

| S. No. | Name of the genotype | Flag leaf angle | Panicle type | Awning | Panicle Threshability | Seed coat colour |
|---|----------------------|-----------------|--------------|--------|-----------------------|------------------|
| Local Landraces | | | | | | |
| 1 | Gulbara | 5 | 3 | 7 | 7 | 2 |
| 2 | Tumlahaal | 5 | 1 | 9 | 3 | 4 |
| 3 | Gurah | 5 | 1 | 5 | 7 | 2 |
| 4 | Baber | 1 | 1 | 0 | 3 | 2 |
| 5 | Wazul krea | 5 | 1 | 0 | 3 | 1 |
| 6 | Mushkbudji | 5 | 1 | 0 | 1 | 3 |
| 7 | Niver Zag | 5 | 3 | 0 | 7 | 2 |
| 8 | Local Budgam 1 | 3 | 1 | 0 | 3 | 1 |
| 9 | Local Budgam 2 | 3 | 2 | 0 | 5 | 4 |
| 10 | Local Budgam 3 | 1 | 1 | 0 | 3 | 2 |
| Released Varieties/Genotypes of SKUAST | | | | | | |
| 1 | SKAU-429 | 3 | 2 | 0 | 7 | 2 |
| 2 | SK-356 | 5 | 3 | 0 | 7 | 2 |
| 3 | Shalimar Rice-1 | 5 | 2 | 0 | 5 | 2 |
| 4 | KHD/GP/120 | 3 | 3 | 0 | 7 | 2 |
| 5 | KHD/GP/27 | 5 | 3 | 0 | 7 | 2 |
| 6 | Shalimar Rice-3 | 5 | 2 | 0 | 5 | 4 |
| 7 | China-1031 | 5 | 2 | 0 | 5 | 2 |
| 8 | SKAU-336 | 1 | 3 | 0 | 5 | 2 |
| 9 | SKAU-2 | 1 | 1 | 0 | 5 | 2 |
| 10 | SKAU-337 | 1 | 1 | 0 | 5 | 2 |
| 11 | Pusa Sugand-3 | 1 | 1 | 0 | 1 | 2 |
| 12 | SKAU-408 | 1 | 2 | 0 | 1 | 2 |
| 13 | SKAU-389 | 1 | 1 | 0 | 5 | 2 |
| 14 | Shalimar Rice -2 | 3 | 2 | 0 | 5 | 2 |
| 15 | KHD/GP/150 | 1 | 1 | 0 | 3 | 2 |
| 16 | Shalkew | 3 | 1 | 0 | 5 | 3 |
| 17 | K-332 | 3 | 1 | 1 | 3 | 1 |
| 18 | K-116 | 5 | 5 | 5 | 5 | 2 |

Contd.

Table-8 contd..

| S. No. | Name of the genotype | Flag leaf angle | Panicle type | Awning | Panicle Threshability | Seed coat colour |
|---------------------------------------|----------------------|-----------------|--------------|--------|-----------------------|------------------|
| 19 | Jehlum | 1 | 1 | 0 | 5 | 1 |
| 20 | SKAU-403 | 3 | 2 | 0 | 5 | 1 |
| 21 | Chenab | 3 | 1 | 0 | 1 | 1 |
| 22 | SKAU-339 | 3 | 1 | 0 | 5 | 2 |
| 23 | China-1039 | 3 | 1 | 5 | 7 | 1 |
| 24 | SKAU-292 | 5 | 2 | 0 | 7 | 4 |
| 25 | China-988 | 5 | 1 | 0 | 7 | 1 |
| 26 | SK-338 | 1 | 1 | 0 | 3 | 4 |
| 27 | SKAU-39 | 3 | 1 | 0 | 5 | 2 |
| 28 | China-1007 | 5 | 1 | 0 | 7 | 1 |
| 29 | Pusa sugand-5 | 1 | 1 | 0 | 1 | 1 |
| 30 | SKAU-46 | 1 | 1 | 0 | 3 | 1 |
| 31 | SKAU-402 | 1 | 1 | 0 | 3 | 4 |
| 32 | SKAU-404 | 3 | 2 | 0 | 5 | 2 |
| 33 | SKAU-3 | 1 | 1 | 0 | 3 | 1 |
| 34 | SKAU-98 | 1 | 1 | 0 | 5 | 4 |
| 35 | SKAU-405 | 3 | 2 | 0 | 5 | 4 |
| 36 | China-972 | 5 | 1 | 0 | 7 | 1 |
| 37 | Kamad | 3 | 1 | 0 | 3 | 1 |
| Other genotypes from IRRI etc. | | | | | | |
| 1 | Calaro | 1 | 1 | 0 | 5 | 2 |
| 2 | C101A51 | 1 | 1 | 0 | 5 | 4 |
| 3 | C101PKT | 1 | 1 | 0 | 5 | 4 |
| 4 | IR61728-413-2-1-1 | 3 | 1 | 0 | 5 | 2 |
| 5 | Shai Tai Tsau | 1 | 1 | 0 | 5 | 4 |
| 6 | C101A51/ARBN 152 | 5 | 3 | 0 | 3 | 4 |
| 7 | BL-122` | 5 | 1 | 0 | 3 | 2 |
| 8 | Rasi | 1 | 2 | 0 | 5 | 2 |
| 9 | IR64 | 5 | 3 | 7 | 7 | 2 |
| 10 | IR68333-RR-B-19 | 5 | 3 | 0 | 5 | 2 |

Contd...

Table-8 contd...

| S. No. | Name of the genotype | Flag leaf angle | Panicle type | Awning | Panicle Threshability | Seed coat colour |
|--------|----------------------|-----------------|--------------|--------|-----------------------|------------------|
| 11 | Kanto-51 | 3 | 2 | 0 | 5 | 1 |
| 12 | RIL-10 | 5 | 3 | 0 | 5 | 4 |
| 13 | IRBL1-CL/EC 565160 | 5 | 3 | 0 | 7 | 4 |
| 14 | IRBL 5M/EC 565166 | 5 | 3 | 0 | 5 | 2 |
| 15 | A57-115-4/ARBN 148 | 5 | 2 | 0 | 5 | 2 |
| 16 | B-4 | 3 | 3 | 0 | 3 | 1 |
| 17 | IRBN KS-S/EC 565178 | 1 | 2 | 0 | 3 | 2 |
| 18 | C104PKT | 1 | 2 | 0 | 3 | 2 |
| 19 | A-57 | 3 | 2 | 0 | 5 | 2 |
| 20 | B-9 | 3 | 2 | 0 | 5 | 4 |
| 21 | B-14 | 3 | 2 | 1 | 5 | 2 |
| 22 | RIL-29 | 1 | 1 | 0 | 5 | 4 |
| 23 | Usen | 1 | 2 | 0 | 3 | 2 |
| 24 | Raminad Str 3 | 1 | 1 | 0 | 1 | 3 |
| 25 | BL 245 | 1 | 1 | 1 | 3 | 4 |
| 26 | BL-42 | 3 | 2 | 0 | 3 | 1 |
| 27 | SAFED BREZ | 3 | 1 | 9 | 1 | 1 |
| 28 | KHD/GP/154 | 1 | 1 | 0 | 5 | 4 |
| 29 | T2 (71) | 1 | 1 | 0 | 3 | 2 |
| 30 | NP 125 | 1 | 1 | 0 | 5 | 4 |
| 31 | IRBN 2008 V-87 | 5 | 1 | 0 | 3 | 4 |
| 32 | Dular | 5 | 3 | 0 | 5 | 4 |
| 33 | CO-39 | 5 | 1 | 0 | 7 | 1 |
| 34 | BL-122/ARBN 141 | 1 | 1 | 0 | 3 | 2 |

Contd...

Table-8 contd...

| S. No. | Name of the genotype | Flag leaf angle | Panicle type | Awning | Panicle Threshability | Seed coat colour |
|--------|----------------------|-----------------|--------------|--------|-----------------------|------------------|
| 35 | BL-142/ARBN 142 | 1 | 1 | 0 | 3 | 1 |
| 36 | IRBL Z-FU/EC 565188 | 3 | 2 | 0 | 5 | 1 |
| 37 | KHD/GP/208 | 1 | 1 | 0 | 5 | 4 |
| 38 | T1 (MAS 70) | 1 | 1 | 0 | 3 | 2 |
| 39 | B-26 | 1 | 1 | 1 | 3 | 4 |
| 40 | B-10 | 1 | 1 | 0 | 5 | 4 |
| 41 | B-13 | 3 | 2 | 0 | 5 | 1 |
| 42 | B-5 | 1 | 1 | 1 | 3 | 4 |
| 43 | B-3 | 3 | 2 | 0 | 5 | 2 |
| 44 | B-19 | 3 | 2 | 0 | 5 | 2 |
| 45 | B-1509 | 1 | 1 | 1 | 3 | 4 |
| 46 | B-28 | 3 | 2 | 0 | 5 | 4 |
| 47 | B-32 | 1 | 1 | 0 | 5 | 4 |
| 48 | B-8 | 1 | 1 | 1 | 3 | 1 |
| 49 | B-11 | 3 | 2 | 0 | 5 | 2 |
| 50 | B-24 | 1 | 3 | 0 | 3 | 2 |
| 51 | YUNLEN-19 | 1 | 1 | 1 | 3 | 1 |
| 52 | A3(IRRI LINE) | 3 | 2 | 0 | 5 | 2 |
| 53 | C105TTP.4L23 | 1 | 1 | 0 | 5 | 4 |
| 54 | IR63347-AL-201-1 | 3 | 1 | 0 | 5 | 2 |
| 55 | K-225 | 1 | 1 | 0 | 3 | 2 |

4.2.1 Analysis of variance

Data were recorded on eleven quantitative traits viz., plant height (cm), panicle length (cm), leaf length (cm), leaf width (cm), tillering ability, days to 50% flowering, days to maturity, grain length (mm), grain width (mm), 1000 seed weight (g), grain yield ha^{-1} .

Analysis of variance revealed significant variation among all the genotypes for all the eleven characters studied. Mean sum of squares due to genotypes for all the characters were found highly significant (Table-9).

4.2.2 Variability and genetic components of variation

The genetic variability components like genotypic mean, range, confidence interval (CI), genotypic variance (GV), phenotypic variance (PV), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (bs) and genetic advance as a per cent of mean were estimated for each qualitative character as well. A comparative performance of all the genotypes under study has been described as follows:

The estimates were computed on single analysis data (Table 10). Plant height ranged from 120.00 to 45.66 cm with a mean of 93.16, panicle length ranged from 24.66 to 14.66 cm with a mean of 19.76, leaf length ranged from 50.66 to 24.00 cm with a mean of 33.98, leaf width ranged from 1.43 to 0.40 cm with a mean of 0.60 cm, tillering ability ranged from 26.66 to 7.00 with a mean of 15.54, days to 50% flowering ranged from 125.00 to 87.33 days with a mean of 104.46, days to maturity ranged from 147.66 to 124.00 days with a mean of 135.37 days, grain length ranged from 8.54 to 6.31 mm with a mean of 7.75 mm, grain width ranged from 3.18 to 2.04 mm with a mean of 2.81 mm, 1000-seed weight ranged from 31.83 to 20.43 g with a mean of 28.43 g and grain yield q ha^{-1} ranged from 131.92 to 16.85 q with a mean of 92.33 q ha^{-1} .

Table 9: Analysis of variance for yield and yield component traits of 102 rice (*O. sativa* L.) genotypes

| S. No. | Source of variation | d.f. | Mean squares | | | | | | | | | | |
|--------------------|---------------------|------|-------------------|---------------------|------------------|-----------------|-------------------|-----------------------|------------------|-------------------|------------------|----------------------|-----------------------------------|
| | | | Plant height (cm) | Panicle length (cm) | Leaf length (cm) | Leaf width (cm) | Tillering ability | Days to 50% flowering | Days to maturity | Grain length (mm) | Grain width (mm) | 1000-seed weight (g) | Grain yield plot ⁻¹ kg |
| 1. | Replication | 2 | 16.74** | 0.421 | 9.558 | 0.005* | 1.39 | 0.59 | 2.83* | 0.028 | 0.13* | 13.50** | 43.01 |
| 2. | Treatment | 101 | 944.23** | 17.04** | 88.65** | 0.12** | 77.87** | 208.47** | 73.19** | 0.40** | 0.263* | 26.32** | 2612.44** |
| 3. | Error | 202 | 1.66 | 0.99 | 9.03 | 0.001 | 0.79 | 0.92 | 0.64 | 0.01 | 0.009 | 0.94 | 23.18 |
| S.E.Diff from Mean | | | 0.74 | 0.57 | 1.72 | 0.02 | 0.51 | 0.55 | 0.46 | 0.07 | 0.05 | 0.55 | 2.76 |
| CD (p≤0.05) | | | 2.74 | 2.11 | 6.38 | 0.08 | 1.88 | 2.04 | 1.70 | 0.27 | 0.20 | 2.05 | 10.22 |

*Significant at 0.05 probability level

**Significant at 0.01 probability level

Table 10: Magnitude of variability for morphological maturity, yield and yield component traits in rice (*O. sativa* L.)

| S. No. | Trait | 95% CI | Mean | Range |
|---------------|-----------------------------------|---------------|-------------|---------------|
| 1. | Plant height (cm) | 91.17-95.15 | 93.16 | 45.66-120.00 |
| 2. | Panicle length (cm) | 19.48-20.04 | 19.76 | 14.66-24.66 |
| 3. | Leaf length (cm) | 33.31-34.64 | 33.98 | 24.00-50.66 |
| 4. | Leaf width (cm) | 0.57-0.62 | 0.60 | 0.40-1.43 |
| 5. | Tillering ability | 14.96-16.12 | 15.54 | 7.00-26.66 |
| 6. | Days to 50% flowering | 103.52-105.40 | 104.46 | 87.33-125.00 |
| 7. | Days to maturity | 134.81-135.93 | 135.37 | 124.00-147.00 |
| 8. | Grain length (mm) | 7.714-7.80 | 7.75 | 6.31-8.54 |
| 9. | Grain width (cm) | 2.77-2.84 | 2.81s | 2.04-3.18 |
| 10. | 100-seed weight (g) | 27.76-28.45 | 28.10 | 20.43-31.83 |
| 11. | Grain yield (q ha ⁻¹) | 88.99-95.67 | 92.33 | 16.85-131.92 |

The estimates of phenotypic coefficient of variance were observed to be higher in magnitude than the corresponding estimates of genotypic coefficient of variance (Table 11). The magnitude of phenotypic and genotypic coefficient of variance was low (<10.0 %) for grain length (mm), days to maturity and days to 50% flowering; it was moderate (10.0-20.0 %) for grain width (mm), 1000-seed weight (g), leaf length (cm), panicle length (cm) and plant height (cm) and high (>20%) for leaf width (cm), tillering ability and grain yield ($q\ ha^{-1}$).

Estimates of heritability (broad sense) were high (>60%) for all the traits (Table 12). Very high value of heritability of 99.0 per cent was exhibited by plant height (cm) whereas, leaf length (cm) exhibited 74 per cent in the same environment. Genetic advance was estimated at 5 per cent of selection intensity (Table 12) and converted into expected genetic gain (per cent of mean). The estimates revealed that the expected genetic gain was high (>30%) for plant height (cm) and grain yield ($q\ ha^{-1}$) whereas, it was low (20.0%) for panicle length (cm), leaf length (cm), leaf width (cm), tillering ability, days to 50% flowering, days to maturity, grain length (mm), grain width (mm), 1000- seed weight (g).

4.2.3 Correlation coefficient

The Correlation coefficients were determined using variances and co-variances to obtain relationship among various characters and their relationship with grain yield ha^{-1} , at both genotypic and phenotypic level. Genotypic correlation coefficient, were by and large, higher in magnitude, though similar in direction than their corresponding correlation coefficients at phenotypic level. The correlation coefficients both genotypic (r_g) and phenotypic (r_p), among the various growth characters of rice genotypes are presented in Table-13. Correlation coefficients revealed that the economically important trait i.e. grain yield ($q\ ha^{-1}$) exhibited significant positive association with plant height ($r_g=0.21$, $r_p = 0.21$), panicle length ($r_g=0.21$, $r_p= 0.21$), leaf length ($r_g = 0.28$, $r_p= 0.23$), leaf width ($r_g =0.21$, $r_p0.20$), tillering ability ($r_g=0.26$, $r_p=0.23$), grain length ($r_g=0.24$, $r_p=0.23$), grain width ($r_g = 0.32$, $r_p = 0.23$), 1000-seed weight ($r_g=0.32$, $r_p=0.29$) both at

Table 11: Phenotypic and genotypic coefficient of variation for different traits in rice (*O. sativa* L.)

| S. No. | Trait | PCV | GCV |
|--------|-----------------------------------|-------|-------|
| 1. | Plant height (cm) | 19.07 | 19.02 |
| 2. | Panicle length (cm) | 12.74 | 11.70 |
| 3. | Leaf length (cm) | 17.55 | 15.16 |
| 4. | Leaf width (cm) | 34.81 | 34.16 |
| 5. | Tillering ability | 33.10 | 32.60 |
| 6. | Days to 50% flowering | 8.01 | 7.96 |
| 7. | Days to maturity | 3.68 | 3.63 |
| 8. | Grain length (mm) | 4.92 | 4.63 |
| 9. | Grain width (cm) | 10.90 | 10.34 |
| 10. | 1000-seed weight (g) | 10.90 | 10.34 |
| 11. | Grain yield (q ha ⁻¹) | 32.24 | 31.81 |

Table 12: Estimates of variability, heritability, genetic advance and expected genetic gain for different traits of rice (*O. sativa* L.)

| S. No. | Traits | Phenotypic variance | Genotypic variance | Heritability (broad sense) | Genetic advance | Expected genetic (% of mean) |
|---------------|-----------------------------------|----------------------------|---------------------------|-----------------------------------|------------------------|-------------------------------------|
| 1. | Plant height (cm) | 315.85 | 314.18 | 0.99 | 36.41 | 39.08 % |
| 2. | Panicle length (cm) | 6.34 | 5.35 | 0.84 | 4.37 | 22.14 % |
| 3. | Leaf length (cm) | 35.57 | 26.53 | 0.74 | 9.16 | 26.97% |
| 4. | Leaf width (cm) | 0.04 | 0.04 | 0.96 | 0.41 | 69.06 % |
| 5. | Tillering ability | 26.48 | 25.69 | 0.97 | 10.28 | 66.15 % |
| 6. | Days to 50% flowering | 70.10 | 69.18 | 0.98 | 17.02 | 16.29 % |
| 7. | Days to maturity | 24.83 | 24.18 | 0.97 | 9.99 | 7.38 % |
| 8. | Grain length (mm) | 0.14 | 0.12 | 0.88 | 0.69 | 8.96 % |
| 9. | Grain width (cm) | 0.09 | 0.08 | 0.90 | 0.56 | 20.22 % |
| 10. | 1000-seed weight (g) | 9.40 | 8.46 | 0.90 | 5.68 | 20.22% |
| 11. | Grain yield (q ha ⁻¹) | 886.26 | 863.08 | 0.97 | 59.72 | 64.68 % |

Table 13: Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficient of yield and yield component traits in 102 rice (*O. sativa* L.) genotypes

| S. No. | Traits | Plant height (cm) | Panicle length (cm) | Leaf length (cm) | Leaf width (cm) | Tillering ability | Days to 50% flowering | Days to maturity | Grain length (mm) | Grain width (mm) | 100-seed weight (g) | Grain yield ha ⁻¹ q |
|--------|-----------------------------------|-------------------|---------------------|------------------|-----------------|-------------------|-----------------------|------------------|-------------------|------------------|---------------------|--------------------------------|
| 1. | Plant height (cm) | 314.18 | 0.37 | 0.08 | -0.31 | -0.35 | -0.49 | -0.41 | -0.03 | -0.06 | -0.06 | 0.20* |
| 2. | Panicle length (cm) | 0.34 ** | 5.35 | 0.20 | 0.007 | -0.33 | -0.35 | -0.25 | 0.04 | 0.29 | 0.29 | 0.21 ** |
| 3. | Leaf length (cm) | 0.07 | 0.21 ** | 26.53 | 0.19 | -0.06 | 0.11 | 0.30 | 0.24 | 0.02 | 0.02 | 0.28** |
| 4. | Leaf width (cm) | -0.30 ** | 0.01 | 0.16 ** | 0.04 | 0.07 | 0.53 | 0.48 | -0.27 | 0.06 | 0.06 | 0.21** |
| 5. | Tillering ability | -0.34** | -0.30 ** | -0.06 | 0.07 | 25.69 | 0.29 | 0.56 | -0.05 | -0.08 | -0.08 | 0.26** |
| 6. | Days to 50% flowering | -0.49** | -0.32 ** | 0.09 | 0.52 ** | 0.29 ** | 69.18 | 0.41 | -0.14 | -0.06 | -0.06 | -0.15 |
| 7. | Days to maturity | -0.40 ** | -0.22 ** | 0.25 ** | 0.47 ** | 0.54 ** | 0.41 ** | 24.18 | -0.001 | 0.19 | 0.19 | 0.13 |
| 8. | Grain length (mm) | -0.03 | 0.03 | 0.18* | -0.24 ** | -0.05 | -0.13 * | -0.002 | 0.12 | -0.17 | -0.17 | 0.24** |
| 9. | Grain width (cm) | -0.05 | 0.25 ** | 0.03 | 0.05 | -0.07 | -0.06 | 0.18 ** | -0.14 * | 0.08 | 1.00 | 0.32** |
| 10. | 100-seed weight (g) | -0.05 | 0.25** | 0.03 | 0.05 | -0.07 | -0.06 | 0.18 ** | -0.14 * | 1.00* | 8.46 | 0.32** |
| 11. | Grain yield plot ⁻¹ kg | 0.21** | 0.21** | 0.23 ** | 0.20 ** | 0.23 * | -0.15 * | -0.13 * | 0.23 * | 0.29 ** | 0.29 ** | 863.08 |

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

genotypic and phenotypic levels. Days to 50% flowering ($r_g = -0.153$, $r_p = -0.151$), days to maturity ($r_g = -0.134$, $r_p = -0.131$) exhibited negative and non-significant association with fruit yield plant⁻¹. It was noticed that grain length was positively and significantly associated with leaf length and leaf width and grain width with panicle length.

4.2.4 Genetic divergence

Analysis of variance for dispersion revealed that the genotypes expressed significant variability for various traits under study. Genetic divergence was estimated for one hundred two genotypes of rice.

Based upon the performance of genotypes, 102 genotypes were grouped into eight clusters (Table-14) as per Mahalanobis D^2 analysis employing Tochers method (Rao, 1952). The cluster diagram and dendrogram (Fig.1 and 2) indicated that the maximum number of genotypes fall in cluster IV (40) followed by cluster II (23), cluster VII (16), cluster VI (14), cluster VIII (3) and cluster I, cluster III and cluster V (two each).

The mean intra and inter-cluster distance (D^2) values for the same environment (Table 15) revealed that cluster V had highest intra-cluster distance (D^2) value of (1271.69) followed by cluster I (601.56). The inter-cluster distances (D^2) value was highest (1146.52) between cluster VIII and VII followed by cluster VII and VI (823.12), cluster VI and I (668.36), cluster V and III (599.95). The minimum inter- cluster distance was observed between cluster II and IV (376.39).

Cluster mean for different traits in the same environment (Table 16) revealed that the magnitude of differences among the mean of the traits for clusters was significant. The range of variation in cluster means for plant height (cm) was 114.04 in cluster VII to 64.00 in cluster VI. Panicle length (cm) was 21.06 in cluster II to 16.83 in cluster I. Leaf length (cm) was 41.67 in cluster VIII to 30.36 in cluster VI. Leaf width (cm) was 1.43 in cluster VIII to 0.50 in cluster III. Tillering ability was 20.17 in cluster I to 13.38 in cluster II. Days to 50%

Table 14: Distribution of different rice genotypes into clusters based on D² statistics (clustering by Tocher Method)

| S. No. | Cluster | No. of genotypes | Variety/Accession No. of genotypes |
|--------|---------|------------------|--|
| 1. | I | 2 | Calaro, Kamad |
| 2. | II | 23 | SKAU-429, B-11, B-24, B-4, B-26, B-3, SKAU-3, SKAU-46, Mushkbudji, B-5, China-988, China-972, B-32, Jehlum, Dular, IRBL Z-FU/EC 565188, Chenab, K-116, Shalimar Rice-2, IRBL5M/EC565166, Shalimar Rice-3, C101A51/ARBN152, Shalimar Rice-1 |
| 3. | III | 2 | B-8, SKAU-356 |
| 4. | IV | 40 | IR64, Tumlaaal, K-225, SKAU-336, C104PKT, A57, SK-2, SKAU-337, B-9, B-14, SKAU-408, IR61728-413-2-1-1, IR68333-RR-B-19, T2(MAS 71), Raminad Str 3, CO.39, Local Budgam-3, IR63347-AL-201-1, IRBN 2008 V-87, Shalkew, BL-42, K-332, Wazul Krew, Kanto-51, RIL-10, BL-142/ARBN 142, T1, SK-339, China-1039, SKAU-338, China-1007, B-10, B-13, B-19, B-1509, SKAU-98, B-28, A3(IRRI LINE), Local Budgam-1, Local Budgam-2 |
| 5. | V | 2 | KHD/GP/27, Pusa Susand-3 |
| 6. | VI | 14 | SKAU-405, Safed Brez, C105TTP.4L23, BL-122, Shai Tai Tsau, Usen, SKAU-389, Baber, RIL-29, IRBLKS-S/EC565178, Gulbara, Rasi, C101PKT, C101A51 |
| 7. | VII | 16 | Niver Zag, IRBL1-CL/EC565160, Gurah, KHD/GP/120, China-1031, A57-115-4/ARBN148, KHD/GP/150, NP 125, BL 245, Yunlen-19, KHD/GP/154, SKAU-403, SKAU-292, SKAU-39, SKAU-402, SKAU-404 |
| 8. | VIII | 3 | BL-122/ARBN 141, KHD/GP/208, Pusa Sugand-5 |

Table 15 : Average inter-cluster and intra-cluster D^2 -values among rice (*O. sativa* L.) genotypes

| S. No. | ↓Cluster→ | I | II | III | IV | V | VI | VII | VIII |
|--------|-----------|--------|--------|--------|--------|---------|--------|--------|---------|
| 1. | I | 601.56 | 376.39 | 431.65 | 305.03 | 599.95 | 364.32 | 621.91 | 593.00 |
| 2. | II | | 74.67 | 177.62 | 193.55 | 505.93 | 545.17 | 187.82 | 874.66 |
| 3. | III | | | 435.27 | 220.46 | 518.98 | 533.96 | 250.13 | 791.78 |
| 4. | IV | | | | 149.36 | 429.71 | 370.68 | 338.67 | 503.01 |
| 5. | V | | | | | 1271.69 | 668.36 | 633.46 | 635.85 |
| 6. | VI | | | | | | 261.09 | 823.12 | 449.53 |
| 7. | VII | | | | | | | 131.99 | 1146.52 |
| 8. | VIII | | | | | | | | 0.00 |

Table 16 : Cluster means for yield and yield component traits in different clusters of 102 rice genotypes (*O. sativa* L.)

| S. No. | Cluster | Plant height (cm) | Panicle length (cm) | Leaf length (cm) | Leaf width (cm) | Tillering ability | Days to 50% flowering | Days to maturity | Grain length (mm) | Grain width (mm) | 1000-seed weight (g) | Grain yield ha ⁻¹ q |
|--------|---------|-------------------|---------------------|------------------|-----------------|-------------------|-----------------------|------------------|-------------------|------------------|----------------------|--------------------------------|
| 1. | I | 77.00 | 16.83 | 31.83 | 0.63 | 20.17 | 104.67 | 138.83 | 7.83 | 2.50 | 24.97 | 120.40 |
| 2. | II | 102.36 | 21.06 | 32.55 | 0.51 | 13.38 | 95.49 | 133.93 | 7.79 | 2.90 | 29.05 | 104.72 |
| 3. | III | 98.67 | 19.83 | 31.50 | 0.50 | 15.33 | 103.00 | 132.83 | 7.90 | 2.84 | 28.38 | 82.20 |
| 4. | IV | 91.57 | 20.00 | 35.91 | 0.61 | 16.68 | 107.17 | 137.68 | 7.74 | 2.96 | 29.63 | 86.73 |
| 5. | V | 95.00 | 19.83 | 34.67 | 0.97 | 17.17 | 109.50 | 140.00 | 7.86 | 2.50 | 25.03 | 66.10 |
| 6. | VI | 64.00 | 17.43 | 30.36 | 0.60 | 16.52 | 109.14 | 134.14 | 7.76 | 2.62 | 26.21 | 89.07 |
| 7. | VII | 114.04 | 19.73 | 33.44 | 0.54 | 13.75 | 102.21 | 129.77 | 7.74 | 2.52 | 25.21 | 101.16 |
| 8. | VIII | 74.67 | 19.67 | 41.67 | 1.43 | 18.00 | 125.00 | 147.67 | 7.66 | 2.90 | 28.97 | 45.88 |

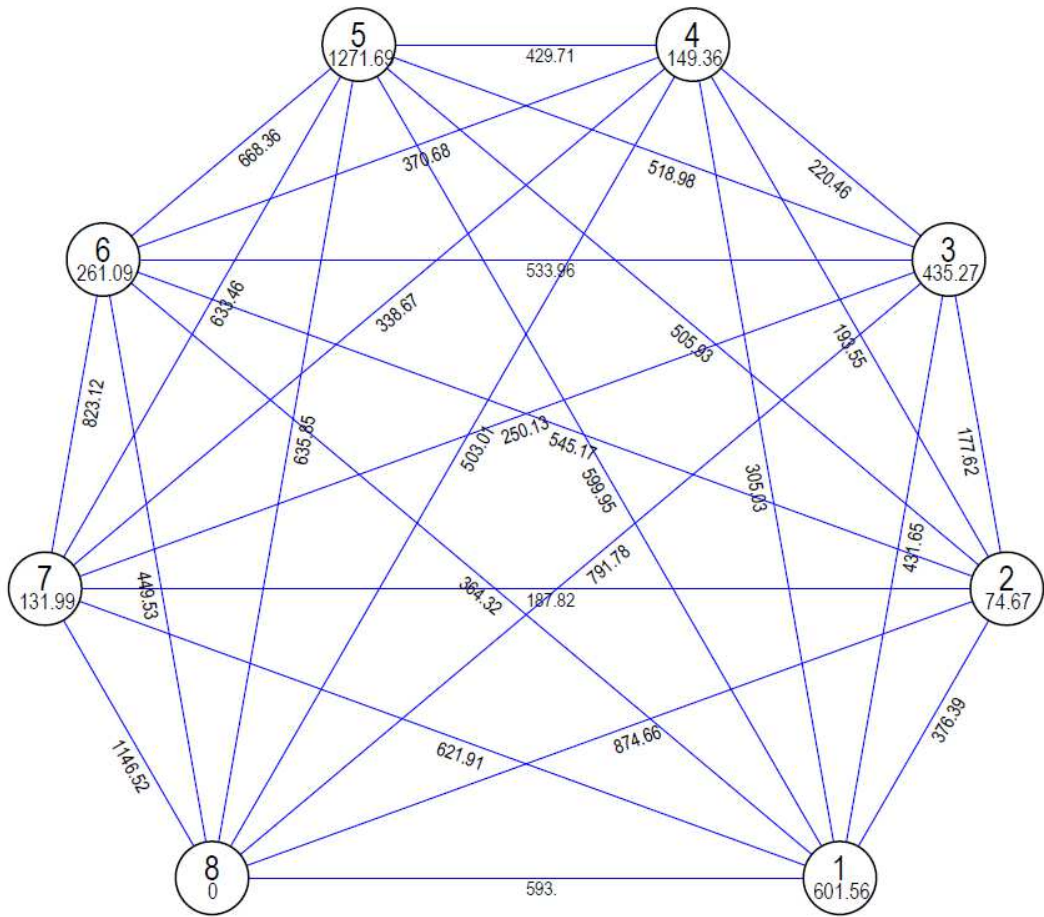


Fig. 1 : Mahalanobis Euclidean Distance (Not to the Scale)

Clustering by Tocher Method

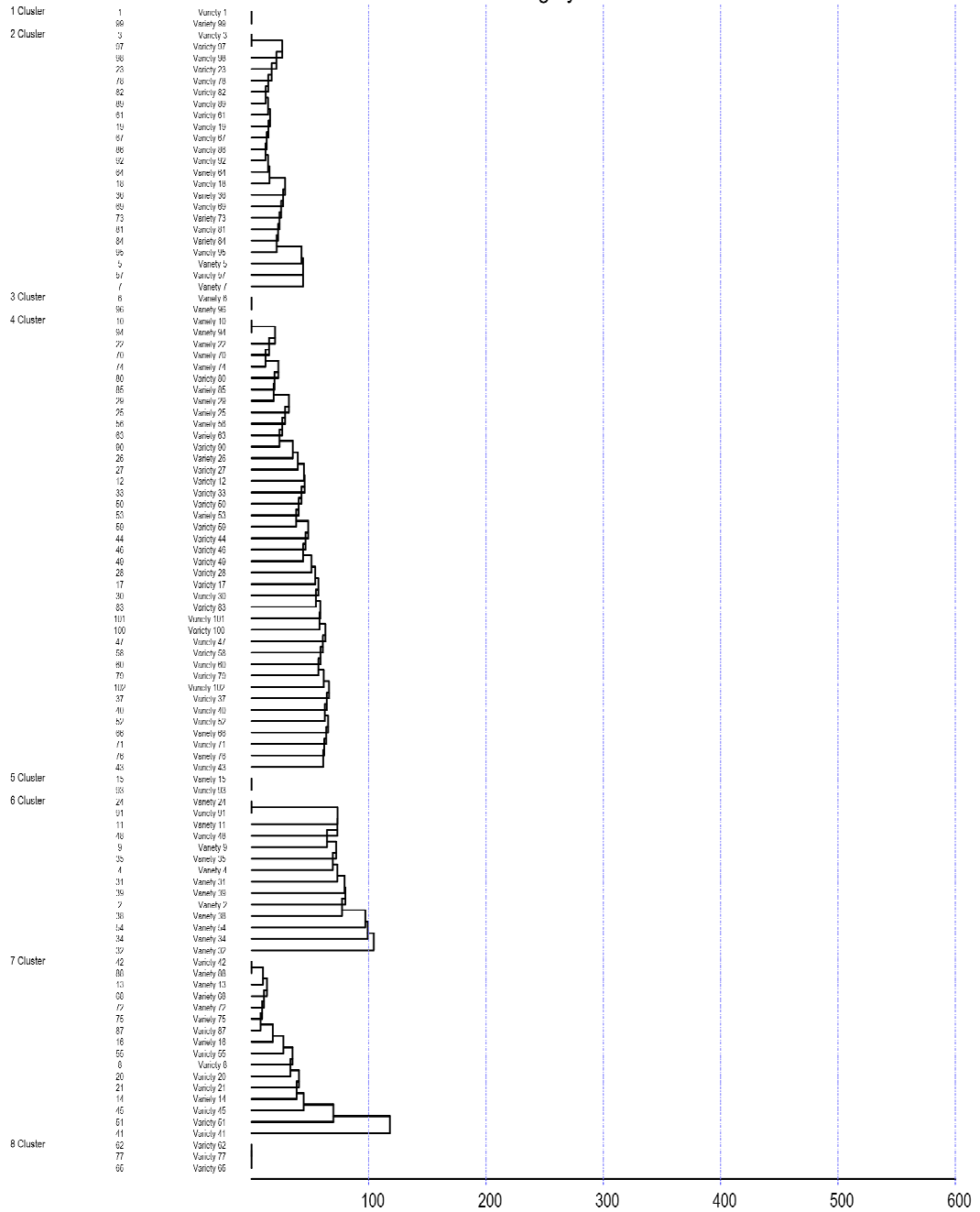


Fig. 2 :Clustering by Tocher Method (Dendrogram)

flowering was 125.00 in cluster VIII to 95.49 in cluster II. Days to maturity was 147.67 in cluster VIII to 129.77 in cluster VII. Grain length (mm) was 7.90 in cluster III to 7.66 in cluster VIII. Grain width (mm) was 2.96 in cluster IV to 2.50 in cluster I. 1000-seed weight (g) was 29.63 in cluster IV to 24.97 in cluster I. Grain yield qha^{-1} was 120.40 in cluster I to 45.88 in cluster VII.

The per cent contributions of the traits towards total genetic divergence (Table-17) revealed that plant height was the main factor contributing to divergence accounting for 41.16% followed by days to 50% flowering (18.17%), grain yield q ha^{-1} (12.02%), days to maturity (9.98%), grain length (9.98%), tillering ability (7.98%), leaf width (5.75%), and grain width (1.13). The minimum contribution towards divergence was from grain width (1.13%) followed by leaf width (5.75%) and tillering ability (7.98%).

4.3 Molecular characterization

Thirty two genotypes of rice were selected in the present study to evaluate their genetic diversity at the molecular level using micro-satellite primers. The genotypes used for this study were C101PKT, C101A51/ARBN152, SKAU-356, SR-1, IR64, Tumlahaal, Niver Zag, IRBL1-CL/EC565160, KHD/GP/27, Gurah, IRBL5M/EC565166, Shalimar Rice-3, China-1031, B-4, A57, SK-337, B-9, B-14, Baber, SKAU-408, Shalimar Rice-2, IR61728-413-2-1-1, IR68333-RR-B-19, KHD/GP/150, RIL-10, A57-115-4/ARBN148, IRBL1-CL/EC565160, IRBL Z-FU/EC 565188, Rasi, Calaro, SKAU-389 and Pusa Sugand-3. The experimental results obtained from the SSR marker analysis of these genotypes are elaborated as under:

4.3.1 Genetic polymorphism

A total of eight simple sequence repeat (SSR) markers were utilized to characterize and assess genetic diversity of 32 genotypes of rice. All these markers generated good and reproducible products for all of the 32 genotypes. The level of polymorphism among the genotypes was evaluated by calculating

Table- 17 : Contribution of different traits to total genetic divergence

| S. No. | Trait | Times ranked 1st | Contribution (%) |
|---------------|-----------------------------------|------------------------------------|-------------------------|
| 1. | Plant height (cm) | 2120 | 41.16 |
| 2. | Days to 50% flowering | 936 | 18.17 |
| 3. | Grain yield (q ha ⁻¹) | 619 | 12.02 |
| 4. | Days to maturity | 514 | 9.98 |
| 5. | Tillering ability | 411 | 7.984 |
| 6. | Leaf width (cm) | 296 | 5.75 |
| 7. | Grain length (mm) | 96 | 9.98 |
| 8. | Grain width (cm) | 58 | 1.13 |

polymorphic bands, polymorphic information content, effective multiplex ratio and marker index for each of the 8 SSR loci evaluated. Each of the 8 loci differed significantly in their ability to determine variability among the genotypes. The size of most intensely amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, 100 base pairs (bp) DNA ladder. A total of 22 alleles were detected across the 32 genotypes through the use of these 8 SSR markers (Table 18). The number of alleles per locus generated by these SSR markers varied from 2 (RM 104) (RM 202) (RM 530) (RM 287) and (RM 125) to 5 (RM 55) (Plate-4-8). The average alleles per locus were 2.87.

Polymorphism index content value is the reflection of allelic diversity and frequency among the genotypes. The PIC values varied from 0.22 (RM 202) and (RM 245) to 0.67 (RM55) with an average of 0.42 per locus. Significant correlation existed between the PIC values and the number of alleles at SSR loci. PIC value of more than 0.60 was found for SSR markers RM55 and RM 555 and it was less than 0.6 in RM 104, RM 530, RM 245, RM287, RM 125 and RM 202.

4.3.2 Similarity coefficients between genotypes increasing genetic distance

All the genotypes were scored for the presence and absence of the SSR bands throughout 32 genotypes and the data were exported to binary data for the presence (1) or absence (0) or as a missing observation (9) for further analysis with NTSYS-pc version 2.2 (Rohlf, 1997). NTSYS-pc was used to construct a UPGMA (unweighted pair group method with arithmetic averages) dendrogram showing the distance-based inter-relationship among the genotypes. A similarity coefficients matrix based on the proportion of shared SSR alleles was used to establish the level of relatedness between each pair of the genotypes. The similarity index coefficient values obtained for each pair wise comparison among 32 genotypes are presented in Table 20. Relationships among 32 genotypes revealed a varying degree of genetic relationship. Pairwise genetic similarity coefficients varied from 0.00 to 0.94, with an average of 0.62. Highest genetic

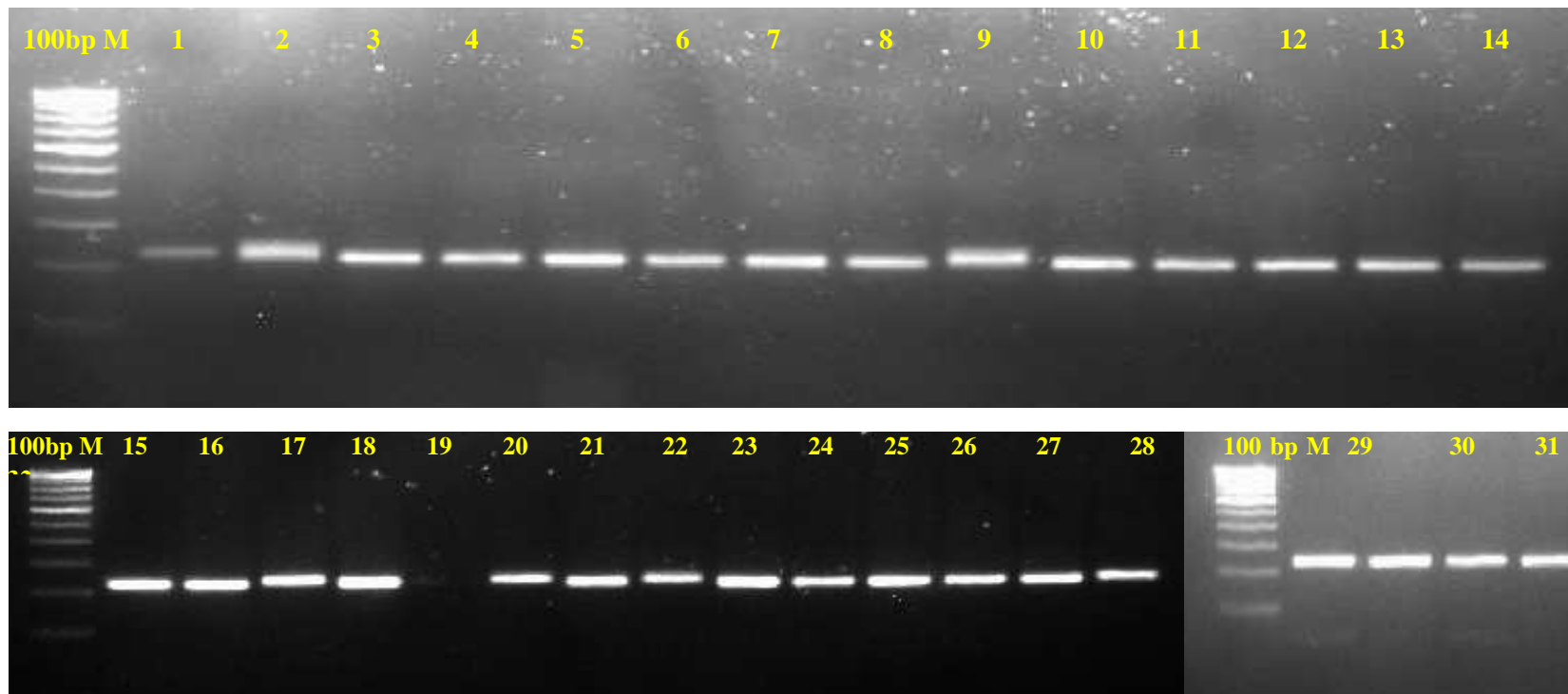


Plate- 4: Ethidium bromide gel picture showing genetic relationship among 32 rice (*Oryza sativa* L.) genotypes using SSR primer RM 555

Coding for genotypes shown in plate

1. Calaro, 2. Tumlahaal, 3. C101A51/ARBN 152, 4. SK-336, 5. A-57, 6. Niver Zag, 7. IRBN5M/EC 565166, 8. KHD/GP/27, 9. IR61728-413-2-1-1, 10. IR64, 11. KHD/GP/150, 12. B-14, 13. B-4, 14. IRBN 1-CL/EC 565160, 15. A57-115-4/ARBN 148, 16. China-1031, 17. Baber, 18. Shalimar Rice -2, 19. IR68333-RR-B-19, 20. SKAU-337, 21. IRBL Z-FU/EC 565188, 22. Shalimar Rice -3, 23. Gureh, 24. B-9, 25. Pusa Sugand-3, 26. SKAU-389, 27. RIL-10, 28. Shalimar Rice-1, 29. SKAU-408, 30. Rasi, 31. SKAU 356 and 32. C101PKT

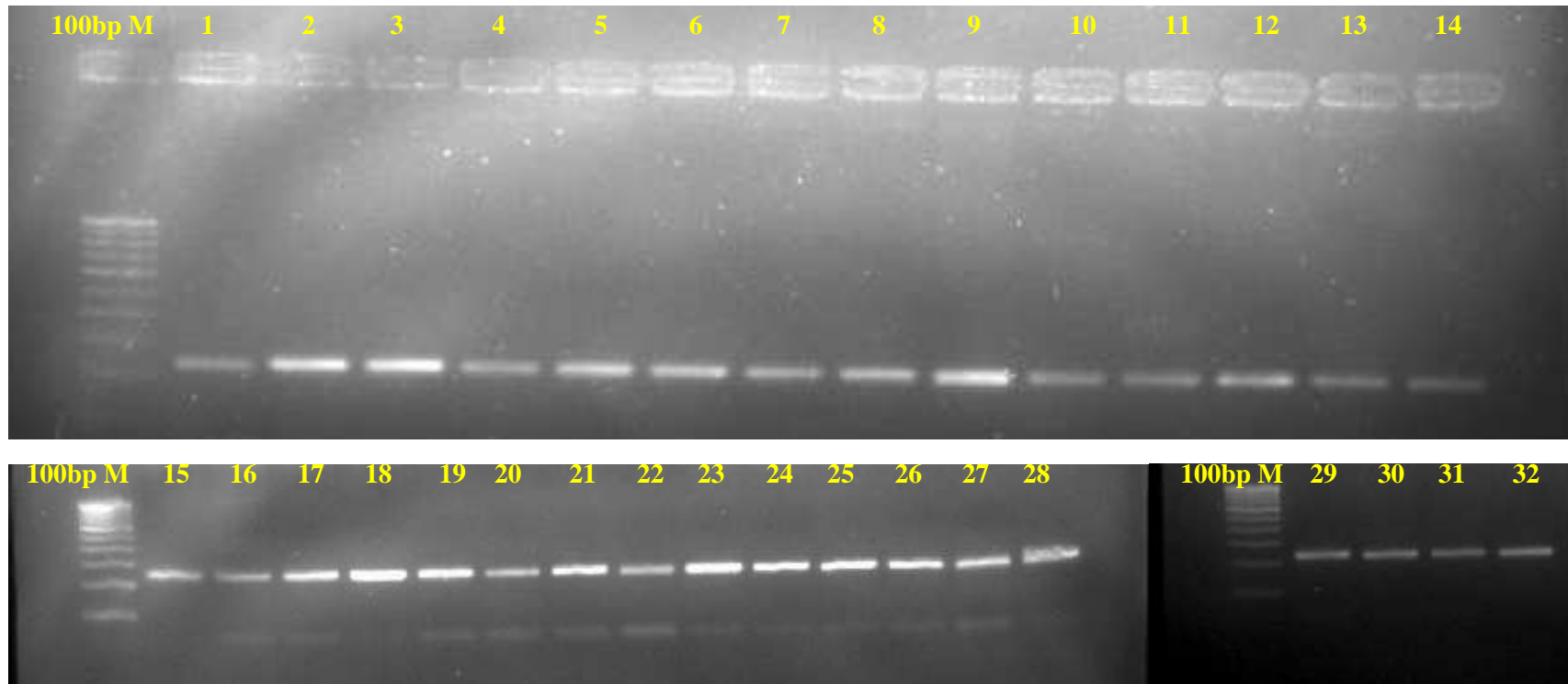


Plate-5: Ethidium bromide gel picture showing genetic relationship among 32 rice (*Oryza sativa* L.) genotypes using SSR primer RM 555

Coding for genotypes shown in plate

1. Calaro, 2. Tumlahaal, 3. C101A51/ARBN 152, 4. SK-336, 5. A-57, 6. Niver Zag, 7. IRBN5M/EC 565166, 8. KHD/GP/27, 9. IR61728-413-2-1-1, 10. IR64, 11. KHD/GP/150, 12. B-14, 13. B-4, 14. IRBN 1-CL/EC 565160, 15. A57-115-4/ARBN 148, 16. China-1031, 17. Baber, 18. Shalimar Rice -2, 19. IR68333-RR-B-19, 20. SKAU-337, 21. IRBL Z-FU/EC 565188, 22. Shalimar Rice -3, 23. Gureh, 24. B-9, 25. Pusa Sugand-3, 26. SKAU-389, 27. RIL-10, 28. Shalimar Rice-1, 29. SKAU-408, 30. Rasi, 31. SKAU 356 and 32. C101PKT

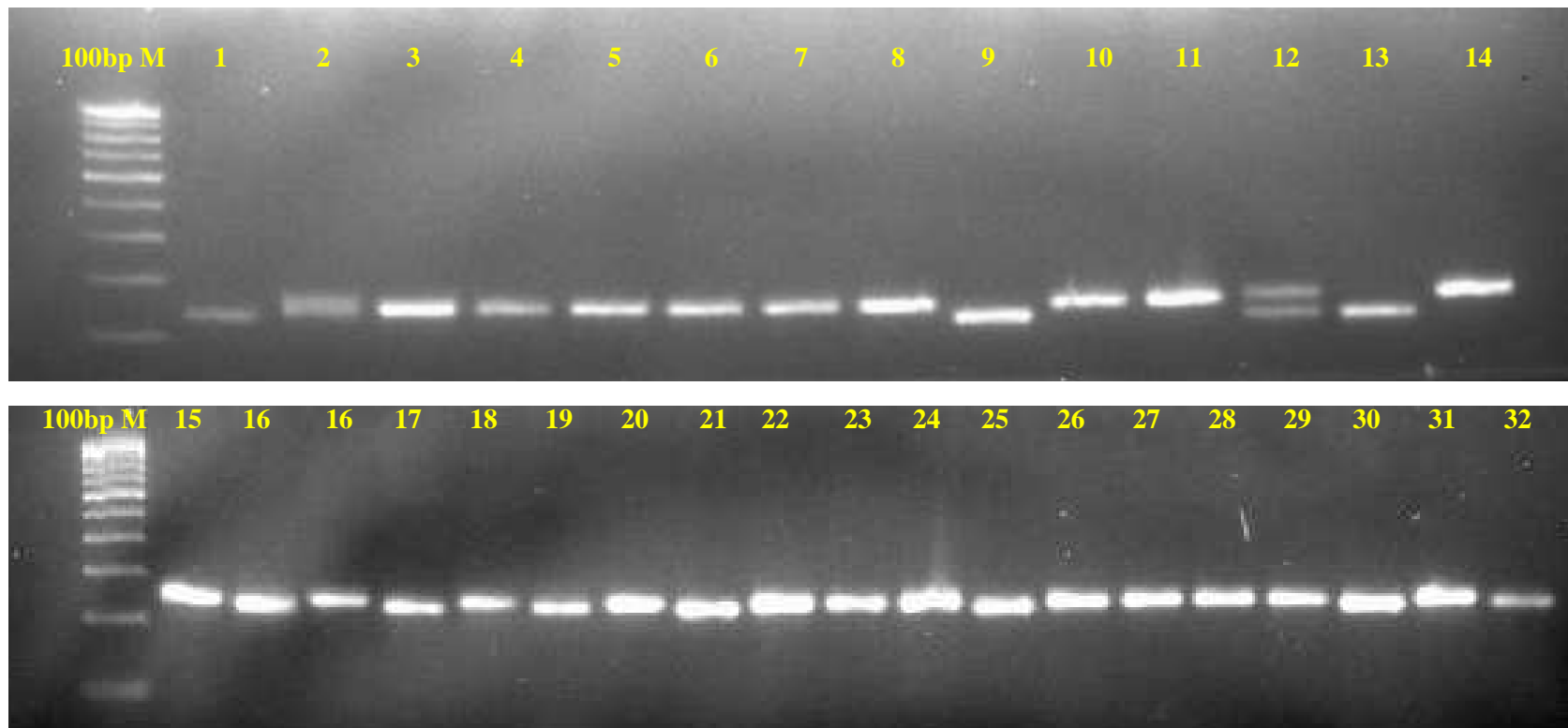


Plate-6 : Ethidium bromide gel picture showing genetic relationship among 32 rice (*Oryza sativa* L.) genotypes using SSR primer RM 104

Coding for genotypes shown in plate

1. Calaro, 2. Tumlahaal, 3. C101A51/ARBN 152, 4. SK-336, 5. A-57, 6. Niver Zag, 7. IRBN5M/EC 565166, 8. KHD/GP/27, 9. IR61728-413-2-1-1, 10. IR64, 11. KHD/GP/150, 12. B-14, 13. B-4, 14. IRBN 1-CL/EC 565160, 15. A57-115-4/ARBN 148, 16. China-1031, 17. Baber, 18. Shalimar Rice -2, 19. IR68333-RR-B-19, 20. SKAU-337, 21. IRBL Z-FU/EC 565188, 22. Shalimar Rice -3, 23. Gureh, 24. B-9, 25. Pusa Sugand-3, 26. SKAU-389, 27. RIL-10, 28. Shalimar Rice-1, 29. SKAU-408, 30. Rasi, 31. SKAU 356 and 32. C101PKT

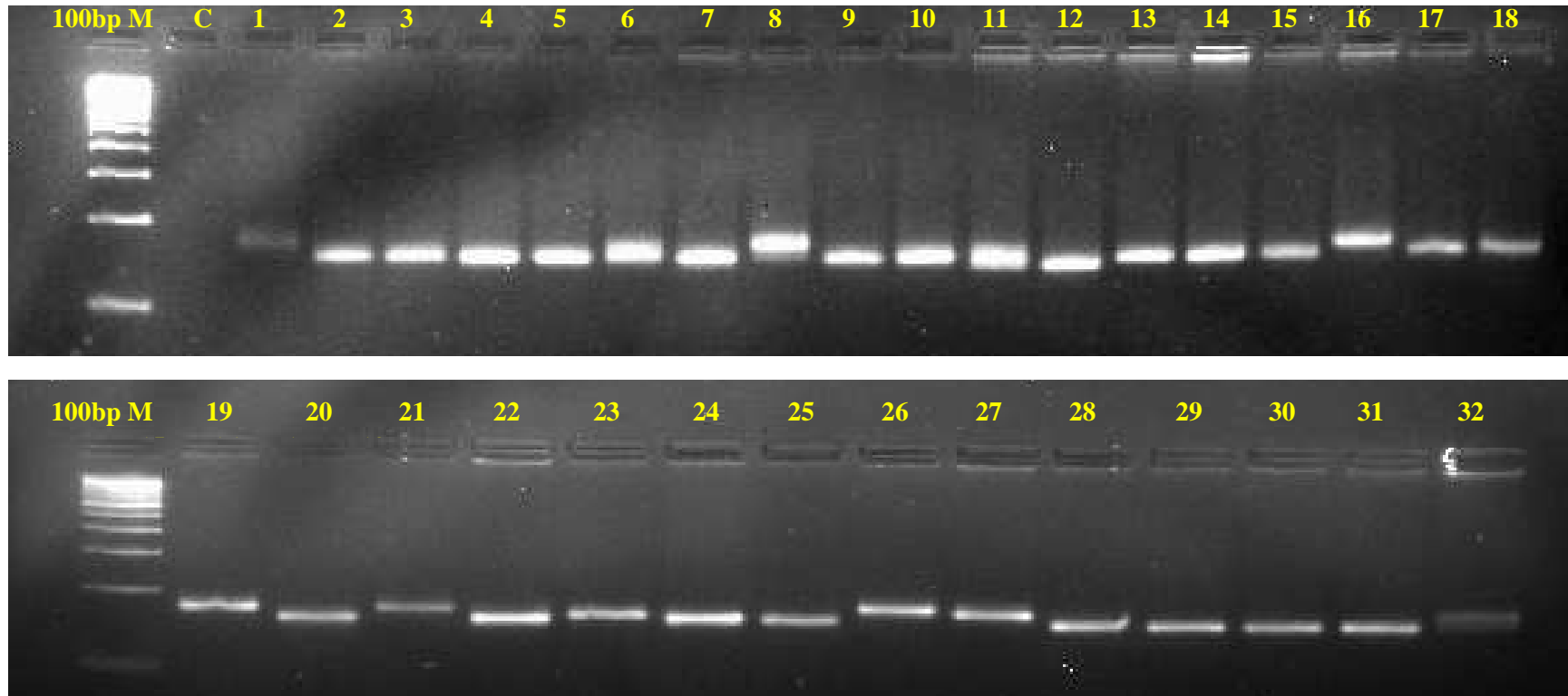


Plate-7: Ethidium bromide gel picture showing genetic relationship among 32 rice (*Oryza sativa* L.) genotypes using SSR primer RM 530

Coding for genotypes shown in plate

1. Calaro, 2. Tumlahaal, 3. C101A51/ARBN 152, 4. SK-336, 5. A-57, 6. Niver Zag, 7. IRBN5M/EC 565166, 8. KHD/GP/27, 9. IR61728-413-2-1-1, 10. IR64, 11. KHD/GP/150, 12. B-14, 13. B-4, 14. IRBN 1-CL/EC 565160, 15. A57-115-4/ARBN 148, 16. China-1031, 17. Baber, 18. Shalimar Rice -2, 19. IR68333-RR-B-19, 20. SKAU-337, 21. IRBL Z-FU/EC 565188, 22. Shalimar Rice -3, 23. Gureh, 24. B-9, 25. Pusa Sugand-3, 26. SKAU-389, 27. RIL-10, 28. Shalimar Rice-1, 29. SKAU-408, 30. Rasi, 31. SKAU 356 and 32. C101PKT

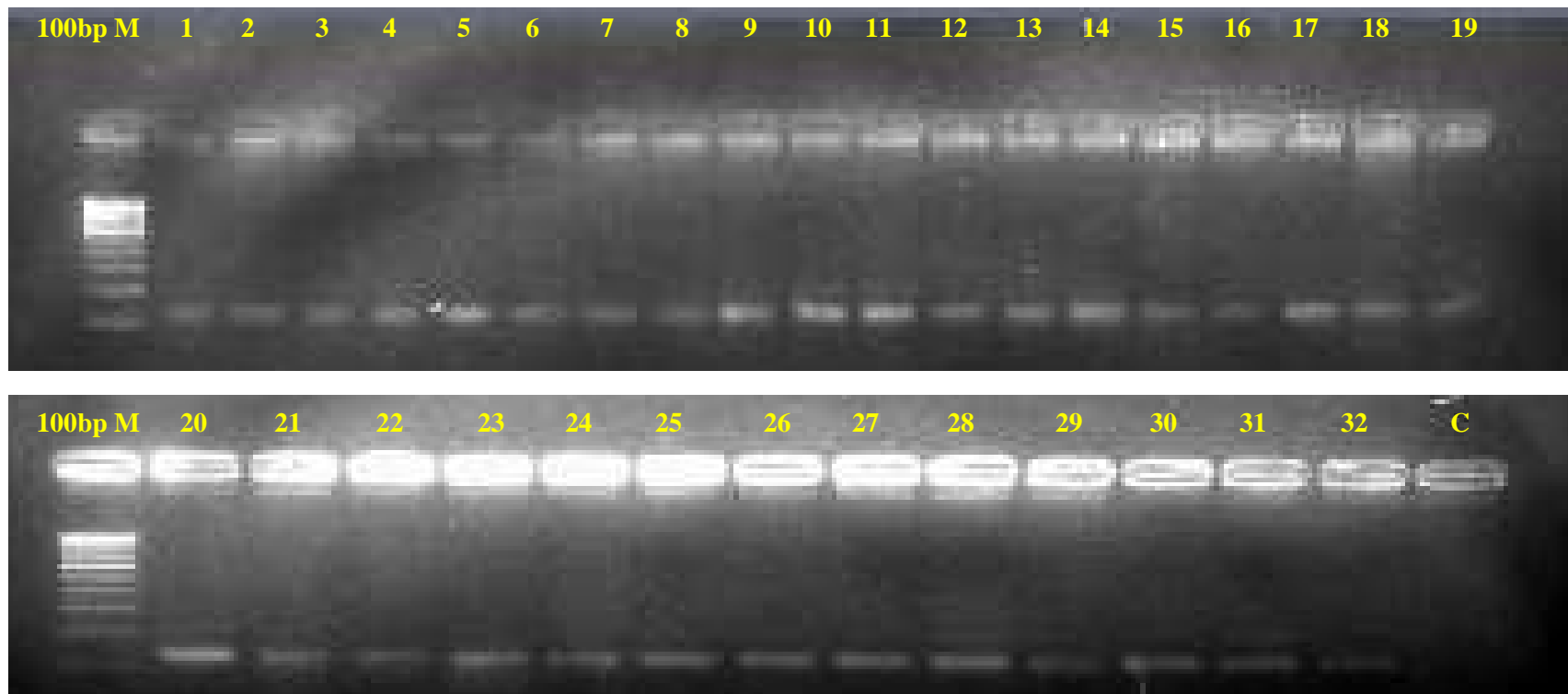


Plate-8: Ethidium bromide gel picture showing genetic relationship among 32 rice (*Oryza sativa* L.) genotypes using SSR primer RM 287

Coding for genotypes shown in plate

1. Calaro, 2. Tumlahaal, 3. C101A51/ARBN 152, 4. SK-336, 5. A-57, 6. Niver Zag, 7. IRBN5M/EC 565166, 8. KHD/GP/27, 9. IR61728-413-2-1-1, 10. IR64, 11. KHD/GP/150, 12. B-14, 13. B-4, 14. IRBN 1-CL/EC 565160, 15. A57-115-4/ARBN 148, 16. China-1031, 17. Baber, 18. Shalimar Rice -2, 19. IR68333-RR-B-19, 20. SKAU-337, 21. IRBL Z-FU/EC 565188, 22. Shalimar Rice -3, 23. Gureh, 24. B-9, 25. Pusa Sugand-3, 26. SKAU-389, 27. RIL-10, 28. Shalimar Rice-1, 29. SKAU-408, 30. Rasi, 31. SKAU 356 and 32. C101PKT

Table 18: Total percentage of polymorphism detected in 32 rice (*O. sativa* L.) genotypes by SSR Markers

| S. No. | Primer Name | Ch. No. | Total bands | Total no. of monomorphic Bands | Total No. of Polymorphic Bands | Percentage of polymorphism |
|------------------------|-------------|---------|--------------|--------------------------------|--------------------------------|----------------------------|
| 1 | RM 145 | 9 | 28 | 26 | 2 | 7.14 |
| 2 | RM 555 | 2 | 31 | 24 | 7 | 22.58 |
| 3 | RM 55 | 3 | 32 | 25 | 7 | 21.87 |
| 4 | RM 104 | 1 | 32 | 27 | 5 | 15.62 |
| 5 | RM 202 | 11 | 24 | 20 | 4 | 16.66 |
| 6 | RM 530 | 2 | 31 | 24 | 7 | 22.58 |
| 7 | RM 287 | 11 | 32 | 29 | 3 | 9.37 |
| 8 | RM 125 | 7 | 32 | 27 | 5 | 15.62 |
| Total | | | 242 | 202 | 40 | |
| Mean per primer | | | 30.25 | 25.25 | 5 | |

Table 19: Allele variation and polymorphism information content (PIC) for SSR loci across 32 selected rice (*O. sativa* L.) genotypes

| S. No | SSR marker | Primer sequence | | Annealing temperature (°C) | No. of alleles | Pic value | Effective multiplex ratio (EMR) | Marker index (MI) |
|----------------|------------|-----------------|----------------------------|----------------------------|----------------|-------------|---------------------------------|-------------------|
| 1. | RM 245 | F | ATGCCGCCAGTGAATAGC | 50 | 3 | 0.22 | 0.14 | 0.03 |
| | | R | CTGAGAATCCAATTATCTGGGG | | | | | |
| 2. | RM 555 | F | TTGGATCAGCCAAAGGAGAC | 52 | 4 | 0.65 | 1.58 | 1.02 |
| | | R | CAGCATTGTGGCATGGATAC | | | | | |
| 3. | RM 55 | F | CCGTCGCCGTAGTAGAGAAG | 52 | 5 | 0.67 | 1.53 | 1.02 |
| | | R | TCCCGGTTATTTTAAGGCG | | | | | |
| 4. | RM 104 | F | GGAAGAGGAGAGAAAGATGTGTGTCG | 50 | 2 | 0.48 | 0.78 | 0.37 |
| | | R | TCAACAGACACACCGCCACCGC | | | | | |
| 5 | RM 202 | F | CAGATTGGAGATGAAGTCCTCC | 45 | 2 | 0.22 | 0.66 | 0.14 |
| | | R | CCAGCAAGCATGTCAATGTA | | | | | |
| 6 | RM 530 | F | GCACTGACCACGACTGTTTG | 55 | 2 | 0.43 | 1.58 | 0.67 |
| | | R | ACCGTAACCCGGATCTATCC | | | | | |
| 7 | RM 287 | F | TTCCCTGTTAAGAGAGAAATC | 60 | 2 | 0.50 | 0.28 | 0.14 |
| | | R | GTGTATTTGGTGAAAGCAAC | | | | | |
| 8 | RM 125 | F | ATCAGCAGCCATGGCAGCGACC | 55 | 2 | 0.26 | 0.78 | 0.20 |
| | | R | AGGGGATCATGTGCCGAAGGCC | | | | | |
| Total | | | | | 22 | 3.43 | | |
| Average | | | | | 2.87 | 0.42 | | |

Table- 20 : Grouping of 32 rice (*O. sativa* L.) genotypes into different clusters based on SSR markers

| Cluster No. | sub-clusters | Number of genotypes | Name of genotypes |
|-------------|--------------|---------------------|---|
| I | Ia | 4 | Calaro, Tumlahaal, Baber, Shalimar Rice-2 |
| | Ib | 4 | IR68333-RR-B-19, SK-337, IRBL Z-FU/EC 565188, Shalimar Rice-3 |
| II | IIa | 4 | C101A51/ARBN 152,SKAU-336, A57-115-4/ARBN 148, China-1031 |
| | IIb | 4 | A-57, Niver Zag, IRBN5M/EC 565166, KHD/GP/27 |
| | IIc | 4 | IR64, KHD/GP/150, B-14, B-4 |
| | IId | 1 | IRBN 1-CL/EC 565160 |
| III | IIIa | 3 | SKAU-408, Rasi, C101PKT |
| | IIIb | 1 | SKAU-356 |
| IV | Iva | 2 | IR61728-413-2-1-1, SR-1 |
| | IVb | 2 | Gureh, Pusa Sugand-3 |
| | IVc | 3 | B-9, SKAU-389, RIL-10 |

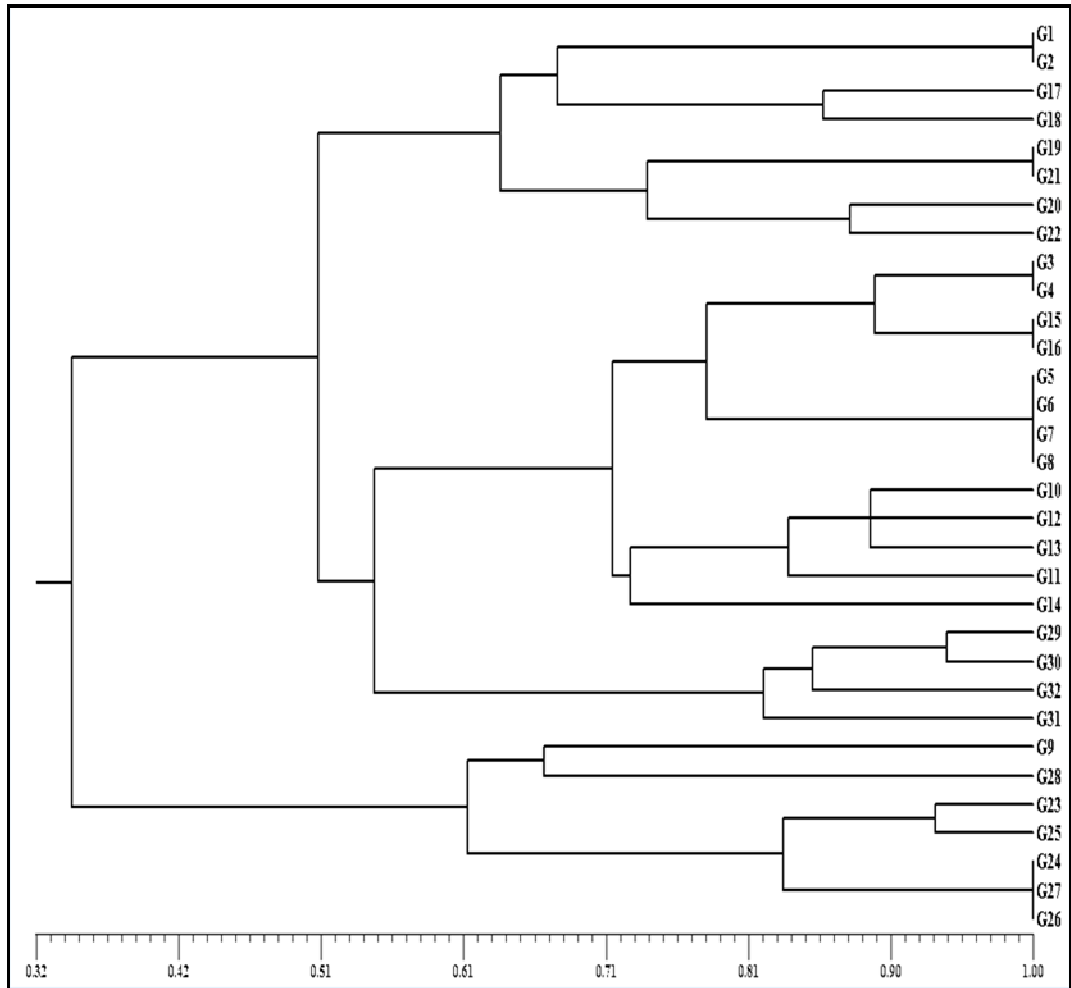


Fig. 3 : Similarity coefficient (Dice)

similarity coefficient was found as 0.84 followed by 0.83 which is further followed by 0.82. The least similarity coefficients found was 0.00 followed by 0.55, which was further followed by 0.61.

4.3.3 Cluster analysis and genetic divergence pattern

The input matrix for the genetic diversity/relatedness among the genotypes was prepared for SSR markers alleles at different loci that led to generation of similarity values among the genotypes studied. Based on information generated, the genotypes got separated into four main clusters which were further sub-clustered into various sub-groups (Table 20). Cluster I had 8 genotypes, Cluster II had 13 genotypes, Cluster III had 4 genotypes and Cluster IV had 7 genotypes.

The UPGMA dendrogram based on the cluster analysis and using unbiased genetic similarity coefficients of the SSR alleles were constructed for the 32 genotypes. The dendrogram revealed a peculiar picture showing the four clusters. The genotypes assigned to different clusters were as under:

- Cluster I consisted of eight genotypes (Calaro, Tumlahaal, Baber, Shalimar Rice-2, IR68333-RR-B-19, SKAU-337, IRBL Z-FU/EC 565188 and Shalimar Rice-3) and was further divided into two sub-clusters (Ia and Ib) separated from each other at a similarity value of 0.67 (Table 21). Among these sub-clusters, both the cluster contained four genotype each.
- Cluster II consisted of thirteen genotypes (C101A51/ARBN 152,SKAU-336, A57-115-4/ARBN 148, China-1031, A-57, Niver Zag, IRBN5M/EC 565166, KHD/GP/27, IR64, KHD/GP/150, B-14, B-4 and IRBN 1-CL/EC 565160) and was further divided into four sub-clusters IIa, IIb, IIc and IIId separated from each other at a similarity value of 0.0.71. Among these sub-cluster, the cluster IIa, IIb, IIc contained four genotypes each and IIId contained only one genotype.

Table-21 : Similarity matrix for 32 rice (*O. sativa* L.) genotypes based on SSR data

| | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 |
|------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|------------|
| G1 | 1.00 | | | | | | | | | | |
| G2 | 1.00 | 1.00 | | | | | | | | | |
| G3 | 0.87 | 0.66 | 1.00 | | | | | | | | |
| G4 | 0.80 | 0.66 | 1.00 | 1.00 | | | | | | | |
| G5 | 0.66 | 0.50 | 0.85 | 0.83 | 1.00 | | | | | | |
| G6 | 0.6 | 0.50 | 0.85 | 0.83 | 1.00 | 1.00 | | | | | |
| G7 | 0.66 | 0.50 | 0.82 | 0.83 | 1.00 | 1.00 | 1.00 | | | | |
| G8 | 0.66 | 0.500 | 0.82 | 0.83 | 1.00 | 1.00 | 1.00 | 1.00 | | | |
| G9 | 0.5 | 0.46 | 0.33 | 0.15 | 0.40 | 0.40 | 0.47 | 0.47 | 1.00 | | |
| G10 | 0.62 | 0.46 | 0.77 | 0.76 | 0.80 | 0.80 | 0.82 | 0.82 | 0.44 | 1.00 | |
| G11 | 0.71 | 0.54 | 0.75 | 0.72 | 0.76 | 0.76 | 0.80 | 0.80 | 0.37 | 0.87 | 1.00 |

| | G12 | G13 | G14 | G15 | G16 | G17 | G18 | G19 | G20 | G21 | G22 |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| G12 | 1.00 | | | | | | | | | | |
| G13 | 0.88 | 1.00 | | | | | | | | | |
| G14 | 0.70 | 0.70 | 1.00 | | | | | | | | |
| G15 | 0.62 | 0.50 | 0.80 | 1.00 | | | | | | | |
| G16 | 0.62 | 0.50 | 0.80 | 1.00 | 1.00 | | | | | | |
| G17 | 0.25 | 0.12 | 0.40 | 0.57 | 0.57 | 1.00 | | | | | |
| G18 | 0.37 | 0.25 | 0.53 | 0.71 | 0.71 | 0.85 | 1.00 | | | | |
| G19 | 0.66 | 0.53 | 0.42 | 0.61 | 0.61 | 0.61 | 0.76 | 1.00 | | | |
| G20 | 0.47 | 0.35 | 0.25 | 0.40 | 0.40 | 0.66 | 0.53 | 0.85 | 1.00 | | |
| G21 | 0.58 | 0.47 | 0.37 | 0.53 | 0.53 | 0.66 | 0.80 | 1.00 | 0.75 | 1.00 | |
| G22 | 0.47 | 0.47 | 0.12 | 0.26 | 0.26 | 0.53 | 0.40 | 0.71 | 0.87 | 0.62 | 1.00 |

| | G23 | G24 | G25 | G26 | G27 | G28 | G29 | G30 | G31 | G32 |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| G23 | 1.00 | | | | | | | | | |
| G24 | 0.80 | 1.00 | | | | | | | | |
| G25 | 0.93 | 0.87 | 1.00 | | | | | | | |
| G26 | 0.76 | 1.00 | 0.85 | 1.00 | | | | | | |
| G27 | 0.80 | 1.00 | 0.87 | 1.00 | 1.00 | | | | | |
| G28 | 0.61 | 0.71 | 0.57 | 0.71 | 0.71 | 1.00 | | | | |
| G29 | 0.53 | 0.37 | 0.50 | 0.46 | 0.37 | 0.46 | 1.00 | | | |
| G30 | 0.57 | 0.40 | 0.53 | 0.50 | 0.40 | 0.50 | 0.94 | 1.00 | | |
| G31 | 0.50 | 0.35 | 0.47 | 0.42 | 0.35 | 0.42 | 0.82 | 0.87 | 1.00 | |
| G32 | 0.42 | 0.53 | 0.40 | 0.66 | 0.53 | 0.66 | 0.82 | 0.87 | 0.75 | 1.00 |

- Cluster III consisted of four genotypes (SKAU-408, Rasi, C101PKT and SKAU- 356) and was divided into two sub-clusters (IIIa and IIIb) separated from each other at a similarity value of 0.55. Among these sub-clusters IIIa contained three genotypes and IIIb only one genotype.
- Cluster IV consisted of seven genotypes (IR61728-413-2-1-1, Shalimar Rice-1, Gurah, Pusa Sugand-3, B-9, SKAU-389 and RIL-10) and was further divided into three sub-clusters separated from each other at a similarity value of 0.61. Among these sub-clusters IVa, IVb contained two genotypes each and IVc contained three genotypes.

Table- 22: Comparative analysis of genetic diversity among 32 rice (*O. sativa* L.) genotypes on the molecular characterization (using SSR markers) and phenotypic diversity (using D²-statistics)

| SSR marker diversity | | | Phenotypic diversity | | |
|----------------------|---------------------|---|----------------------|------------------|--|
| Cluster No. | Number of genotypes | Name of genotypes | Cluster No. | No. of genotypes | Variety/Accession No. of genotypes |
| I | 4 | Calaro, Tumlahaal, Baber, Shalimar Rice-2 | I | 2 | Calaro, Kamad |
| | 4 | IR68333-RR-B-19, SKAU-337, IRBL Z-FU/EC 565188, Shalimar Rice-3 | | | |
| II | 4 | C101A51/ARBN 152, SKAU-336, A57-115-4/ARBN 148, China-1031 | II | 23 | SKAU-429, B-11, B-24, B-4, B-26, B-3, SKAU-3, SKAU-46, Mushkbudji, B-5, China-988, China-972, B-32, Jehlum, Dular, IRBL Z-FU/EC 565188, Chenab, K-116, Shalimar Rice-2, IRBL5M/EC565166, Shalimar Rice-3, C101A51/ARBN152, Shalimar Rice-1 |
| | 4 | A-57, Niver Zag, IRBN5M/EC 565166, KHD/GP/27 | | | |
| | 4 | IR64, KHD/GP/150, B-14, B-4 | | | |
| | 1 | IRBN 1-CL/EC 565160 | | | |
| III | 3 | SKAU-408, Rasi, C101PKT | III | 2 | B-8, SKAU-356 |
| | 1 | SKAU-356 | | | |
| IV | 2 | IR61728-413-2-1-1, SR-1 | IV | 40 | IR64, Tumlahaal, K-225, SKAU-336, C104PKT, A57, SK-2, SKAU-337, B-9, B-14, SKAU-408, IR61728-413-2-1-1, IR68333-RR-B-19, T2(MAS 71), Raminad |

| | | | | | |
|--|---|-----------------------|------|----|--|
| | | | | | Str 3, CO.39, Local Budgam-3 , IR63347-AL-201-1, IRBN 2008 V-87, Shalkew, BL-42, K-332, Wazul Krew, Kanto-51, RIL-10, BL-142/ARBN 142, T1, SK-339, China-1039, SKAU-338, China-1007,B-10,B-13,B-19, B-1509, SKAU-98, B-28, A3(IRRI LINE), Local Budgam-1, Local Budgam-2 |
| | 2 | Gureh, Pusa Sugand-3 | V | 2 | KHD/GP/27, Pusa Susand-3 |
| | 3 | B-9, SKAU-389, RIL-10 | VI | 14 | SKAU-405, Safed Brez, C105TTP.4L23, BL-122, Shai Tai Tsau,Usen, SKAU-389, Baber, RIL-29, IRBLKS-S/EC565178, Gulbara, Rasi, C101PKT, C101A51 |
| | | | VII | 16 | Niver Zag, IRBL1-CL/EC565160, Gurah, KHD/GP/120,China-1031, A57-115-4/ARBN148, KHD/GP/150, NP 125, BL 245, Yunlen-19, KHD/GP/154,SKAU-403, SKAU-292, SKAU-39, SKAU-402, SKAU-404 |
| | | | VIII | 3 | BL-122/ARBN 141, KHD/GP/208, Pusa Sugand-5 |

Chapter - 5

DISCUSSION

Rice (*oryza sativa* L.) is one of the world's most important cereal crop. It is the staple food of over half of the world's population, with much higher dependence as a source of food energy in Asia. It is life for Asians in general and Indians in particular. Rice (*Oryza sativa* L.) is a popular cereal crop that belongs to family Poaceae and includes other cereals such as wheat and corn. It is an excellent source of complex carbohydrates and is the best source of energy. Rice is rich in nutrients and contains a number of vitamins and minerals. Two species of rice are considered important *Oryza sativa* and *Oryza glaberrima* and grown worldwide. Despite the continuous increase in global demand of rice, it is subjected to various diseases that often place major constraints on its production. Among the major fungal diseases of rice, Rice sheath blight is one of the most devastating fungal disease of rice. It is second only to and often rivals rice blast in importance. Its causal agent is the necrotrophic fungus *Rhizoctonia solani* Kühn [teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk] anastomosis group (AG) 1, intraspecific group IA (Webster *et al.*, 1992).

Rice sheath blight (RSB) is difficult to control due to the low level of inherent resistance of rice against this disease (Anderson *et al.*, 1972; Khodayari *et al.*, 2009). Although partial genetic resistance to RSB has been reported and major genes responsible for resistance have been found (Pan *et al.*, 1999). RSB is still not efficiently controlled by resistance breeding (Cubeta and Vilgalys, 1997; Wu *et al.*, 2003). Sheath blight (ShB) management typically has relied on the application of fungicides in combination with cultural practices (Willoquet *et al.*, 2000). Hence, development of resistant varieties has a major role to play to control RSB is desirable, but greatly dependent on an understanding of the genetic diversity of the rice genotypes and population structure of the pathogen. Better understanding on the genetic diversity ensures the breeder in planning crosses for hybrid and line development, in assigning lines to heterotic groups and in plant

variety protection. Thus the success of breeding programme depends mainly upon choosing breeding stocks that have sufficient variability. Classification of total variability into its heritable and non-heritable components such as phenotypic and genotypic coefficient of variations, heritability estimates and expected genetic advance is of paramount importance in understanding the genetic make-up of any breeding material under improvement.

Genetic diversity is normally assessed by common morphological traits. However, such traits are affected by environment, development stage of the plant, type of plant material and require several replications to establish the genotypic contributions. Molecular markers are expected to bring information complimentary to the morphological descriptors, and could be potentially exploited for crop improvement as they provide excellent opportunities to assess genetic diversity more accurately due to their high informative index values (Wong *et al.*, 2009). Molecular genetic markers have brought phenomenal changes in the area of plant breeding and management of germplasm collections (Hoxha *et al.*, 2004) through their ability to produce unique DNA profiles in various crops. The use of molecular markers allows the direct assessment of genotypic variation at the DNA level. As genes involved in traits of agronomic importance are mapped and tagged, markers assist in breeding programs and even to isolate the gene. Marker analysis helps to understand the genetic makeup of the accessions and also make it possible to analyze the global organization of genetic diversity within a species (Welish and McCland, 1990). DNA fingerprinting technology in rice, physical mapping and genome sequencing in rice and combining of two genes for bacterial leaf blight resistance in Basmati rice variety through marker assisted selection has been successfully achieved at NRCPB, New Delhi (Joseph *et al.*, 2004; Gopalakrishnan *et al.*, 2008; Parida *et al.*, 2009; Ngangkham *et al.*, 2010).

Besides linkage mapping, gene targeting and assisted breeding, the DNA polymorphism assays are powerful tool for characterization and investigating

germplasm resources and genetic relatedness (Powell *et al.*, 1996). Several classes of DNA markers are available, but SSR markers are considered as most robust and amenable for genetic divergence studies due to their multiallelic nature, high reproducibility, co-dominant inheritance and extensive genomic coverage (McCouch *et al.*, 1998), exhibition of high degree of allelic variation (Temnykh *et al.*, 2000) and ability to detect genetic variation within and between accessions (Gilbert *et al.*, 1999; Zeng *et al.*, 2007 and Zheng *et al.*, 2011). Thousands of potential SSRs have been identified in rice and 35,000 have been developed as molecular markers (Temnykh *et al.*, 2000; McCouch *et al.*, 2002). These markers are being used in rice to develop high density genetic maps, characterize genotypic accessions and determine their genetic structure, optimize the assembly of core collections and strengthen marker assisted breeding techniques (McCouch *et al.*, 2002; Yu *et al.*, 2002; Garris *et al.*, 2005). SSR markers detect polymorphism by assaying subsets of the total amounts of the DNA sequence variation in a genome. PCR based polymorphisms result from DNA sequence variation at the primer binding sites and from DNA length differences between primer binding sites. The SSR assay utilizes pairs of primers flanking each simple sequence repeat and polymorphism differ for the number of repetitive di-tri or tetranucleotides units present at one locus. Polymorphism information content (PIC) value is reflection of allelic diversity and frequency among the genotypes, any value exceeding 0.5 reflects higher degree of polymorphism (Dewoody *et al.*, 1995).

5.1 Screening of genotypes for disease resistance/tolerance

In the present study 102 genotypes were screened for resistance/tolerance to rice sheath blight disease. Among all the genotypes screened the highest disease incidence (100%) and intensity (91.34%) was recorded in Pusa Sugand-3 followed by the genotype Pusa Sugand-5 (100% and 90.12% respectively) while the lowest disease incidence (10%) and intensity (1.11) was recorded in Niver Zag. The screening of the genotypes revealed that none of the genotype was immune, while 34 genotypes were resistant and 9 were

moderately resistant. Thus about 35.53 per cent genotypes showed resistance. Of the remaining 78 genotypes 12 were moderately susceptible, 46 were susceptible and 20 were highly susceptible. Several researchers have attempted in the past to identify sources of resistance to sheath blight (ShB) by screening a large number of wild species, landraces, local and improved cultivars, advanced breeding lines using different screening techniques (Srinivasachary *et al.*, 2011). They broadly included use of colonized typha bits (Bhaktavatsalam *et al.*, 1978), a toothpicks infected with *R. solani* (Zou *et al.*, 2000), broadcasting of inoculum on rice plants (Li *et al.*, 1995; Savary *et al.*, 1995; Singha and Borah 2000 and Han *et al.*, 2002), infected rice grain-hull mixtures (Pan *et al.*, 1999; Willocquet *et al.*, 2000), detached leaf technique (Prasad and Eizenga, 2008) and micro-chamber method (Jia *et al.*, 2009) under controlled greenhouse conditions. In comparison with field ShB evaluation, the micro-chamber and mist-chamber assays were simple, precise and more reliable assays methods in tagging ShB resistance (Liu *et al.*, 2009; Jia *et al.*, 2009). In the present study controlled chamber method was adopted by keeping the potted plants inoculated with typha bits under greenhouse condition, maintained at optimum humidity (90%) and temperature (28-30°C) for disease development.

5.2 Genetic variability, heritability and expected genetic gain

Extent of genetic variation was estimated in 102 genotypes. Morphological characterization of these genotypes, as per the Standard Evaluation System of IRRI, revealed that the flag leaf angle (a measure of photosynthetic efficiency) was erect flag leaf angle in 45 (44.11%), intermediate in 32 (31.37) and horizontal in 25 (24.50%) genotypes. Thus majority (76.37) had desirable (erect/intermediate) flag leaf angle. Panicle type was compact in 56 (54.90%) and intermediate in 32 (31.37%) genotypes. It was open in 14 (13.72%) genotypes. Awning pattern revealed that it was absent in 87 (85.29%), short and partly awned in 9 (8.82%), short and fully awned in 3 (2.94%) and long and partially awned in 1 (0.98%). Seed coat colour was white in 23 (22.54%), light brown in 47

(39.16%) speckled brown colour in 3 (2.94%) genotypes and brown in 27 (26.47). Panicle threshability was difficult in 7 (6.86%) genotypes. It was moderately in 31 (30.39%), intermediate in 49 (48.03%) and loose in 15 (14.70%) genotypes.

Genetic variability for 11 different quantitatively inherited traits viz., plant height (cm), panicle length (cm), leaf length (cm), leaf width (cm), tillering ability, days to 50% flowering, days to maturity, grain length (mm), grain width (mm), 1000-seed weight (g), grain yield (qha^{-1}). Analysis of quantitative traits revealed the presence of significant genetic variation for all the traits. Mean plant height of the genotypes was recorded as 93.16 ± 0.74 cm, with the range of variability from 120.00 to 45.66 cm. Panicle length had a population mean of 19.76 ± 0.57 cm, with variability range of 24.66 to 14.66 cm. Leaf length and its width ranged from 50.66 to 24.00 cm and 1.43 to 0.40 cm with a population mean of 33.98 ± 1.72 and 0.60 ± 0.02 . Tillering ability exhibited from 26.66 to 7.00, with a mean of 15.54 ± 0.51 . Days to 50% flowering revealed that on an average the mean days taken to 50% flowering were 104.46 ± 0.55 days with the variability range of 125.00 to 87.33. Days to maturity revealed that early, medium and late maturing genotypes were present in the population studied. On an average, the mean days taken to maturity were 135.37 ± 0.46 with the variability range of 147.66 to 124.00 days. Grain length and its width also exhibited significant variation in the genotypes. The minimum grain length recorded was 7.75 cm and grain width 2.04 cm, while maximum grain length was 8.54 cm and grain width 3.18 cm. The population mean for Grain length was 7.75 ± 0.07 cm and for grain width 2.81 ± 0.05 cm. 1000-seed recorded a population mean weight (g) of 28.43 ± 0.55 with a variability range of 31.83 to 20.43. Grain yield qha^{-1} revealed tremendous variability range of 131.92 to 16.85 q with a mean population grain yield of 10.22 ± 2.76 q ha^{-1} .

Estimates of coefficient of variation (phenotypic) and (genotypic) were derived for each quantitative trait. Over all magnitude of coefficient of variation (phenotypic and genotypic) was low (>10.0) for days to maturity,

grain length (mm) and days to 50% flowering. It was moderate (10.0-30.0%) for plant height (cm), panicle length (cm), leaf length (cm), grain width (mm) and 1000-seed weight (g) and high (>30%) for leaf width (cm), tillering ability and grain yield $q\ ha^{-1}$. Characters which possessed moderate to high coefficients of variation suggested that there is better potential for improvement through selection. A wide range of variability along with high estimates of phenotypic and genotypic coefficients of variation further indicate that these attributes would respond to selection. Characterization of crop germplasm through different morphological traits is an important step for assessment of its genetic potential. Our present finding shows great genetic potential of the studied genotypes. The promising genotypes identified during the current study have the potential to be used in future breeding programs for getting productive and quality results. During the current study for most of the qualitative and quantitative traits highly significant and positive differences were found. Our results are in close agreement with those of Pandey *et al.* (2009), who recorded highly significant variability among the different rice genotypes. Similarly the finding of Wang *et al.* (2006) also gives support to the current findings. The findings of Chandra *et al.* (2007) and Abarshahr *et al.* (2011) further strengthen the current findings, who also found valuable and highly significant and positive variability among their studied genotypes.

Estimates of heritability are of considerable importance to breeders as they help in information of an efficient and pragmatic breeding programme. Heritability (broad sense) estimates are informative as they indicate relative importance of genotypic and environmental contribution to variability exhibited and reliance that can be placed on phenotypic value during selection. It is considered in conjunction with the predicated genetic advance as suggested by Panse and Sukhatme (1957) and Johnson *et al.* (1955) as the heritability is influenced by bio-metrical method, generation of hybrid, sample size of experimental material and environment. Heritability (broad sense) were high

(>60%) for all the traits in environment taken. The estimated genetic advance and heritability (bs) was utilized to calculate expected genetic gain (% of mean). Therefore, the effect of selection is realized more quickly in those characters which have high heritability as well as high genetic gain. Normally in self-pollinating crops like rice estimation of expected genetic gain is not having that much significance. However, identification of a few high yielding varieties in yield traits and their selection *per se* from a large number of genotypes can provide a logistic approach to increase production/productivity of that crop species.

When high heritability is accompanied with high Genetic advance as per cent of mean (GAM), it indicates additive gene effects and selection may be effective. High heritability with low GAM indicates importance of non-additive gene action where high heritability is exhibited due to favourable influence of environment rather than genotype and the selection for such traits may not be rewarding. Low heritability with high GAM is governed by additive gene effects in which low heritability is exhibited due to high environmental effects and the selection may be effective in such cases. Low heritability coupled with low GAM indicates that character is highly influenced by environmental effects and selection would be ineffective. The expected genetic gain revealed that it was high (>30%) for plant height (cm) and grain yield (q ha^{-1}) whereas, it was low (20.0%) for panicle length (cm), leaf length (cm), leaf width (cm), tillering ability, days to 50% flowering, days to maturity, grain length (mm), grain width (mm), 1000-seed weight (g).

The magnitude of PCV was higher than the GCV for all the characters which may be due to higher degree of interaction of genotypes with the environment (Kavitha & Reddy, 2002). Katoch *et al.* (1993) and Sawant & Patil (1995) reported high GCV for plant height, whereas Ganesan and Subramanian (1994) reported moderate GCV for this trait. High GCV for tillers plant⁻¹ (Ganesan and Subramanian, 1994) and for panicle length (Lokaprakash *et al.*,

1992) has been reported. Similarly, several research workers have reported high GCV for yield and yield component traits..High magnitude of genetic variability, heritability and genetic gain has been reported in this crop. High heritability for tillers/plant (Sarvanan and Senthil, 1994) and grain yield plant⁻¹ (Sawant and Patil, 1995; Choudhury and Das 1998) have been reported. High expected genetic gain for tillers plant⁻¹ and yield components have been reported by Selvarani and Rangaswamy (1997). Most of the above results in respect of heritability and genetic advance were in agreement with reports on rice by Sarawgi and Rastogi (2000), Vange (2009) and Pandey and Anurag (2010). Similarly, Anjaneyulu *et al.* (2010) studied on 50 germplasm lines of rice revealed that high PCV and GCV were recorded for number of grains per panicle, fertility percentage and grain yield per plant.

5.3 Correlation coefficients

Yield is an ultimate criterion which a plant breeder has always to keep in view for evolving improved cultivars of any crop. However, yield is a polygenic character and highly influenced by environment. Knowledge of the association of quantitative characters specifically for yield and its attributes is of immense practical value during selection. Variability studies provide information on the extent of improvement possible in different characters, but they do not throw light on the extent and nature of relationship existing between various contributing characters and economically important characters. Hence, a knowledge regarding association of various characters among themselves and with economic character is necessary for making indirect selection for improvement of economical characters. Correlation studies pave way to know the association prevailing between highly heritable characters with most economic characters and gives better understanding of the contribution of each trait in building up the genetic make up of the crop. The phenotypic correlations indicate the extent of the observed relationship between two characters. This does not give true genetic picture of the relationship because it indicates both heritability as well as

environmental influences. Genotypic correlations provide an estimate of inherent association between genes controlling any two characters. Hence, it is of greater significance and could be effectively utilized in formulating an effective selection scheme. Perusal of Table-13 indicated that in the present investigation, the estimates of phenotypic correlation were in general slightly higher than genotypic correlation. In all instances, however, more impetus may be placed on the genotypic correlations. The nature of genotypic correlation was more or less similar to phenotypic correlation under study. Correlation coefficients revealed that the economically important trait i.e. grain yield (qha^{-1}) exhibited significant positive association with plant height ($r_g=0.211$, $r_p = 0.210$), panicle length ($r_g=0.214$, $r_p= 0.212$), leaf length ($r_g = 0.287$, $r_p= 0.232$), leaf width ($r_g =0.211$, $r_p0.203$), tillering ability ($r_g=0.261$, $r_p=0.234$), grain length ($r_g=0.242$, $r_p=0.230$), grain width ($r_g =0.322$, $r_p = 0.231$), 1000-seed weight ($r_g=0.322$, $r_p=0.293$) both at genotypic and phenotypic levels. A similar result was also reported by Sarkar (2006) and Sharifi *et al.* (2013). The finding of present investigation is agreement with the earlier worker viz., Laxmi *et al.* (2014), Shankar *et al.* (2006) and Ravindra *et al.* (2012); they have also recorded the positive correlation of effective tiller with grain yield per plant.

Days to 50% flowering ($r_g = -0.153$, $r_p = - 0.151$), Days to maturity ($r_g = - 0.134$, $r_p = -0.131$) exhibited negative and non-significant association with grain yield plant^{-1} . It was noticed that grain length was positively and significantly associated with leaf length and leaf width and grain width with panicle length. Similar result also estimated by Sarawgi *et al.* (1997) with leaf length and leaf width on grain yield which showed positive direct effect. The harmony conclusion of negative direct effect of days to maturity on grain yield was exposed by Hasan *et al.* (2012). The correlation between plant height and grain yield was significant and positive. The positive direct effect of plant height on grain yield also explained by Choudhury and Das (1998), Akhtar *et al.* (2011) and Ravindra (2012).

5.4 Genetic diversity

Analysis of genetic diversity is a platform for stratified sampling of breeding population. Involving genetically diverse parents is known to provide an opportunity for bringing together gene constellations yielding desirable transgressive segregants in advanced generations. Superior genotypes are selected and used as parents in hybridization programmes. However, the selection of superior parents from a large number of genotypes is a difficult task to perform. Genetic divergence analysis among genotypes is helpful to screen the genetically diverse parents that are likely to produce high heterotic effects among crosses and also generate large spectrum of variability during segregation and recombination of genes at heterozygous polygenic blocks. Multivariate technique using D^2 statistics (Mahalanobis, 1928) is a powerful tool in quantifying the degree of divergence among the genotypes. This would help to identify putative parents for executing an effective breeding strategy to obtain high heterotic response and transgressive segregants. Estimation of genetic divergence helps in reducing the large data of genotypes to manageable proportions. It is assumed that the parents showing wide genetic divergence are best suited for being used in the hybridization programme. In the process of formulating the rice improvement programme through hybridization and creating variability for the improvement of yield and other desirable traits, it is essential to understand the nature and degree of genetic divergence present in the available germplasm.

The utility of multivariate analysis in quantifying the degree of divergence between populations so as to understand the trend of their evolutionary pattern and assess the relative contribution of different components to the total divergence together with the nature of forces operating at intra and inter cluster levels had greatly been emphasized (Murty and Qadri, 1966; Anand and Murty, 1968; Mishra *et al.*, 1994). High genetic variability for different quantitative traits in rice has been reported earlier (Khan *et al.*, 2009; Ullah *et al.*, 2011; Seyoum *et al.*, 2012).

In the present study, 102 genotypes of rice were evaluated to estimate the genetic divergence for identification of potential parents using Mahalanobis D^2 statistics. Analysis of variance for divergence revealed that the value of V-statistic were significant indicating substantial genetic diversity in the material. Classification of the 102 genotypes led to formation of eight clusters (Table-14) as per Mahalanobis D^2 analysis employing Tochers method (Rao, 1952). Cluster IV comprised of maximum number of genotypes (40) followed by cluster II (23), cluster VII (16), cluster VI (14), cluster VIII (3) and cluster I, cluster III and cluster V (two each).

The pattern of group constellations in the present study, suggested that geographical diversity was not an essential factor to group the genotypes from a particular source or origin into one particular cluster. This means that, geographical diversity, though important, was not the only factor in determining the genetic divergence. Genetic diversity is the outcome of several factors, including geographical diversity. Therefore selection of parents should be based on genetic diversity rather than geographical diversity and statistical distances (D^2) presented the index of genetic diversity among these clusters. According to Rao (1952), no formal rules can be laid down for forming a cluster, yet any two genotypes belonging to the same cluster should atleast, on an average, show a smaller D^2 value as compared to two genotypes falling into different clusters.

Clustering together of genotypes from different sources is probably due to free exchange of germplasm among breeders of different regions and/or unidirectional selection practised by breeders while tailoring promising cultivars for different regions (Chaturvedi and Maurya, 2005; Sabesan *et al.*, 2008). Similarly, genotypes from the same source of origin getting grouped into different clusters may be due to differential adaptation to varied agro-ecosystems (Kadamoorthy and Govindarasu, 2005; Senapati and Sarkar, 2005). The mean inter- and intra-cluster distances was measured to identify the most divergent clusters and also the genotypes within a cluster. The maximum intra-cluster

distance (D^2) of (1271.69) was in cluster V had followed by cluster I (601.56) and the minimum intra-cluster distance (D^2) of (0.00) was in cluster VIII. The maximum inter-cluster distances (D^2) value was (1146.52) between cluster VIII and VII followed by cluster VII and VI (823.12), cluster VI and I (668.36), cluster V and III (599.95). The minimum inter- cluster distance was observed between cluster II and IV (376.39). Genotypes included in a particular cluster indicated their close relationship among themselves as compared to the other clusters. Therefore, it could be expected that genotypes within a cluster were less genetically different with each other, and were diverse from the accessions belonging to other clusters. Sohrabi *et al.* (2012) clustered 35 upland rice into six clusters, cluster III contains 27 accessions and clusters I, II, IV and V comprised 6, 10, 2 and 4 accessions, respectively, and cluster VI was monogenic.

Cluster mean for different traits (Table-15) revealed that the magnitude of differences among the mean of the traits for clusters was significant. The highest cluster means for plant height cm (114.04) was found in cluster VII, for panicle length (cm) was 21.06 in cluster II, for leaf length (cm) was 41.67 in cluster VIII, for leaf width (cm) was 1.43 in cluster VIII, for tillering ability was 20.17 in cluster I, for days to 50% flowering was 125.00 in cluster VIII, for days to maturity was 147.67 in cluster VIII, for grain length (mm) was 7.90 in cluster III, for grain width (mm) was 2.96 in cluster IV, for 1000-seed weight (g) was 29.63 in cluster IV and for grain yield $q\ ha^{-1}$ was 120.40 in cluster I. Cluster mean of different characters help in choosing the diverse parents for hybridization and these divergent parents are likely to broaden the genetic base (variability) and make available transgressive segregants with high heterotic effects (Qian and He, 1991; Rao and Gomathinayagam, 1997). Sardana *et al.* (1997) observed that cluster means and genotypic coefficient variation reveal interesting picture about nature of diversity.

The maximum intra cluster distance (D^2) (cluster V) indicated high heterogeneity in genetic constitution of genotypes in that cluster while minimum

intra cluster distance (D^2) (cluster VIII) indicated homogeneity in genetic constitution of genotypes in that cluster. As well as the highest value of intercluster distance (cluster VIII and VII) indicated also more heterogeneous genetic constitution of genotypes included in both clusters. Selection of the parents for hybridization should be done from different clusters having wide inter-cluster distance and those selected parents should have high *per se* performance for the traits contributing maximum towards divergence (Singh *et al.*, 1996). Clusters consisting of only one genotype with specific traits could be used in hybridisation programme for the exploitation of heterosis mainly as testers for expression of maximum heterosis. Singh *et al.* (2011) reported that highest mean values for days to maturity, plant height, total number of tillers per plant and panicle length and lowest mean values for test weight and kernel length.

The per cent contributions of the traits towards total genetic divergence (Table-17) revealed that plant height was the main factor contributing to divergence accounting for 41.16% followed by days to 50% flowering (18.17%), gain yield $q\ ha^{-1}$ (12.02%), days to maturity (9.98%), grain length (9.98%), tillering ability (7.98%), leaf width (5.75%), and grain width (1.13). The minimum contribution towards divergence was from grain width (1.13%) followed by leaf width (5.75%) and tillering ability (7.98%). The traits contributing maximum towards the divergence should be given great emphasis for deciding the clusters to be chosen for hybridisation and the subsequent selection of the parents from the clusters be based on their *per se* performance. In rice crop significant contribution towards divergence has been reported from plant height (Karthikeyan, 2002); and grain yield (Singh *et al.*, 1996; Ahmad and Borah, 1999; Karthikeyan, 2002; Datt and Mani, 2003). The important characters reported by some researchers were plant height, panicle length, number of unfilled grains per panicle, 1000-grain weight (Latif *et al.*, 2011 and Sabesan *et al.*, 2009), grain yield, number of effective tillers (Baradhan and Thangavel, 2011), harvest index (Ahmed *et al.*, 2010), days to maturity (Kanwal *et al.*, 1983) and number of

secondary branches per panicle (Sinha *et al.*, 1991).

5.5 Genetic diversity studies using SSR markers

Genetic diversity assessment of the rice genotypes is an essential component in germplasm characterization and conservation to identify potential parents. Morphological and seed traits have long been the means of studying taxonomy and variability among plant species. Microsatellites are among the most widely used DNA marker for many purposes such as diversity, genome mapping, varietal identification, etc. (Teixeira da Silva, 2005). Simple sequence repeat is an important tool for genetic variation identification of germplasm (Powell *et al.*, 1996; Ma *et al.*, 2011). SSR marker have some merits such a quickness, simplicity, rich polymorphism and stability, thus being widely applied in genetic diversity analysis, molecular map construction and gene mapping (Zhang *et al.*, 2007; Ma *et al.*, 2011), construction of fingerprints (Xiao *et al.*, 2006; Ma *et al.*, 2011), genetic purity test (Peng *et al.*, 2003; Ma *et al.*, 2011), analysis of germplasm diversity (Zhou *et al.*, 2003; Jin *et al.*, 2010; Ma *et al.*, 2011) utilization of heterosis, especially in identification of species with closer genetic relationship. A total of 18,828 Class 1 di-, tri- and tetra-nucleotide SSRs, representing 47 distinctive motif families, were identified and annotated on the rice genome. An abundance of microsatellite markers is now available through the published high-density linkage map; there was an average of 51 hypervariable SSRs per Mb, with the highest density of markers occurring on chromosome 3 (55.8 SSRMb-1) and the lowest occurring on chromosome 4 (41.0 SSRMb-1) (IRGSP, 2005).

The present investigation addresses the utilization of 8 sheath blight linked microsatellite markers to reveal genetic polymorphism and ensures unambiguous identification of sheath blight QTLS. The 8 SSR markers used in this study were mapped previously by Liu *et al.* (2013). The total number of alleles produced by these primers was 22 and ranged from 2-5 with an average of 2.87 alleles per primer. Among the primers used RM 55 produced a maximum of

5 alleles. This number is smaller than that reported for average number of alleles 7.8 alleles/locus by Jain *et al.* (2006); as recorded from a set of Indian aromatic and quality rice accessions or Yu *et al.* (2003) reported (6.3 alleles/locus) from 193 rice accessions drawn from 26 countries using 101 SSR primer pairs and detected an average allele number of 6.3 per locus, which is also higher than the value reported here. Pervaiz *et al.* (2009) used 32 SSR markers to determine the genetic diversity of 35 cultivars of Asian rice and showed a clear division of cultivars into aromatic and non-aromatic groups. In their experiment, the number of alleles detected by microsatellite markers varied from 2 to 13 with an average of 4.5 alleles per locus, which is higher than our study (3.33 alleles per locus). Such variability exist in the number of alleles detected per locus might be due to the diverse germplasm used and selection of SSR primers with scorable alleles. However the average number of allele per locus (2.87 alleles) obtained in our study is comparable with the result reported by Cho *et al.*, (2000) detecting 2.0-5.5 alleles per SSR locus for various classes of microsatellites. Wong *et al.* (2009) reported the genetic relationship and diversity analysis among 8 Bario rice cultivars using 12 SSR primers, detecting a total of 31 alleles.

Polymorphism index content value is the reflection of allelic diversity and frequency among the genotypes. The PIC values varied from 0.22 (RM 202) and (RM 245) to 0.67 (RM55) with an average of 0.42 per locus. Significant correlation existed between the PIC values and the number of alleles at SSR loci. PIC value of >0.60 was found for SSR markers RM55and RM 555 and it was < than 0.6 in RM 104, RM 530, RM 245, RM287, RM 125 and RM 202. Ram *et al.* (2007) studied genetic diversity among 35 rice accession (landraces, cultivars and wild relatives) using SSR markers. Mean number of alleles per locus identified were 4.86 with a mean PIC value of 0.70.

The maximum number of alleles (5) were recorded by SSR marker RM 55 with high PIC values of around 0.67. The PIC value of SSR marker that recorded 4 alleles was 0.65 and that recorded 3 alleles was 0.22, while those that

recorded 2 alleles was from 0.22-0.50. PIC value of >0.60 was found for SSR markers RM55 and RM 555 and it was $<$ than 0.6 in RM 104, RM 530, RM 245, RM287, RM 125 and RM 202. In the present investigation, comparatively low mean PIC value (0.67) could be due to: (1) only limited number of genotypes which are very well acclimatized to the local environment were used; (2) less differences in the contribution of the marker DNA regions; (3) gene pool with narrow genetic base; (4) high selection pressure of the genotypes that might have resulted in more similarity of the genotypes (Giarrocco *et al.*, 2005). In the present study, the level of polymorphism determined by the PIC value (mean= 0.42) is consistent with the reported PIC value in previous works (Lu *et al.*, 2005; Wong *et al.*, 2009; Hossain *et al.*, 2012). According to the early reports on the PIC values ranged from a low of 0.24 to a high of 0.92 and averaged 0.61 (Jain *et al.*, 2004), 0.19 to 0.90 with an average of 0.75 (Borba *et al.*, 2009), which is markedly higher than the result in our study. Upadhyay *et al.* (2011) also reported the average PIC value of 0.78.

These result revealed that markers RM55 would be best in screening rice genotypes followed by RM555, RM104 and so on. Thus, the PIC value indicates that all these primers were highly informative and capable of distinguishing between genotypes.

5.6 Estimation of genetic distance

Dendrogram from SSR markers put the rice genotypes in slightly different groups, thereby, indicating these markers vary in their efficiency in extracting similarities and differences among these types. Pair-wise comparisons between the tested genotypes were used to calculate the genetic similarity. All the genotypes were scored for the presence and absence of the SSR bands throughout all 32 genotypes and the data were exported to binary data for the presence (1) or absence (0) or as a missing observation (9) for further analysis with NTSYS-pc version 2.2 (Rohlf, 1997). NTSYS-pc was used to construct a UPGMA (unweighted pair group method with arithmetic averages) dendrogram showing

the distance-based interrelationship among the genotypes. A similarity coefficients matrix based on the proportion of shared SSR alleles was used to establish the level of relatedness between each pair of the genotypes using JACCARD's coefficient. The similarity index values obtained for each pair wise comparison among 32 genotypes are presented in Table-20. Relationships among 32 genotypes revealed a varying degree of genetic relationship. Pairwise genetic similarity coefficients varied from 0.00 to 0.94, with an average of 0.62. Highest genetic similarity coefficient was found as 0.84 followed by 0.83 which is further followed by 0.82. The least similarity coefficients found was 0.00 followed by 0.55, which was further followed by 0.61. Above 75% genetic similarity between genotypes in cluster analysis for 48 traditional aromatic rice collected from different parts of Odisha using 12 SSR and 24 ISSR markers were obtained by Meti *et al.* (2013) and Samal *et al.* (2014) respectively, which was comparable to current results obtained in present study. High similarity coefficient indicate genotypes belonging to similar genetic background. This arises possibly by selective crossing wherein alleles of a source are assembled or occasional out crossing common events occurring spontaneously (Chakravarthi and Naravaneni, 2006). Contrarily, magnitude and spectrum of lower value similarity coefficient among genotypes is indication of higher genetic diversity among the genotypes. In this study, the larger range of similarity values for cultivars revealed by microsatellite markers provides greater confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programs. With the aid of microsatellite makers and clustering data, different distantly related rice genotypes may be combined by intercrossing genotypes, for instance, aromatic rice genotypes with non-aromatic rice genotypes from different clusters to get hybrid varieties with highest heterosis. Many studies have also reported significantly greater allelic diversity of microsatellite markers than other molecular markers (McCouch *et al.*, 2001). Marker-aided backcrossing (MAB), enabled by advances in genomics and molecular mapping in recent years, is more

precise, time-saving, and cost-effective way to develop rice varieties that can withstand these stresses than conventional breeding.

5.7 Cluster analysis and genetic diversity pattern

The input matrix for the genetic diversity/relatedness among the genotypes was prepared for SSR markers alleles at different loci that led to generation of similarity values among the genotypes studied. Based on information generated, the genotypes got separated into four main clusters which were further sub-clustered into various sub-groups (Table). Cluster I had 8 genotypes, Cluster II had 13 genotypes, Cluster III had 4 genotypes and Cluster IV had 7 genotypes. The dendrogram reveals that the genotypes that are derivatives of the genetically common background tend to cluster together or nearer. The clustering pattern may be due to the substantial variation in geographic and climatic conditions that house different agro-ecosystems (Zhu *et al.*, 2004). Similar to current clustering pattern was also obtained by Meti *et al.* (2013) for 48 aromatic rice collection from Odisha using 12 SSR markers, which separated the genotypes into 2 major clusters at 49% genetic similarity using jaccard's similarity index. Comparative studies in different rice species using SSR were successfully conducted by other researchers Nagaraju *et al.* (2002), Rahman *et al.* (2002), Wong *et al.* (2009), Prabhakaran *et al.* (2010), Singh and Singh. (2012), Zhu *et al.* (2013). Herrera *et al.* (2008) used 48 simple-sequence-repeat (SSR) markers to assess the genetic diversity of 11 Venezuelan rice cultivars released by the National Rice Breeding Program between 1978 and 2007 and detected 203 alleles. Pervaiz *et al.* (2010) used 35 SSR markers to detect genetic diversity in 75 rice landraces and identified 142 alleles. The polymorphism information content (PIC) ranged from 0.124 to 0.836, with an average of 0.569. Behera *et al.* (2012) used 36 microsatellite markers to assess genetic diversity in a set of 33 medicinal rice genotypes and detected 166 polymorphic loci. The PIC values ranged between 0.24 and 0.956 with an average of 0.811 per locus.

The gene diversity detected in this study (0.55) is comparable to overall gene diversity of rice core collection (0.544) from China, North Korea, Japan, Philippines, Brazil, Celebes, Java, Oceanina and Vietnam (Zhang *et al.*, 2011) and it is higher than US accession panel with average gene diversity of 0.43 (Agrama and Eizenga 2008) and Chinese rice accession panel by Jin *et al.* (2010) with the average gene diversity of 0.47. The gene diversity reported in our study is lesser than gene diversity (0.68) reported by (Liakat Ali *et al.*, 2011). Most of the diversity panel with global accessions has the gene diversity of 0.5 to 0.7 (Garris *et al.*, 2005; Liakat Ali *et al.*, 2011; Ni *et al.*, 2002). These results on global accessions help to infer that this diversity panel of germplasm lines represents a large proportion of the genetic diversity that exists in major rice growing Asian continent.

Chapter - 6

SUMMARY AND CONCLUSION

The present investigation entitled “Identification and Morpho-molecular characterization of genotypes of rice (*Oryza sativa* L.) showing resistance to sheath blight disease” was undertaken to obtain information on the variability and genetic divergence of the genotypes studied. Assessment of genetic diversity in crop germplasm is vital for identification of the genotypes to be used in the future breeding programme. Nature and magnitude of genetic variability, genetic diversity (D^2 statistics) at the phenotypic level was estimated in 102 rice genotypes and genetic distance at molecular level (using SSR primers) was assessed in selected 32 genotypes. One hundred two genotypes were screened for resistance/tolerance to rice sheath blight disease under field conditions. The genotypes were evaluated for morphological characteristics as per the standard procedure of the International Rice Research Institute, Philippines. A general scale with index value 0-9 was adopted for most of the traits. The observations were recorded on eleven quantitative traits viz., plant height (cm), panicle length (cm), leaf length (cm), leaf width (cm), tillering ability, days to 50% flowering, days to maturity, grain length (mm), grain width (mm), 1000 seed weight (g), grain yield $q\ ha^{-1}$. Data was subjected to various statistical and biometrical analysis and the results obtained are summarized in the present chapter:

- Screening of the genotypes for resistance/tolerance to rice sheath blight disease revealed that among all the genotypes the highest disease incidence (100%) and intensity (91.34%) was recorded in Pusa Sugand-3 followed by the genotype Pusa Sugand-5 (100% and 90.12% respectively) while the lowest disease incidence (10%) and intensity (1.11) was recorded in Niver Zag. About 35.53 per cent genotypes showed resistance to the disease. Of the remaining 78 genotypes 12 were moderately susceptible, 46 were susceptible and 20 were highly susceptible.

- Morphological traits *vis-a-vis*, flag leaf angle, panicle type, awning, panicle threshability and seed coat colour was characterized using standard procedure of IRRI on the basis of scale (scores 0-9). Flag leaf angle was erect/intermediate in nearly 69.33 per cent genotypes. Panicle type was compact/intermediate in nearly 80.43 genotypes; with panicle threshability as intermediate to easy in nearly 38.37 genotypes, seed coat colour was white/brown in nearly 73.43 genotypes. Awning was absent in 84.31 genotypes.
- Analysis of variance revealed significant differences among genotypes for all the traits studied. The highest phenotypic and genotypic coefficients of variability were observed for leaf width (cm) followed by tillering ability, grain yield ha^{-1} (q), plant height (cm), leaf length (cm) and panicle length (cm). In general the phenotypic coefficients of variation were higher than genotypic coefficients of variation which indicates the role of environment in the expression of traits under observation.
- The estimates of heritability in broad sense was high for all the characters. The present investigation indicates a great scope in the improvement of these traits as these characters in general possessed high estimates of heritability coupled with high genetic advancement except for days to maturity, grain length (mm) and days to 50% flowering (high heritability but moderate genetic gain) indicating the preponderance of additive gene action for control of these traits.
- Genotypic correlation coefficient, were by and large, higher in magnitude, though similar in direction than their corresponding correlation coefficients at phenotypic level. Correlation coefficients revealed that the economically important trait i.e. grain yield (q ha^{-1}) exhibited significant positive association with plant height ($r_g=0.211$, $r_p = 0.210$), panicle length ($r_g=0.214$, $r_p= 0.212$), leaf length ($r_g = 0.287$, $r_p=$

0.232), leaf width ($r_g = 0.211$, $r_p = 0.203$), tillering ability ($r_g = 0.261$, $r_p = 0.234$), grain length ($r_g = 0.242$, $r_p = 0.230$), grain width ($r_g = 0.322$, $r_p = 0.231$), 1000-seed weight ($r_g = 0.322$, $r_p = 0.293$) both at genotypic and phenotypic levels.

- Estimates of divergence among 102 rice genotypes revealed that significant divergence existed among them. The genotypes under study were grouped into 8 clusters as per Mahalanobis D^2 (1928) analysis employing Tochers method; with maximum number of genotypes in cluster IV (40) followed by cluster II (23), cluster VII (16), cluster VI (14), cluster VIII (3) and cluster I, cluster III and cluster V (two each).
- The mean intra and inter-cluster distance (D^2) values for the same environment (Table 16) revealed that cluster V had highest intra-cluster distance (D^2) value of (1271.69) followed by cluster I (601.56). The inter-cluster distances (D^2) value was highest (1146.52) between cluster VIII and VII followed by cluster VII and VI (823.12), cluster VI and I (668.36), cluster V and III (599.95). The minimum inter-cluster distance was observed between cluster II and IV (376.39).
- The per cent contribution towards the total genetic divergence revealed that plant height (cm), days to 50% flowering, grain yield ha^{-1} (q), days to maturity, tillering ability and leaf width (cm) were the main contributing characters towards total genetic divergence.
- Molecular characterization of selected 32 genotypes from amongst the 102 genotypes was carried out using SSR markers. A total of eight simple sequence repeat (SSR) markers were utilized to characterize and assess genetic diversity of 32 genotypes of rice. All these markers generated good and reproducible products for all of the 32 genotypes. The level of polymorphism among the genotypes was evaluated by calculating polymorphic bands, polymorphic information content,

effective multiplex ratio and marker index for each of the 8 SSR loci evaluated. A total of 22 alleles were detected across the 32 genotypes through the use of these 8 SSR markers. The number of alleles per locus generated by these SSR markers varied from 2 (RM 104) (RM 202) (RM 530) (RM 287) and (RM 125) to 5 (RM 55), which also stands clearly indicated from the fingerprinting (Plate 4-8). The average alleles per locus were 2.87.

- Polymorphism information content value which is the reflection of allelic diversity and frequency among the genotypes, revealed that the PIC values varied from 0.22 (RM 202) and (RM 245) to 0.67 (RM55) with an average of 0.42 per locus. Significant correlation existed between the PIC values and the number of alleles at SSR loci. PIC value of >0.60 was found for SSR markers RM 55 and RM 555 and it was < 0.6 in RM 104, RM 530, RM 245, RM287, RM 125 and RM 202.
- Similarity coefficients (measuring relatedness) were used to construct the dendrogram and clustering pattern of the genotypes. Pair wise similarity coefficients ranged from 0.10-0.93. Similarity coefficient value revealed presence of good genetic diversity among these genotypes at the molecular level. All the 32 genotypes got clustered into four clusters which were further sub-clustered into various sub-groups, with maximum number of genotypes (13) in cluster III followed by cluster I. Cluster I had 8 genotypes, Cluster II had 13 genotypes, Cluster III had 4 genotypes and Cluster IV had 7 genotypes.

Based on the findings of the present investigation the following conclusion could be drawn as under:

- 1) Genotypes which were screened for sheath blight disease resistance, exhibit substantial genetic diversity at morphological level.
- 2) For future breeding programme parents can be selected from most

divergent clusters showing high performance and can be utilized in hybridization programme for creating variability in the genetically narrow crop species. The genotypes can also be utilised for the transfer of specific traits into the commercial variety/ies.

- 3) Marker analysis of the 32 selected genotypes revealed that SSR markers were very robust in studying the genetic polymorphism. However an elaborate study is suggested to confirm the present findings through the use of large number of SSR markers that would cover whole genome and also identify rare/unique alleles
- 4) Forty resistant genotypes were identified under field conditions, which need to be studied under artificial epiphytotic conditions. The genotypes can then be used for the development of mapping population for the retrogression of sheath blight resistance gene(s) or QTL(s) into commercial cultivars.

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Appendix - 1**Raw Morphological data of 102 rice (*Oryza sativa* L.) genotypes**

| | | Plant height (cm) | Penicle length (cm) | Leaf length (cm) | Leaf width (cm) | Tillering ability | Days to 50% flowering | Days to maturity | Grain length (mm) | Grain width (mm) | 1000 seed weight (g) | Grain yield ha ⁻¹ (q) |
|-----------------|------|-------------------|---------------------|------------------|-----------------|-------------------|-----------------------|------------------|-------------------|------------------|----------------------|----------------------------------|
| Calaro | R1 | 54 | 15 | 33 | 0.8 | 24 | 110 | 142 | 7.99 | 2.03 | 20.3 | 125.39 |
| | R2 | 52 | 14 | 33 | 0.8 | 25 | 109 | 142 | 7.93 | 2.06 | 20.6 | 126.98 |
| | R3 | 54 | 15 | 34 | 0.7 | 24 | 110 | 142 | 7.97 | 2.06 | 20.6 | 126.98 |
| | Mean | 53.3333 | 14.6667 | 33.3333 | 0.76667 | 24.3333 | 109.667 | 142 | 7.96333 | 2.05 | 20.5 | 126.45 |
| C101A51 | R1 | 59 | 15 | 31 | 0.6 | 17 | 102 | 135 | 6.36 | 2.85 | 28.5 | 69.57 |
| | R2 | 60 | 14 | 33 | 0.5 | 16 | 102 | 135 | 6.36 | 2.86 | 28.6 | 72.59 |
| | R3 | 60 | 15 | 33 | 0.5 | 15 | 103 | 136 | 6.23 | 2.76 | 27.6 | 72.56 |
| | Mean | 59.6667 | 14.6667 | 32.3333 | 0.53333 | 16 | 102.333 | 135.333 | 6.31667 | 2.82333 | 28.2333 | 71.5733 |
| SKAU-429 | R1 | 115 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.23 | 2.05 | 20.5 | 130.87 |
| | R2 | 117 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.41 | 2.27 | 22.7 | 132.45 |
| | R3 | 115 | 18 | 37 | 0.5 | 17 | 106 | 132 | 7.43 | 2.29 | 22.9 | 132.45 |
| | Mean | 115.67 | 18.67 | 37.67 | 0.57 | 16.33 | 106.67 | 132.67 | 7.36 | 2.20 | 22.03 | 131.92 |
| C101PKT | R1 | 56 | 17 | 29 | 0.6 | 18 | 109 | 130 | 6.88 | 2.99 | 29.9 | 98.99 |
| | R2 | 56 | 17 | 27 | 0.5 | 15 | 110 | 129 | 6.76 | 3.09 | 30.9 | 99.78 |
| | R3 | 58 | 18 | 27 | 0.6 | 19 | 109 | 131 | 6.79 | 2.87 | 28.7 | 99.67 |
| | Mean | 56.6667 | 17.3333 | 27.6667 | 0.56667 | 17.3333 | 109.333 | 130 | 6.81 | 2.98333 | 29.8333 | 99.48 |
| C101A51/ARBN152 | R1 | 115 | 20 | 33 | 0.5 | 16 | 102 | 135 | 7.71 | 2.99 | 29.9 | 56.98 |
| | R2 | 115 | 22 | 24 | 0.5 | 18 | 104 | 138 | 7.79 | 2.98 | 29.8 | 66.34 |
| | R3 | 111 | 22 | 36 | 0.5 | 16 | 100 | 136 | 7.66 | 2.88 | 28.8 | 63.67 |
| | Mean | 113.667 | 21.3333 | 31 | 0.5 | 16.6667 | 102 | 136.333 | 7.72 | 2.95 | 29.5 | 62.33 |
| SKAU-356 | R1 | 108 | 20 | 29 | 0.5 | 7 | 102 | 124 | 8.05 | 2.66 | 26.6 | 76.34 |
| | R2 | 110 | 22 | 30 | 0.5 | 8 | 102 | 124 | 7.99 | 2.99 | 29.9 | 78.34 |
| | R3 | 112 | 23 | 29 | 0.5 | 7 | 101 | 124 | 7.99 | 2.58 | 25.8 | 76.99 |
| | Mean | 110 | 21.6667 | 29.3333 | 0.5 | 7.33333 | 101.667 | 124 | 8.01 | 2.74333 | 27.4333 | 77.2233 |
| Shalimar Rice-1 | R1 | 90 | 22 | 33 | 0.5 | 16 | 107 | 133 | 7.99 | 2.89 | 28.9 | 123.78 |

| | | | | | | | | | | | | |
|-------------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | R2 | 93 | 23 | 32 | 0.5 | 15 | 106 | 132 | 8.05 | 2.85 | 28.5 | 127.65 |
| | R3 | 94 | 23 | 34 | 0.5 | 17 | 107 | 133 | 8.05 | 2.89 | 28.9 | 127.56 |
| | Mean | 92.3333 | 22.6667 | 33 | 0.5 | 16 | 106.667 | 132.667 | 8.03 | 2.87667 | 28.7667 | 126.33 |
| KHD/GP/120 | R1 | 117 | 18 | 35 | 0.6 | 9 | 102 | 126 | 7.33 | 2.76 | 27.6 | 100.01 |
| | R2 | 119 | 17 | 33 | 0.6 | 8 | 104 | 129 | 7.56 | 3.07 | 30.7 | 101.45 |
| | R3 | 117 | 18 | 35 | 0.6 | 7 | 104 | 127 | 7.39 | 2.75 | 27.5 | 111.03 |
| | Mean | 117.667 | 17.6667 | 34.3333 | 0.6 | 8 | 103.333 | 127.333 | 7.42667 | 2.86 | 28.6 | 104.163 |
| Rasi | R1 | 61 | 17 | 25 | 0.5 | 16 | 105 | 133 | 7.99 | 2.77 | 27.7 | 99.34 |
| | R2 | 64 | 16 | 27 | 0.5 | 16 | 104 | 134 | 7.67 | 3.06 | 30.6 | 100.23 |
| | R3 | 62 | 17 | 25 | 0.5 | 18 | 104 | 134 | 7.68 | 2.81 | 28.1 | 99.67 |
| | Mean | 62.3333 | 16.6667 | 25.6667 | 0.5 | 16.6667 | 104.333 | 133.667 | 7.78 | 2.88 | 28.8 | 99.7467 |
| IR64 | R1 | 92 | 21 | 24 | 0.9 | 17 | 109 | 136 | 6.33 | 3.09 | 30.9 | 87.99 |
| | R2 | 93 | 22 | 23 | 0.9 | 16 | 108 | 135 | 7.03 | 3.23 | 32.3 | 88.77 |
| | R3 | 95 | 21 | 25 | 0.9 | 17 | 110 | 137 | 6.54 | 3.23 | 32.3 | 88.56 |
| | Mean | 93.3333 | 21.3333 | 24 | 0.9 | 16.6667 | 109 | 136 | 6.63333 | 3.18333 | 31.8333 | 88.44 |
| Gulbara | R1 | 68 | 18 | 29 | 0.5 | 16 | 110 | 133 | 8.01 | 2.65 | 26.5 | 109.23 |
| | R2 | 67 | 16 | 28 | 0.5 | 17 | 110 | 134 | 8.02 | 2.51 | 25.1 | 107.89 |
| | R3 | 69 | 18 | 29 | 0.5 | 18 | 111 | 133 | 7.99 | 2.71 | 27.1 | 107.99 |
| | Mean | 68 | 17.3333 | 28.6667 | 0.5 | 17 | 110.333 | 133.333 | 8.00667 | 2.62333 | 26.2333 | 108.37 |
| Tumlahall | R1 | 95 | 21 | 33 | 0.5 | 16 | 110 | 135 | 7.22 | 3.01 | 30.1 | 126.77 |
| | R2 | 98 | 22 | 34 | 0.6 | 17 | 110 | 134 | 7.34 | 3.05 | 30.5 | 126.67 |
| | R3 | 97 | 21 | 33 | 0.5 | 17 | 111 | 134 | 7.41 | 3.03 | 30.3 | 126.44 |
| | Mean | 96.6667 | 21.3333 | 33.3333 | 0.53333 | 16.6667 | 110.333 | 134.333 | 7.32333 | 3.03 | 30.3 | 126.627 |
| Niver Zag | R1 | 118 | 18 | 35 | 0.5 | 17 | 101 | 134 | 8.09 | 2.01 | 20.1 | 115.34 |
| | R2 | 119 | 19 | 34 | 0.4 | 18 | 101 | 133 | 7.99 | 2.07 | 20.7 | 115.54 |
| | R3 | 120 | 20 | 32 | 0.5 | 17 | 101 | 133 | 8.02 | 2.05 | 20.5 | 116.34 |
| | Mean | 119 | 19 | 33.6667 | 0.46667 | 17.3333 | 101 | 133.333 | 8.03333 | 2.04333 | 20.4333 | 115.74 |
| IRBL1-CL/EC565160 | R1 | 120 | 24 | 33 | 0.5 | 8 | 94 | 125 | 8.02 | 2.95 | 29.5 | 76.67 |
| | R2 | 121 | 13 | 33 | 0.5 | 8 | 94 | 126 | 7.99 | 2.95 | 29.5 | 78.05 |
| | R3 | 119 | 24 | 32 | 0.5 | 7 | 95 | 126 | 7.99 | 2.77 | 27.7 | 78.04 |

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|-------------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | Mean | 120 | 20.3333 | 32.6667 | 0.5 | 7.66667 | 94.3333 | 125.667 | 8 | 2.89 | 28.9 | 77.5867 |
| KHD/GP/27 | R1 | 115 | 20 | 28 | 0.5 | 16 | 93 | 133 | 8.11 | 2.04 | 20.4 | 85.78 |
| | R2 | 116 | 20 | 27 | 0.5 | 17 | 94 | 132 | 8.01 | 2.23 | 22.3 | 87.06 |
| | R3 | 115 | 20 | 28 | 0.5 | 16 | 95 | 132 | 8.09 | 2.06 | 20.6 | 86.08 |
| | Mean | 115.333 | 20 | 27.6667 | 0.5 | 16.3333 | 94 | 132.333 | 8.07 | 2.11 | 21.1 | 86.3067 |
| Gurah | R1 | 120 | 22 | 32 | 0.6 | 16 | 101 | 134 | 7.91 | 3.05 | 30.5 | 117.67 |
| | R2 | 120 | 23 | 33 | 0.5 | 17 | 100 | 131 | 7.87 | 3.06 | 30.6 | 116.34 |
| | R3 | 120 | 22 | 33 | 0.6 | 17 | 102 | 134 | 7.99 | 3.02 | 30.2 | 118.09 |
| | Mean | 120 | 22.3333 | 32.6667 | 0.56667 | 16.6667 | 101 | 133 | 7.92333 | 3.04333 | 30.4333 | 117.367 |
| K-225 | R1 | 72 | 18 | 29 | 0.5 | 16 | 110 | 135 | 7.03 | 3.03 | 30.3 | 89.99 |
| | R2 | 71 | 17 | 30 | 0.5 | 17 | 110 | 134 | 7.23 | 3.21 | 32.1 | 87.99 |
| | R3 | 70 | 18 | 31 | 0.4 | 16 | 111 | 136 | 7.22 | 3.09 | 30.9 | 89.56 |
| | Mean | 71 | 17.6667 | 30 | 0.46667 | 16.3333 | 110.333 | 135 | 7.16 | 3.11 | 31.1 | 89.18 |
| IRBL5M/EC565166 | R1 | 100 | 18 | 32 | 0.5 | 14 | 94 | 131 | 8.44 | 2.88 | 28.8 | 97.99 |
| | R2 | 101 | 17 | 31 | 0.5 | 15 | 94 | 130 | 8.04 | 2.21 | 22.1 | 96.89 |
| | R3 | 100 | 18 | 30 | 0.6 | 16 | 95 | 131 | 8.35 | 2.65 | 26.5 | 96.99 |
| | Mean | 100.333 | 17.6667 | 31 | 0.53333 | 15 | 94.3333 | 130.667 | 8.27667 | 2.58 | 25.8 | 97.29 |
| Shalimar Rice-3 | R1 | 100 | 20 | 30 | 0.5 | 16 | 93 | 133 | 7.66 | 2.99 | 29.9 | 99.45 |
| | R2 | 101 | 21 | 31 | 0.5 | 17 | 94 | 132 | 7.59 | 2.89 | 28.9 | 100.11 |
| | R3 | 100 | 20 | 30 | 0.5 | 16 | 93 | 132 | 7.75 | 3.03 | 30.3 | 99.89 |
| | Mean | 100.333 | 20.3333 | 30.3333 | 0.5 | 16.3333 | 93.3333 | 132.333 | 7.66667 | 2.97 | 29.7 | 99.8167 |
| China-1031 | R1 | 115 | 17 | 34 | 0.5 | 7 | 101 | 126 | 7.55 | 3.04 | 30.4 | 120.44 |
| | R2 | 116 | 18 | 33 | 0.5 | 8 | 103 | 128 | 7.78 | 3.03 | 30.3 | 119.67 |
| | R3 | 118 | 17 | 34 | 0.5 | 6 | 101 | 128 | 7.65 | 3.01 | 30.1 | 120.22 |
| | Mean | 116.333 | 17.3333 | 33.6667 | 0.5 | 7 | 101.667 | 127.333 | 7.66 | 3.02667 | 30.2667 | 120.11 |
| A57-115-4/ARBN148 | R1 | 115 | 19 | 33 | 0.5 | 7 | 101 | 128 | 7.79 | 3.03 | 30.3 | 67.66 |
| | R2 | 116 | 19 | 34 | 0.5 | 7 | 102 | 129 | 8.01 | 2.56 | 25.6 | 69.67 |
| | R3 | 115 | 19 | 33 | 0.5 | 7 | 100 | 126 | 7.61 | 2.99 | 29.9 | 68.55 |
| | Mean | 115.333 | 19 | 33.3333 | 0.5 | 7 | 101 | 127.667 | 7.80333 | 2.86 | 28.6 | 68.6267 |
| SKAU-336 | R1 | 86 | 18 | 34 | 0.5 | 24 | 105 | 142 | 7.77 | 2.94 | 29.4 | 96.77 |

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|-------------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | R2 | 88 | 18 | 33 | 0.5 | 23 | 104 | 142 | 7.87 | 2.89 | 28.9 | 67.88 |
| | R3 | 88 | 18 | 34 | 0.5 | 23 | 104 | 141 | 7.73 | 2.97 | 29.7 | 96.88 |
| | Mean | 87.3333 | 18 | 33.6667 | 0.5 | 23.3333 | 104.333 | 141.667 | 7.79 | 2.93333 | 29.3333 | 87.1767 |
| B-4 | R1 | 100 | 20 | 30 | 0.5 | 15 | 99 | 136 | 7.77 | 2.95 | 29.5 | 114.03 |
| | R2 | 102 | 19 | 31 | 0.5 | 16 | 100 | 136 | 7.76 | 2.89 | 28.9 | 115.23 |
| | R3 | 100 | 18 | 30 | 0.5 | 17 | 100 | 135 | 7.57 | 2.99 | 29.9 | 113.78 |
| | Mean | 100.667 | 19 | 30.3333 | 0.5 | 16 | 99.6667 | 135.667 | 7.7 | 2.94333 | 29.4333 | 114.347 |
| IRBLKS-S/EC565178 | R1 | 60 | 17 | 27 | 0.6 | 15 | 110 | 135 | 8.14 | 2.33 | 23.3 | 86.88 |
| | R2 | 63 | 18 | 28 | 0.6 | 17 | 112 | 134 | 8.19 | 2.77 | 27.7 | 87.44 |
| | R3 | 64 | 17 | 28 | 0.6 | 15 | 113 | 134 | 8.12 | 2.36 | 23.6 | 87.01 |
| | Mean | 62.3333 | 17.3333 | 27.6667 | 0.6 | 15.6667 | 111.667 | 134.333 | 8.15 | 2.48667 | 24.8667 | 87.11 |
| C104PKT | R1 | 95 | 19 | 32 | 0.5 | 23 | 110 | 141 | 7.99 | 3.01 | 30.1 | 120.43 |
| | R2 | 93 | 20 | 31 | 0.5 | 24 | 111 | 141 | 7.88 | 3.04 | 30.4 | 119.78 |
| | R3 | 92 | 19 | 30 | 0.5 | 24 | 112 | 142 | 7.93 | 2.99 | 29.9 | 120.34 |
| | Mean | 93.3333 | 19.3333 | 31 | 0.5 | 23.6667 | 111 | 141.333 | 7.93333 | 3.01333 | 30.1333 | 120.183 |
| A57 | R1 | 93 | 19 | 32 | 0.4 | 13 | 113 | 139 | 7.93 | 2.99 | 29.9 | 89.45 |
| | R2 | 94 | 20 | 33 | 0.5 | 14 | 112 | 140 | 7.77 | 3.02 | 30.2 | 90.33 |
| | R3 | 93 | 19 | 32 | 0.5 | 15 | 110 | 140 | 7.85 | 3.03 | 30.3 | 90.56 |
| | Mean | 93.3333 | 19.3333 | 32.3333 | 0.46667 | 14 | 111.667 | 139.667 | 7.85 | 3.01333 | 30.1333 | 90.1133 |
| SKAU-2 | R1 | 78 | 18 | 34 | 0.5 | 15 | 107 | 138 | 7.88 | 3.03 | 30.3 | 119.34 |
| | R2 | 78 | 19 | 35 | 0.5 | 16 | 109 | 139 | 7.45 | 3.04 | 30.4 | 118.45 |
| | R3 | 78 | 18 | 34 | 0.5 | 15 | 108 | 139 | 7.88 | 3.03 | 30.3 | 118.98 |
| | Mean | 78 | 18.3333 | 34.3333 | 0.5 | 15.3333 | 108 | 138.667 | 7.73667 | 3.03333 | 30.3333 | 118.923 |
| SKAU-337 | R1 | 73 | 20 | 40 | 0.6 | 16 | 113 | 137 | 8.13 | 2.99 | 29.9 | 98.67 |
| | R2 | 74 | 20 | 42 | 0.6 | 16 | 113 | 139 | 8.14 | 2.98 | 29.8 | 99.07 |
| | R3 | 74 | 20 | 44 | 0.6 | 16 | 114 | 138 | 8.12 | 2.99 | 29.9 | 98.87 |
| | Mean | 73.6667 | 20 | 42 | 0.6 | 16 | 113.333 | 138 | 8.13 | 2.98667 | 29.8667 | 98.87 |
| B-9 | R1 | 85 | 18 | 38 | 0.8 | 22 | 98 | 142 | 7.66 | 3.01 | 30.1 | 114.75 |
| | R2 | 85 | 19 | 39 | 0.8 | 23 | 98 | 142 | 7.89 | 3.03 | 30.3 | 115.56 |
| | R3 | 89 | 19 | 38 | 0.8 | 24 | 99 | 143 | 7.99 | 3.03 | 30.3 | 115.23 |

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|-------------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | Mean | 86.3333 | 18.6667 | 38.3333 | 0.8 | 23 | 98.3333 | 142.333 | 7.84667 | 3.02333 | 30.2333 | 115.18 |
| B-14 | R1 | 95 | 22 | 40 | 0.9 | 8 | 102 | 135 | 7.79 | 2.69 | 26.9 | 104.67 |
| | R2 | 94 | 21 | 19 | 0.9 | 7 | 103 | 136 | 8.01 | 2.78 | 27.8 | 103.99 |
| | R3 | 95 | 22 | 40 | 1 | 7 | 104 | 136 | 7.77 | 2.72 | 27.2 | 104.89 |
| | Mean | 94.6667 | 21.6667 | 33 | 0.93333 | 7.33333 | 103 | 135.667 | 7.85667 | 2.73 | 27.3 | 104.517 |
| RIL-29 | R1 | 62 | 17 | 34 | 0.8 | 8 | 113 | 133 | 8.14 | 2.69 | 26.9 | 76.45 |
| | R2 | 64 | 18 | 44 | 0.8 | 7 | 112 | 134 | 7.99 | 2.99 | 29.9 | 78.23 |
| | R3 | 63 | 17 | 34 | 0.8 | 9 | 112 | 134 | 8.11 | 2.73 | 27.3 | 77.05 |
| | Mean | 63 | 17.3333 | 37.3333 | 0.8 | 8 | 112.333 | 133.667 | 8.08 | 2.80333 | 28.0333 | 77.2433 |
| Baber | R1 | 60 | 20 | 30 | 1 | 7 | 113 | 129 | 7.49 | 2.49 | 24.9 | 115.56 |
| | R2 | 62 | 21 | 30 | 1 | 7 | 112 | 127 | 7.55 | 2.88 | 28.8 | 114.99 |
| | R3 | 60 | 21 | 34 | 1 | 8 | 114 | 129 | 7.53 | 2.55 | 25.5 | 115.98 |
| | Mean | 60.6667 | 20.6667 | 31.3333 | 1 | 7.33333 | 113 | 128.333 | 7.52333 | 2.64 | 26.4 | 115.51 |
| SKAU-408 | R1 | 83 | 24 | 40 | 0.6 | 14 | 114 | 138 | 7.72 | 2.71 | 27.1 | 87.05 |
| | R2 | 84 | 23 | 39 | 0.6 | 15 | 112 | 135 | 7.77 | 2.87 | 28.7 | 90.56 |
| | R3 | 84 | 24 | 40 | 0.6 | 15 | 114 | 138 | 7.75 | 2.77 | 27.7 | 92.32 |
| | Mean | 83.6667 | 23.6667 | 39.6667 | 0.6 | 14.6667 | 113.333 | 137 | 7.74667 | 2.78333 | 27.8333 | 89.9767 |
| SKAU-389 | R1 | 45 | 17 | 29 | 0.4 | 24 | 111 | 140 | 8.81 | 2.18 | 21.8 | 130.53 |
| | R2 | 47 | 18 | 30 | 0.4 | 23 | 110 | 140 | 8.02 | 2.34 | 23.4 | 131.23 |
| | R3 | 45 | 17 | 29 | 0.4 | 24 | 110 | 139 | 8.79 | 2.22 | 22.2 | 132.04 |
| | Mean | 45.6667 | 17.3333 | 29.3333 | 0.4 | 23.6667 | 110.333 | 139.667 | 8.54 | 2.24667 | 22.4667 | 131.267 |
| Usen | R1 | 55 | 20 | 29 | 0.5 | 23 | 108 | 126 | 8.01 | 2.49 | 24.9 | 76.88 |
| | R2 | 55 | 19 | 28 | 0.5 | 23 | 109 | 127 | 8.04 | 2.52 | 25.2 | 76.88 |
| | R3 | 56 | 20 | 29 | 0.5 | 22 | 107 | 127 | 7.99 | 2.56 | 25.6 | 75.99 |
| | Mean | 55.3333 | 19.6667 | 28.6667 | 0.5 | 22.6667 | 108 | 126.667 | 8.01333 | 2.52333 | 25.2333 | 76.5833 |
| Shalimar Rice-2 | R1 | 105 | 25 | 39 | 0.5 | 8 | 87 | 133 | 7.99 | 2.99 | 29.9 | 111.44 |
| | R2 | 105 | 25 | 39 | 0.5 | 8 | 88 | 133 | 7.84 | 2.98 | 29.8 | 113.67 |
| | R3 | 105 | 24 | 28 | 0.5 | 7 | 87 | 133 | 7.81 | 2.98 | 29.8 | 111.34 |
| | Mean | 105 | 24.6667 | 35.3333 | 0.5 | 7.66667 | 87.3333 | 133 | 7.88 | 2.98333 | 29.8333 | 112.15 |
| IR61728-413-2-1-1 | R1 | 89 | 17 | 33 | 0.5 | 17 | 102 | 140 | 7.87 | 3.04 | 30.4 | 20.89 |

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|-----------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | R2 | 89 | 17 | 38 | 0.5 | 14 | 102 | 139 | 7.99 | 3.05 | 30.5 | 30.22 |
| | R3 | 94 | 18 | 40 | 0.6 | 14 | 100 | 139 | 7.94 | 3.05 | 30.5 | 34.33 |
| | Mean | 90.6667 | 17.3333 | 37 | 0.53333 | 15 | 101.333 | 139.333 | 7.93333 | 3.04667 | 30.4667 | 28.48 |
| Shai Tai Tsau | R1 | 68 | 17 | 29 | 0.6 | 15 | 104 | 138 | 8.03 | 2.92 | 29.2 | 16.44 |
| | R2 | 68 | 17 | 29 | 0.6 | 16 | 104 | 137 | 8.01 | 2.94 | 29.4 | 16.89 |
| | R3 | 67 | 19 | 29 | 0.6 | 15 | 103 | 138 | 8.01 | 2.94 | 29.4 | 17.22 |
| | Mean | 67.6667 | 17.6667 | 29 | 0.6 | 15.3333 | 103.667 | 137.667 | 8.01667 | 2.93333 | 29.3333 | 16.85 |
| BL-122 | R1 | 62 | 17 | 27 | 0.5 | 14 | 114 | 138 | 7.89 | 3.01 | 30.1 | 17.22 |
| | R2 | 63 | 17 | 29 | 0.5 | 15 | 115 | 135 | 7.91 | 3.02 | 30.2 | 18.09 |
| | R3 | 65 | 18 | 30 | 0.5 | 17 | 115 | 136 | 7.91 | 3.02 | 30.2 | 18.03 |
| | Mean | 63.3333 | 17.3333 | 28.6667 | 0.5 | 15.3333 | 114.667 | 136.333 | 7.90333 | 3.01667 | 30.1667 | 17.78 |
| IR68333-RR-B-19 | R1 | 89 | 17 | 33 | 0.5 | 17 | 102 | 140 | 7.87 | 3.04 | 30.4 | 20.89 |
| | R2 | 89 | 17 | 38 | 0.5 | 14 | 102 | 139 | 7.99 | 3.05 | 30.5 | 30.22 |
| | R3 | 94 | 18 | 40 | 0.6 | 14 | 100 | 139 | 7.94 | 3.05 | 30.5 | 34.33 |
| | Mean | 89 | 17 | 33 | 0.5 | 17 | 102 | 140 | 7.87 | 3.04 | 30.4 | 20.89 |
| NP 125 | R1 | 85 | 24 | 32 | 0.6 | 26 | 108 | 127 | 8.14 | 2.69 | 26.9 | 76.45 |
| | R2 | 87 | 24 | 32 | 0.6 | 27 | 109 | 125 | 7.99 | 2.99 | 29.9 | 78.23 |
| | R3 | 86 | 22 | 33 | 0.6 | 27 | 108 | 125 | 8.01 | 2.99 | 29.9 | 76.45 |
| | Mean | 86 | 23.3333 | 32.3333 | 0.6 | 26.6667 | 108.333 | 125.667 | 8.04667 | 2.89 | 28.9 | 77.0433 |
| KHD/GP/150 | R1 | 115 | 20 | 28 | 0.5 | 16 | 93 | 133 | 8.11 | 2.04 | 20.4 | 85.78 |
| | R2 | 116 | 20 | 27 | 0.5 | 17 | 94 | 132 | 8.01 | 2.23 | 22.3 | 87.06 |
| | R3 | 115 | 20 | 28 | 0.5 | 16 | 95 | 132 | 8.09 | 2.06 | 20.6 | 86.08 |
| | Mean | 115.333 | 20 | 27.6667 | 0.5 | 16.3333 | 94 | 132.333 | 8.07 | 2.11 | 21.1 | 86.3067 |
| MAS-71 | R1 | 99 | 22 | 48 | 0.5 | 14 | 98 | 137 | 7.99 | 2.98 | 29.8 | 34.23 |
| | R2 | 97 | 22 | 51 | 0.6 | 16 | 100 | 135 | 7.98 | 2.99 | 29.9 | 32.66 |
| | R3 | 95 | 24 | 53 | 0.5 | 16 | 98 | 135 | 7.99 | 2.98 | 29.8 | 32.44 |
| | Mean | 97 | 22.6667 | 50.6667 | 0.53333 | 15.3333 | 98.6667 | 135.667 | 7.98667 | 2.98333 | 29.8333 | 33.11 |
| RAMINAD StR 3 | R1 | 92 | 21 | 24 | 0.9 | 17 | 109 | 136 | 6.33 | 3.09 | 30.9 | 87.99 |
| | R2 | 93 | 22 | 23 | 0.9 | 16 | 108 | 135 | 7.03 | 3.23 | 32.3 | 88.77 |
| | R3 | 95 | 21 | 25 | 0.9 | 17 | 110 | 137 | 6.54 | 3.23 | 32.3 | 88.56 |

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|------------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | Mean | 93.3333 | 21.3333 | 24 | 0.9 | 16.6667 | 109 | 136 | 6.63333 | 3.18333 | 31.8333 | 88.44 |
| BL 245 | R1 | 108 | 20 | 29 | 0.5 | 7 | 102 | 124 | 8.05 | 2.66 | 26.6 | 76.34 |
| | R2 | 110 | 22 | 30 | 0.5 | 8 | 102 | 124 | 7.99 | 2.99 | 29.9 | 78.34 |
| | R3 | 112 | 23 | 29 | 0.5 | 7 | 101 | 124 | 7.99 | 2.58 | 25.8 | 76.99 |
| | Mean | 110 | 21.6667 | 29.3333 | 0.5 | 7.33333 | 101.667 | 124 | 8.01 | 2.74333 | 27.4333 | 77.2233 |
| CO.39 | R1 | 92 | 21 | 24 | 0.9 | 17 | 109 | 136 | 6.33 | 3.09 | 30.9 | 87.99 |
| | R2 | 93 | 22 | 23 | 0.9 | 16 | 108 | 135 | 7.03 | 3.23 | 32.3 | 88.77 |
| | R3 | 95 | 21 | 25 | 0.9 | 17 | 110 | 137 | 6.54 | 3.23 | 32.3 | 88.56 |
| | Mean | 93.3333 | 21.3333 | 24 | 0.9 | 16.6667 | 109 | 136 | 6.63333 | 3.18333 | 31.8333 | 88.44 |
| Local Budgam 1 | R1 | 97 | 17 | 34 | 0.6 | 8 | 113 | 133 | 8.14 | 2.69 | 26.9 | 76.45 |
| | R2 | 99 | 18 | 44 | 0.5 | 7 | 112 | 134 | 7.99 | 2.99 | 29.9 | 78.23 |
| | R3 | 97 | 17 | 34 | 0.5 | 9 | 112 | 134 | 8.11 | 2.73 | 27.3 | 77.05 |
| | Mean | 97.6667 | 17.3333 | 37.3333 | 0.53333 | 8 | 112.333 | 133.667 | 8.08 | 2.80333 | 28.0333 | 77.2433 |
| C105TTP.4L23 | R1 | 60 | 17 | 27 | 0.6 | 15 | 110 | 135 | 8.14 | 2.33 | 23.3 | 86.88 |
| | R2 | 63 | 18 | 28 | 0.6 | 17 | 112 | 134 | 8.19 | 2.77 | 27.7 | 87.44 |
| | R3 | 64 | 17 | 28 | 0.6 | 15 | 113 | 134 | 8.12 | 2.36 | 23.6 | 87.01 |
| | Mean | 62.3333 | 17.3333 | 27.6667 | 0.6 | 15.6667 | 111.667 | 134.333 | 8.15 | 2.48667 | 24.8667 | 87.11 |
| IR63347-AL-201-1 | R1 | 92 | 21 | 24 | 0.9 | 17 | 109 | 136 | 6.33 | 3.09 | 30.9 | 87.99 |
| | R2 | 93 | 22 | 23 | 0.9 | 16 | 108 | 135 | 7.03 | 3.23 | 32.3 | 88.77 |
| | R3 | 95 | 21 | 25 | 0.9 | 17 | 110 | 137 | 6.54 | 3.23 | 32.3 | 88.56 |
| | Mean | 93.3333 | 21.3333 | 24 | 0.9 | 16.6667 | 109 | 136 | 6.63333 | 3.18333 | 31.8333 | 88.44 |
| IRBN 2008 V-87 | R1 | 83 | 24 | 40 | 0.6 | 14 | 114 | 138 | 7.72 | 2.71 | 27.1 | 87.05 |
| | R2 | 84 | 23 | 39 | 0.6 | 15 | 112 | 135 | 7.77 | 2.87 | 28.7 | 90.56 |
| | R3 | 84 | 24 | 40 | 0.6 | 15 | 114 | 138 | 7.75 | 2.77 | 27.7 | 92.32 |
| | Mean | 83.6667 | 23.6667 | 39.6667 | 0.6 | 14.6667 | 113.333 | 137 | 7.74667 | 2.78333 | 27.8333 | 89.9767 |
| YUNLEN-19 | R1 | 108 | 20 | 29 | 0.5 | 7 | 102 | 124 | 8.05 | 2.66 | 26.6 | 20.89 |
| | R2 | 110 | 22 | 30 | 0.5 | 8 | 102 | 124 | 7.99 | 2.99 | 29.9 | 30.22 |
| | R3 | 112 | 23 | 29 | 0.5 | 7 | 101 | 124 | 7.99 | 2.58 | 25.8 | 34.33 |
| | Mean | 110 | 21.6667 | 29.3333 | 0.5 | 7.33333 | 101.667 | 124 | 8.01 | 2.74333 | 27.4333 | 28.48 |
| SHALKEW | R1 | 89 | 17 | 33 | 0.5 | 17 | 102 | 140 | 7.87 | 3.04 | 30.4 | 20.89 |

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|------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | R2 | 89 | 17 | 38 | 0.5 | 14 | 102 | 139 | 7.99 | 3.05 | 30.5 | 30.22 |
| | R3 | 94 | 18 | 40 | 0.6 | 14 | 100 | 139 | 7.94 | 3.05 | 30.5 | 34.33 |
| | Mean | 90.6667 | 17.3333 | 37 | 0.53333 | 15 | 101.333 | 139.333 | 7.93333 | 3.04667 | 30.4667 | 28.48 |
| BL-42 | R1 | 83 | 24 | 40 | 0.6 | 14 | 114 | 138 | 7.72 | 2.71 | 27.1 | 87.05 |
| | R2 | 84 | 23 | 39 | 0.6 | 15 | 112 | 135 | 7.77 | 2.87 | 28.7 | 90.56 |
| | R3 | 84 | 24 | 40 | 0.6 | 15 | 114 | 138 | 7.75 | 2.77 | 27.7 | 92.32 |
| | Mean | 83.6667 | 23.6667 | 39.6667 | 0.6 | 14.6667 | 113.333 | 137 | 7.74667 | 2.78333 | 27.8333 | 89.9767 |
| SAFED BREZ | R1 | 54 | 15 | 33 | 0.8 | 24 | 110 | 142 | 7.99 | 2.03 | 20.3 | 125.39 |
| | R2 | 52 | 14 | 33 | 0.8 | 25 | 109 | 142 | 7.93 | 2.06 | 20.6 | 126.98 |
| | R3 | 54 | 15 | 34 | 0.7 | 24 | 110 | 142 | 7.97 | 2.06 | 20.6 | 126.98 |
| | Mean | 53.3333 | 14.6667 | 33.3333 | 0.76667 | 24.3333 | 109.667 | 142 | 7.96333 | 2.05 | 20.5 | 126.45 |
| KHD/GP/154 | R1 | 115 | 20 | 28 | 0.5 | 16 | 93 | 133 | 8.11 | 2.04 | 20.4 | 85.78 |
| | R2 | 116 | 20 | 27 | 0.5 | 17 | 94 | 132 | 8.01 | 2.23 | 22.3 | 87.06 |
| | R3 | 115 | 20 | 28 | 0.5 | 16 | 95 | 132 | 8.09 | 2.06 | 20.6 | 86.08 |
| | Mean | 115.333 | 20 | 27.6667 | 0.5 | 16.3333 | 94 | 132.333 | 8.07 | 2.11 | 21.1 | 86.3067 |
| K-332 | R1 | 95 | 19 | 32 | 0.5 | 23 | 110 | 141 | 7.99 | 3.01 | 30.1 | 120.43 |
| | R2 | 93 | 20 | 31 | 0.5 | 24 | 111 | 141 | 7.88 | 3.04 | 30.4 | 119.78 |
| | R3 | 92 | 19 | 30 | 0.5 | 24 | 112 | 142 | 7.93 | 2.99 | 29.9 | 120.34 |
| | Mean | 93.3333 | 19.3333 | 31 | 0.5 | 23.6667 | 111 | 141.333 | 7.93333 | 3.01333 | 30.1333 | 120.183 |
| K-116 | R1 | 89 | 17 | 33 | 0.5 | 17 | 102 | 140 | 7.87 | 3.04 | 30.4 | 76.45 |
| | R2 | 89 | 17 | 38 | 0.5 | 14 | 102 | 139 | 7.99 | 3.05 | 30.5 | 78.23 |
| | R3 | 94 | 18 | 40 | 0.6 | 14 | 100 | 139 | 7.94 | 3.05 | 30.5 | 77.05 |
| | Mean | 90.6667 | 17.3333 | 37 | 0.53333 | 15 | 101.333 | 139.333 | 7.93333 | 3.04667 | 30.4667 | 77.2433 |
| WAZUL KREA | R1 | 97 | 17 | 34 | 0.6 | 8 | 113 | 133 | 8.14 | 2.69 | 26.9 | 76.45 |
| | R2 | 99 | 18 | 44 | 0.5 | 7 | 112 | 134 | 7.99 | 2.99 | 29.9 | 78.23 |
| | R3 | 97 | 17 | 34 | 0.5 | 9 | 112 | 134 | 8.11 | 2.73 | 27.3 | 77.05 |
| | Mean | 97.6667 | 17.3333 | 37.3333 | 0.53333 | 8 | 112.333 | 133.667 | 8.08 | 2.80333 | 28.0333 | 77.2433 |
| KANTO-51 | R1 | 83 | 24 | 40 | 0.6 | 14 | 114 | 138 | 7.72 | 2.71 | 27.1 | 87.05 |
| | R2 | 84 | 23 | 39 | 0.6 | 15 | 112 | 135 | 7.77 | 2.87 | 28.7 | 90.56 |
| | R3 | 84 | 24 | 40 | 0.6 | 15 | 114 | 138 | 7.75 | 2.77 | 27.7 | 92.32 |

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|------------------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | Mean | 83.6667 | 23.6667 | 39.6667 | 0.6 | 14.6667 | 113.333 | 137 | 7.74667 | 2.78333 | 27.8333 | 89.9767 |
| RIL-10 | R1 | 97 | 17 | 34 | 0.6 | 8 | 113 | 133 | 8.14 | 2.69 | 26.9 | 76.45 |
| | R2 | 99 | 18 | 44 | 0.5 | 7 | 112 | 134 | 7.99 | 2.99 | 29.9 | 78.23 |
| | R3 | 97 | 17 | 34 | 0.5 | 9 | 112 | 134 | 8.11 | 2.73 | 27.3 | 77.05 |
| | Mean | 97.6667 | 17.3333 | 37.3333 | 0.53333 | 8 | 112.333 | 133.667 | 8.08 | 2.80333 | 28.0333 | 77.2433 |
| DULAR | R1 | 100 | 20 | 30 | 0.5 | 15 | 99 | 136 | 7.77 | 2.95 | 29.5 | 87.05 |
| | R2 | 102 | 19 | 31 | 0.5 | 16 | 100 | 136 | 7.76 | 2.89 | 28.9 | 90.56 |
| | R3 | 100 | 18 | 30 | 0.5 | 17 | 100 | 135 | 7.57 | 2.99 | 29.9 | 92.32 |
| | Mean | 100.667 | 19 | 30.3333 | 0.5 | 16 | 99.6667 | 135.667 | 7.7 | 2.94333 | 29.4333 | 89.9767 |
| BL-122/ARBN 141 | R1 | 75 | 20 | 40 | 1.5 | 19 | 125 | 148 | 7.55 | 2.85 | 28.5 | 45.67 |
| | R2 | 75 | 19 | 43 | 1.5 | 18 | 124 | 147 | 7.89 | 2.97 | 29.7 | 46.99 |
| | R3 | 74 | 20 | 42 | 1.3 | 17 | 126 | 148 | 7.53 | 2.87 | 28.7 | 44.99 |
| | Mean | 74.6667 | 19.6667 | 41.6667 | 1.43333 | 18 | 125 | 147.667 | 7.65667 | 2.89667 | 28.9667 | 45.8833 |
| BL-142/ARBN 142 | R1 | 95 | 19 | 32 | 0.5 | 23 | 110 | 141 | 7.99 | 3.01 | 30.1 | 120.43 |
| | R2 | 93 | 20 | 31 | 0.5 | 24 | 111 | 141 | 7.88 | 3.04 | 30.4 | 119.78 |
| | R3 | 92 | 19 | 30 | 0.5 | 24 | 112 | 142 | 7.93 | 2.99 | 29.9 | 120.34 |
| | Mean | 93.3333 | 19.3333 | 31 | 0.5 | 23.6667 | 111 | 141.333 | 7.93333 | 3.01333 | 30.1333 | 120.183 |
| IRBL Z-FU/EC 565188 | R1 | 100 | 20 | 30 | 0.5 | 15 | 99 | 136 | 7.77 | 2.95 | 29.5 | 76.45 |
| | R2 | 102 | 19 | 31 | 0.5 | 16 | 100 | 136 | 7.76 | 2.89 | 28.9 | 78.23 |
| | R3 | 100 | 18 | 30 | 0.5 | 17 | 100 | 135 | 7.57 | 2.99 | 29.9 | 77.05 |
| | Mean | 100.667 | 19 | 30.3333 | 0.5 | 16 | 99.6667 | 135.667 | 7.7 | 2.94333 | 29.4333 | 77.2433 |
| KHD/GP/208 | R1 | 75 | 20 | 40 | 1.5 | 19 | 125 | 148 | 7.55 | 2.85 | 28.5 | 45.67 |
| | R2 | 75 | 19 | 43 | 1.5 | 18 | 124 | 147 | 7.89 | 2.97 | 29.7 | 46.99 |
| | R3 | 74 | 20 | 42 | 1.3 | 17 | 126 | 148 | 7.53 | 2.87 | 28.7 | 44.99 |
| | Mean | 74.6667 | 19.6667 | 41.6667 | 1.43333 | 18 | 125 | 147.667 | 7.65667 | 2.89667 | 28.9667 | 45.8833 |
| MAS-70 | R1 | 99 | 22 | 48 | 0.5 | 14 | 98 | 137 | 7.99 | 2.98 | 29.8 | 34.23 |
| | R2 | 97 | 22 | 51 | 0.6 | 16 | 101 | 135 | 7.98 | 2.99 | 29.9 | 32.66 |
| | R3 | 95 | 24 | 53 | 0.5 | 16 | 98 | 135 | 7.99 | 2.98 | 29.8 | 32.44 |
| | Mean | 97 | 22.6667 | 50.6667 | 0.53333 | 15.3333 | 99 | 135.667 | 7.98667 | 2.98333 | 29.8333 | 33.11 |
| JEHLUM | R1 | 100 | 20 | 30 | 0.5 | 16 | 93 | 133 | 7.66 | 2.99 | 29.9 | 99.45 |

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|------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | R2 | 101 | 21 | 31 | 0.5 | 17 | 94 | 132 | 7.59 | 2.89 | 28.9 | 100.11 |
| | R3 | 100 | 20 | 30 | 0.5 | 16 | 93 | 132 | 7.75 | 3.03 | 30.3 | 99.89 |
| | Mean | 100.333 | 20.3333 | 30.3333 | 0.5 | 16.3333 | 93.3333 | 132.333 | 7.66667 | 2.97 | 29.7 | 99.8167 |
| SKAU-403 | R1 | 115 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.23 | 2.05 | 20.5 | 130.87 |
| | R2 | 117 | 19 | 38 | 0.6 | 17 | 107 | 134 | 7.41 | 2.27 | 22.7 | 132.45 |
| | R3 | 116 | 18 | 37 | 0.5 | 17 | 106 | 132 | 7.43 | 2.29 | 22.9 | 132.45 |
| | Mean | 116 | 18.6667 | 37.6667 | 0.56667 | 16.6667 | 106.667 | 133 | 7.35667 | 2.20333 | 22.0333 | 131.923 |
| CHENAB | R1 | 105 | 25 | 39 | 0.5 | 8 | 87 | 133 | 7.99 | 2.99 | 29.9 | 111.44 |
| | R2 | 105 | 25 | 39 | 0.5 | 8 | 88 | 133 | 7.84 | 2.98 | 29.8 | 113.67 |
| | R3 | 105 | 24 | 28 | 0.5 | 7 | 87 | 133 | 7.81 | 2.98 | 29.8 | 111.34 |
| | Mean | 105 | 24.6667 | 35.3333 | 0.5 | 7.66667 | 87.3333 | 133 | 7.88 | 2.98333 | 29.8333 | 112.15 |
| SKAU-339 | R1 | 86 | 18 | 34 | 0.5 | 24 | 105 | 142 | 7.77 | 2.94 | 29.4 | 96.77 |
| | R2 | 88 | 18 | 33 | 0.5 | 23 | 104 | 142 | 7.87 | 2.89 | 28.9 | 67.88 |
| | R3 | 88 | 18 | 34 | 0.5 | 23 | 104 | 141 | 7.73 | 2.97 | 29.7 | 96.88 |
| | Mean | 87.3333 | 18 | 33.6667 | 0.5 | 23.3333 | 104.333 | 141.667 | 7.79 | 2.93333 | 29.3333 | 87.1767 |
| China-1039 | R1 | 99 | 22 | 48 | 0.5 | 14 | 98 | 137 | 7.99 | 2.98 | 29.8 | 34.23 |
| | R2 | 97 | 22 | 51 | 0.6 | 16 | 101 | 135 | 7.98 | 2.99 | 29.9 | 32.66 |
| | R3 | 95 | 24 | 53 | 0.5 | 16 | 98 | 135 | 7.99 | 2.98 | 29.8 | 32.44 |
| | Mean | 97 | 22.6667 | 50.6667 | 0.53333 | 15.3333 | 99 | 135.667 | 7.98667 | 2.98333 | 29.8333 | 33.11 |
| SKAU-292 | R1 | 118 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.23 | 2.05 | 20.5 | 130.87 |
| | R2 | 117 | 19 | 38 | 0.6 | 17 | 107 | 133 | 7.41 | 2.27 | 22.7 | 132.45 |
| | R3 | 115 | 18 | 37 | 0.5 | 17 | 106 | 132 | 7.43 | 2.29 | 22.9 | 132.45 |
| | Mean | 116.667 | 18.6667 | 37.6667 | 0.56667 | 16.6667 | 106.667 | 132.667 | 7.35667 | 2.20333 | 22.0333 | 131.923 |
| China-988 | R1 | 105 | 25 | 39 | 0.5 | 8 | 87 | 133 | 7.99 | 2.99 | 29.9 | 111.44 |
| | R2 | 105 | 25 | 39 | 0.5 | 8 | 88 | 133 | 7.84 | 2.98 | 29.8 | 113.67 |
| | R3 | 105 | 24 | 28 | 0.5 | 7 | 87 | 133 | 7.81 | 2.98 | 29.8 | 111.34 |
| | Mean | 105 | 24.6667 | 35.3333 | 0.5 | 7.66667 | 87.3333 | 133 | 7.88 | 2.98333 | 29.8333 | 112.15 |
| SKAU-338 | R1 | 86 | 18 | 34 | 0.5 | 24 | 105 | 142 | 7.77 | 2.94 | 29.4 | 96.77 |
| | R2 | 88 | 18 | 33 | 0.5 | 23 | 104 | 142 | 7.87 | 2.89 | 28.9 | 67.88 |
| | R3 | 88 | 18 | 34 | 0.5 | 23 | 104 | 141 | 7.73 | 2.97 | 29.7 | 96.88 |

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|---------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | Mean | 87.3333 | 18 | 33.6667 | 0.5 | 23.3333 | 104.333 | 141.667 | 7.79 | 2.93333 | 29.3333 | 87.1767 |
| SKAU-39 | R1 | 115 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.23 | 2.05 | 20.5 | 130.87 |
| | R2 | 117 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.41 | 2.27 | 22.7 | 132.45 |
| | R3 | 115 | 18 | 37 | 0.5 | 17 | 106 | 132 | 7.43 | 2.29 | 22.9 | 132.45 |
| | Mean | 115.667 | 18.6667 | 37.6667 | 0.56667 | 16.3333 | 106.667 | 132.667 | 7.35667 | 2.20333 | 22.0333 | 131.923 |
| China-1007 | R1 | 99 | 22 | 48 | 0.5 | 14 | 98 | 137 | 7.99 | 2.98 | 29.8 | 34.23 |
| | R2 | 97 | 22 | 51 | 0.6 | 16 | 101 | 135 | 7.98 | 2.99 | 29.9 | 32.66 |
| | R3 | 95 | 24 | 53 | 0.5 | 16 | 98 | 135 | 7.99 | 2.98 | 29.8 | 32.44 |
| | Mean | 97 | 22.6667 | 50.6667 | 0.53333 | 15.3333 | 99 | 135.667 | 7.98667 | 2.98333 | 29.8333 | 33.11 |
| Pusa Sugand-5 | R1 | 75 | 20 | 40 | 1.5 | 19 | 125 | 148 | 7.55 | 2.85 | 28.5 | 45.67 |
| | R2 | 75 | 19 | 43 | 1.5 | 18 | 124 | 147 | 7.89 | 2.97 | 29.7 | 46.99 |
| | R3 | 74 | 20 | 42 | 1.3 | 17 | 126 | 148 | 7.53 | 2.87 | 28.7 | 44.99 |
| | Mean | 74.6667 | 19.6667 | 41.6667 | 1.43333 | 18 | 125 | 147.667 | 7.65667 | 2.89667 | 28.9667 | 45.8833 |
| B-26 | R1 | 100 | 20 | 30 | 0.5 | 15 | 99 | 136 | 7.77 | 2.95 | 29.5 | 114.03 |
| | R2 | 102 | 19 | 31 | 0.5 | 16 | 100 | 136 | 7.76 | 2.89 | 28.9 | 115.23 |
| | R3 | 100 | 18 | 30 | 0.5 | 17 | 100 | 135 | 7.57 | 2.99 | 29.9 | 113.78 |
| | Mean | 100.667 | 19 | 30.3333 | 0.5 | 16 | 99.6667 | 135.667 | 7.7 | 2.94333 | 29.4333 | 114.347 |
| B-10 | R1 | 97 | 17 | 34 | 0.6 | 8 | 113 | 133 | 8.14 | 2.69 | 26.9 | 76.45 |
| | R2 | 99 | 18 | 44 | 0.5 | 7 | 112 | 134 | 7.99 | 2.99 | 29.9 | 78.23 |
| | R3 | 97 | 17 | 34 | 0.5 | 9 | 112 | 134 | 8.11 | 2.73 | 27.3 | 77.05 |
| | Mean | 97.6667 | 17.3333 | 37.3333 | 0.53333 | 8 | 112.333 | 133.667 | 8.08 | 2.80333 | 28.0333 | 77.2433 |
| B-13 | R1 | 91 | 18 | 38 | 0.8 | 22 | 98 | 142 | 7.66 | 3.01 | 30.1 | 114.75 |
| | R2 | 89 | 19 | 39 | 0.8 | 23 | 98 | 142 | 7.89 | 3.03 | 30.3 | 115.56 |
| | R3 | 90 | 19 | 38 | 0.8 | 24 | 99 | 143 | 7.99 | 3.03 | 30.3 | 115.23 |
| | Mean | 90 | 18.6667 | 38.3333 | 0.8 | 23 | 98.3333 | 142.333 | 7.84667 | 3.02333 | 30.2333 | 115.18 |
| B-5 | R1 | 105 | 25 | 39 | 0.5 | 8 | 87 | 133 | 7.99 | 2.99 | 29.9 | 111.44 |
| | R2 | 105 | 25 | 39 | 0.5 | 8 | 88 | 133 | 7.84 | 2.98 | 29.8 | 113.67 |
| | R3 | 105 | 24 | 28 | 0.5 | 7 | 87 | 133 | 7.81 | 2.98 | 29.8 | 111.34 |
| | Mean | 105 | 24.6667 | 35.3333 | 0.5 | 7.66667 | 87.3333 | 133 | 7.88 | 2.98333 | 29.8333 | 112.15 |
| B-3 | R1 | 100 | 20 | 30 | 0.5 | 15 | 99 | 136 | 7.77 | 2.95 | 29.5 | 114.03 |

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|------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | R2 | 102 | 19 | 31 | 0.5 | 16 | 100 | 136 | 7.76 | 2.89 | 28.9 | 115.23 |
| | R3 | 100 | 18 | 30 | 0.5 | 17 | 100 | 135 | 7.57 | 2.99 | 29.9 | 113.78 |
| | Mean | 100.667 | 19 | 30.3333 | 0.5 | 16 | 99.6667 | 135.667 | 7.7 | 2.94333 | 29.4333 | 114.347 |
| B-19 | R1 | 95 | 22 | 40 | 0.9 | 8 | 102 | 135 | 7.79 | 2.69 | 26.9 | 104.67 |
| | R2 | 94 | 21 | 19 | 0.9 | 7 | 103 | 136 | 8.01 | 2.78 | 27.8 | 103.99 |
| | R3 | 95 | 22 | 40 | 1 | 7 | 104 | 136 | 7.77 | 2.72 | 27.2 | 104.89 |
| | Mean | 94.6667 | 21.6667 | 33 | 0.93333 | 7.33333 | 103 | 135.667 | 7.85667 | 2.73 | 27.3 | 104.517 |
| MUSHKBUDJI | R1 | 105 | 25 | 39 | 0.5 | 8 | 87 | 133 | 7.99 | 2.99 | 29.9 | 111.44 |
| | R2 | 105 | 25 | 39 | 0.5 | 8 | 88 | 133 | 7.84 | 2.98 | 29.8 | 113.67 |
| | R3 | 105 | 24 | 28 | 0.5 | 7 | 87 | 133 | 7.81 | 2.98 | 29.8 | 111.34 |
| | Mean | 105 | 24.6667 | 35.3333 | 0.5 | 7.66667 | 87.3333 | 133 | 7.88 | 2.98333 | 29.8333 | 112.15 |
| B-1509 | R1 | 91 | 18 | 38 | 0.8 | 22 | 98 | 142 | 7.66 | 3.01 | 30.1 | 114.75 |
| | R2 | 89 | 19 | 39 | 0.8 | 23 | 98 | 142 | 7.89 | 3.03 | 30.3 | 115.56 |
| | R3 | 90 | 19 | 38 | 0.8 | 24 | 99 | 143 | 7.99 | 3.03 | 30.3 | 115.23 |
| | Mean | 90 | 18.6667 | 38.3333 | 0.8 | 23 | 98.3333 | 142.333 | 7.84667 | 3.02333 | 30.2333 | 115.18 |
| SKAU-46 | R1 | 100 | 20 | 30 | 0.5 | 16 | 93 | 133 | 7.66 | 2.99 | 29.9 | 99.45 |
| | R2 | 101 | 21 | 31 | 0.5 | 17 | 94 | 132 | 7.59 | 2.89 | 28.9 | 100.11 |
| | R3 | 100 | 20 | 30 | 0.5 | 16 | 93 | 132 | 7.75 | 3.03 | 30.3 | 99.89 |
| | Mean | 100.333 | 20.3333 | 30.3333 | 0.5 | 16.3333 | 93.3333 | 132.333 | 7.66667 | 2.97 | 29.7 | 99.8167 |
| SKAU-402 | R1 | 115 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.23 | 2.05 | 20.5 | 130.87 |
| | R2 | 117 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.41 | 2.27 | 22.7 | 132.45 |
| | R3 | 115 | 18 | 37 | 0.5 | 17 | 106 | 132 | 7.43 | 2.29 | 22.9 | 132.45 |
| | Mean | 115.667 | 18.6667 | 37.6667 | 0.56667 | 16.3333 | 106.667 | 132.667 | 7.35667 | 2.20333 | 22.0333 | 131.923 |
| SKAU-404 | R1 | 115 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.23 | 2.05 | 20.5 | 130.87 |
| | R2 | 117 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.41 | 2.27 | 22.7 | 132.45 |
| | R3 | 115 | 18 | 37 | 0.5 | 17 | 106 | 132 | 7.43 | 2.29 | 22.9 | 132.45 |
| | Mean | 115.667 | 18.6667 | 37.6667 | 0.56667 | 16.3333 | 106.667 | 132.667 | 7.35667 | 2.20333 | 22.0333 | 131.923 |
| SKAU-3 | R1 | 100 | 20 | 30 | 0.5 | 15 | 99 | 136 | 7.77 | 2.95 | 29.5 | 114.03 |
| | R2 | 102 | 19 | 31 | 0.5 | 16 | 100 | 136 | 7.76 | 2.89 | 28.9 | 115.23 |
| | R3 | 100 | 18 | 30 | 0.5 | 17 | 100 | 135 | 7.57 | 2.99 | 29.9 | 113.78 |

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|---------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | Mean | 100.667 | 19 | 30.3333 | 0.5 | 16 | 99.6667 | 135.667 | 7.7 | 2.94333 | 29.4333 | 114.347 |
| SKAU-98 | R1 | 95 | 19 | 32 | 0.5 | 23 | 110 | 141 | 7.99 | 3.01 | 30.1 | 120.43 |
| | R2 | 93 | 20 | 31 | 0.5 | 24 | 111 | 141 | 7.88 | 3.04 | 30.4 | 119.78 |
| | R3 | 92 | 19 | 30 | 0.5 | 24 | 112 | 142 | 7.93 | 2.99 | 29.9 | 120.34 |
| | Mean | 93.3333 | 19.3333 | 31 | 0.5 | 23.6667 | 111 | 141.333 | 7.93333 | 3.01333 | 30.1333 | 120.183 |
| SKAU-405 | R1 | 115 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.23 | 2.05 | 20.5 | 130.87 |
| | R2 | 117 | 19 | 38 | 0.6 | 16 | 107 | 133 | 7.41 | 2.27 | 22.7 | 132.45 |
| | R3 | 115 | 18 | 37 | 0.5 | 17 | 106 | 132 | 7.43 | 2.29 | 22.9 | 132.45 |
| | Mean | 115.667 | 18.6667 | 37.6667 | 0.56667 | 16.3333 | 106.667 | 132.667 | 7.35667 | 2.20333 | 22.0333 | 131.923 |
| China-972 | R1 | 100 | 20 | 30 | 0.5 | 16 | 93 | 133 | 7.66 | 2.99 | 29.9 | 99.45 |
| | R2 | 101 | 21 | 31 | 0.5 | 17 | 94 | 132 | 7.59 | 2.89 | 28.9 | 100.11 |
| | R3 | 100 | 20 | 30 | 0.5 | 16 | 93 | 132 | 7.75 | 3.03 | 30.3 | 99.89 |
| | Mean | 100.333 | 20.3333 | 30.3333 | 0.5 | 16.3333 | 93.3333 | 132.333 | 7.66667 | 2.97 | 29.7 | 99.8167 |
| Pusa Sugand-3 | R1 | 75 | 20 | 40 | 1.5 | 19 | 125 | 148 | 7.55 | 2.85 | 28.5 | 45.67 |
| | R2 | 75 | 19 | 43 | 1.5 | 18 | 124 | 147 | 7.89 | 2.97 | 29.7 | 46.99 |
| | R3 | 74 | 20 | 42 | 1.3 | 17 | 126 | 148 | 7.53 | 2.87 | 28.7 | 44.99 |
| | Mean | 74.6667 | 19.6667 | 41.6667 | 1.43333 | 18 | 125 | 147.667 | 7.65667 | 2.89667 | 28.9667 | 45.8833 |
| B-28 | R1 | 86 | 18 | 34 | 0.5 | 24 | 105 | 142 | 7.77 | 2.94 | 29.4 | 96.77 |
| | R2 | 88 | 18 | 33 | 0.5 | 23 | 104 | 142 | 7.87 | 2.89 | 28.9 | 67.88 |
| | R3 | 88 | 18 | 34 | 0.5 | 23 | 104 | 141 | 7.73 | 2.97 | 29.7 | 96.88 |
| | Mean | 87.3333 | 18 | 33.6667 | 0.5 | 23.3333 | 104.333 | 141.667 | 7.79 | 2.93333 | 29.3333 | 87.1767 |
| B-32 | R1 | 105 | 25 | 39 | 0.5 | 8 | 87 | 133 | 7.99 | 2.99 | 29.9 | 111.44 |
| | R2 | 105 | 25 | 39 | 0.5 | 8 | 88 | 133 | 7.84 | 2.98 | 29.8 | 113.67 |
| | R3 | 105 | 24 | 28 | 0.5 | 7 | 87 | 133 | 7.81 | 2.98 | 29.8 | 111.34 |
| | Mean | 105 | 24.6667 | 35.3333 | 0.5 | 7.66667 | 87.3333 | 133 | 7.88 | 2.98333 | 29.8333 | 112.15 |
| B-8 | R1 | 86 | 18 | 34 | 0.5 | 24 | 105 | 142 | 7.77 | 2.94 | 29.4 | 96.77 |
| | R2 | 88 | 18 | 33 | 0.5 | 23 | 104 | 142 | 7.87 | 2.89 | 28.9 | 67.88 |
| | R3 | 88 | 18 | 34 | 0.5 | 23 | 104 | 141 | 7.73 | 2.97 | 29.7 | 96.88 |
| | Mean | 87.3333 | 18 | 33.6667 | 0.5 | 23.3333 | 104.333 | 141.667 | 7.79 | 2.93333 | 29.3333 | 87.1767 |
| B-11 | R1 | 105 | 25 | 39 | 0.5 | 8 | 87 | 133 | 7.99 | 2.99 | 29.9 | 111.44 |

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|----------------|------|---------|----------|----------|----------|----------|---------|----------|---------|----------|----------|----------|
| | R2 | 105 | 25 | 39 | 0.5 | 8 | 88 | 133 | 7.84 | 2.98 | 29.8 | 113.67 |
| | R3 | 105 | 24 | 28 | 0.5 | 7 | 87 | 133 | 7.81 | 2.98 | 29.8 | 111.34 |
| | Mean | 105 | 24.6667 | 35.3333 | 0.5 | 7.66667 | 87.3333 | 133 | 7.88 | 2.98333 | 29.8333 | 112.15 |
| B-24 | R1 | 102 | 18 | 29 | 0.5 | 14 | 104 | 134 | 7.79 | 2.69 | 26.9 | 104.67 |
| | R2 | 102 | 19 | 28 | 0.5 | 13 | 102 | 134 | 8.01 | 2.78 | 27.8 | 103.99 |
| | R3 | 100 | 19 | 28 | 0.6 | 14 | 102 | 135 | 7.77 | 2.72 | 27.2 | 104.89 |
| | Mean | 101.333 | 18.6667 | 28.3333 | 0.53333 | 13.6667 | 102.667 | 134.333 | 7.85667 | 2.73 | 27.3 | 104.517 |
| KAMAD | R1 | 100 | 20 | 30 | 0.5 | 15 | 99 | 136 | 7.77 | 2.95 | 29.5 | 114.03 |
| | R2 | 102 | 19 | 31 | 0.5 | 16 | 100 | 136 | 7.76 | 2.89 | 28.9 | 115.23 |
| | R3 | 100 | 18 | 30 | 0.5 | 17 | 100 | 135 | 7.57 | 2.99 | 29.9 | 113.78 |
| | Mean | 100.667 | 19 | 30.3333 | 0.5 | 16 | 99.6667 | 135.667 | 7.7 | 2.94333 | 29.4333 | 114.347 |
| A3(IRRI LINE) | R1 | 99 | 20 | 35 | 0.5 | 22 | 107 | 131 | 7.99 | 3.01 | 30.1 | 132.45 |
| | R2 | 102 | 24 | 36 | 0.6 | 23 | 105 | 134 | 7.88 | 3.04 | 30.4 | 132.45 |
| | R3 | 103 | 25 | 34 | 0.6 | 20 | 109 | 132 | 7.93 | 2.99 | 29.9 | 99.45 |
| | Mean | 101.333 | 23 | 35 | 0.56667 | 21.6667 | 107 | 132.333 | 7.93333 | 3.01333 | 30.1333 | 121.45 |
| Local Budgam 2 | R1 | 100 | 19 | 37 | 0.6 | 18 | 113 | 135 | 8.11 | 2.73 | 27.3 | 111.44 |
| | R2 | 105 | 22 | 34 | 0.5 | 16 | 109 | 134 | 7.66 | 3.01 | 30.1 | 113.67 |
| | R3 | 106 | 20 | 36 | 0.6 | 15 | 115 | 136 | 7.89 | 3.03 | 30.3 | 111.34 |
| | Mean | 103.667 | 20.3333 | 35.6667 | 0.56667 | 16.3333 | 112.333 | 135 | 7.88667 | 2.92333 | 29.2333 | 112.15 |
| Local Budgam 3 | R1 | 107 | 19 | 35 | 0.6 | 14 | 115 | 138 | 7.89 | 2.97 | 29.7 | 96.77 |
| | R2 | 109 | 18 | 36 | 0.5 | 16 | 113 | 135 | 7.53 | 2.87 | 28.7 | 67.88 |
| | R3 | 108 | 21 | 38 | 0.5 | 17 | 111 | 136 | 7.77 | 2.94 | 29.4 | 96.88 |
| | Mean | 108 | 19.33333 | 36.33333 | 0.533333 | 15.66667 | 113 | 136.3333 | 7.73 | 2.926667 | 29.26667 | 87.17667 |

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. Nasheeman Ashraf, Indian Institute of Integrative Medicine, Govt. of India, Sanantnagar, Srinagar during Viva-Voce examination held on 12-11-2015 have been incorporated in the manuscript entitled **“Identification and Morpho-molecular Characterization of Genotypes of Rice (*Oryza sativa* L.) Showing Resistance to Sheath Blight Disease”** submitted by Ms. Saba Mir (Regd. No. 2013-A-980-M).

(Dr. M. Ashraf Bhat)
Chairman
Advisory Committee